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Interactions, Heterogeneity, and the Determinants of Multispecies Coexistence

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Credits for the illustrations on the cover of each chapter:

- Introduction — Monet, Claude. *Banks of the Seine*. 1880. National Gallery of Art, Washington, DC, USA
- Chapter 1 — Sisley, Alfred. *Meadow*. 1875. National Gallery of Art, Washington, DC, USA
- Chapter 2 — van Gogh, Vincent. *Plain near Auvers*. 1890. Neue Pinakothek, Munich, Germany
- Chapter 3 — van Gogh, Vincent. *Olive trees*. 1889. The Nelson-Atkins Museum of Art, Kansas City, MO, USA
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- Discussion — Corot, Camille. *The Ferryman*. 1865. The Metropolitan Museum of Art, New York, NY, USA

Abstract

No living being is an island. Every creature needs resources from its environment to survive and thrive throughout its life. But in a finite world with over 8.7 million species, acquiring such resources is an inherently competitive endeavour. This has resulted in an extraordinary diversity of interactions, with varying strengths that have either negative or positive consequences for species' performance. Over the last century, ecologists have sought to understand how the balance of these interactions affects a species' ability to persist in a community. Initially, research focused on studying interactions between pairs of species, finding that coexistence is possible when stabilising niche differences, which occur when intraspecific competition exceeds interspecific competition, outweigh fitness differences that favour one competitor over others. However, species typically engage in interactions that go beyond species pairs, making this pairwise framework unsuitable to explore species persistence in complex communities. Besides, one main characteristic of ecological interactions is that they are not set in stone—on the contrary, they vary through space and time in response to modifications in the surrounding conditions. Therefore, new theoretical developments are providing us with tools to comprehend how interactions shape biodiversity in complex, multispecies communities with environmental heterogeneity, which is of paramount importance in the changing world that we inhabit.

In this thesis, I combine the most recent advances in ecological theory with thorough empirical data to explore how different sources of environmental heterogeneity alter biotic interactions between plant species, and in turn how the structure of such interactions shapes the determinants of multispecies coexistence. In the first three chapters, I use a structural stability approach that allows me to investigate the mechanisms of coexistence beyond pairwise combinations of species.

In chapter one, I examine how changes in resources (nitrogen availability) and natural enemies (foliar pathogens) modify the mechanisms of plant diversity and composition. To do that, I quantify the intrinsic growth rates and interaction coefficients between eight common Central European perennial plants in an experiment factorially modifying the nitrogen availability and foliar fungal pathogens of a Swiss perennial grassland. I find that both nitrogen addition and pathogen suppression decrease structural fitness differences that drive competitive dominance but, surprisingly, they also promote niche differences that stabilise the dynamics of interacting species. Interestingly, all effects of resources and enemies on the mechanisms of plant coexistence are dependent on the number of interacting species.

In chapter two, I use data from 8 years and 150 German grasslands to investigate the effects of land use intensification on the growth rates and interactions of 50 perennial plant species. The data shows that increasing land use causes species loss by reducing structural niche differences in a non-linear way, rather than by increasing differences in fitness. However, I also find that niche differences play a role in maintaining coexistence among the species that persist at high land use intensities.

In chapter three, I evaluate the role of different components of interaction networks in shaping structural niche and fitness differences. To do this, I use data from an experiment in a French temperate grassland, where cages to control the abundances and combinations of six grasshopper species were used. I first use group lasso regularisation to distinguish and quantify those pairwise and higher-order interactions between plants and grasshoppers that describe the temporal dynamics of plant species. Detailed analyses show that plant interactions between pairs of species are the main contributor to plant coexistence while the rest of the interaction types (high-order and herbivory) play a little role. In particular, the analysis of different metrics shows that coexistence is more likely in multispecies communities with dominant intraspecific interactions, weak interspecific interactions, and strong facilitation in many cases.

Last, in the fourth chapter, I apply the latest updates in modern coexistence theory that allow me to characterise niche and fitness at the species level, rather than calculating differences between pairs of interacting species. Using five years of data from a salt marsh grassland in Doñana National Park, Spain, I explore the temporal variation of the coexistence mechanisms of 20 annual plants. My results show strong interannual variability in niche and fitness that is independent of species identity or functional traits, and is instead linked to a environmental driver such as rainfall. This finding agrees with the notion that a niche is a multidimensional object but it challenges at the same time the idea of the niche as a purely species-level property, suggesting that it may behave more as a population feature in response to the environment.

Resumen

Ningún ser vivo es una isla. Todas las criaturas necesitan recursos de su entorno para sobrevivir y prosperar a lo largo de su vida. Pero, en un mundo finito con más de 8,7 millones de especies, la obtención de dichos recursos es una tarea competitiva por naturaleza. Esto ha dado lugar a una extraordinaria diversidad de interacciones, con intensidades variadas que tienen consecuencias positivas o negativas para el desempeño de las especies. A lo largo del último siglo, los ecólogos han buscado comprender cómo el balance entre estas interacciones afecta a la capacidad de una especie para persistir en una comunidad. Inicialmente, las investigaciones se centraron en interacciones entre pares de especies, encontrando que la coexistencia es posible cuando las diferencias estabilizadoras de nicho, que ocurren cuando la competencia interespecífica excede la competencia interespecífica, superan las diferencias que favorecen a un competidor sobre otros. Sin embargo, las especies suelen participar en interacciones que van más allá de pares de especies, por lo que este marco conceptual no es adecuado para explorar la coexistencia en comunidades complejas. Además, una de las características principales de las interacciones ecológicas es que no están talladas en piedra, sino que varían en el espacio y en el tiempo en respuesta a modificaciones en las condiciones del entorno. Por lo tanto, recientemente se han desarrollado nuevos marcos conceptuales que nos aportan las herramientas para comprender cómo las interacciones moldean la biodiversidad en comunidades complejas de múltiples especies con heterogeneidad ambiental, lo cual es de suma importancia en el mundo cambiante que habitamos.

En esta tesis, combino los avances más recientes en teoría ecológica con exhaustivos datos empíricos para explorar cómo diferentes fuentes de heterogeneidad ambiental alteran las interacciones bióticas entre especies de plantas y, a su vez, cómo la estructura de dichas interacciones da forma a los determinantes de la coexistencia entre múltiples especies. En los primeros tres capítulos, utilizo un enfoque de estabilidad estructural que me permite investigar los mecanismos de coexistencia más allá de las combinaciones entre pares de especies.

En el primer capítulo, examino cómo cambios en recursos (disponibilidad de nitrógeno) y enemigos naturales (patógenos foliares) modifican los mecanismos de la diversidad y composición de plantas. Para hacerlo, cuantifico las tasas de crecimiento intrínsecas y los coeficientes de interacción entre ocho plantas perennes comunes de Europa Central en un experimento que modifica de manera factorial la disponibilidad de nitrógeno y los hongos patógenos foliares en praderas de Suiza. Descubro que tanto la adición de nitrógeno como la supresión de patógenos disminuyen las diferencias estructurales de capacidad competitiva, pero, sorprendentemente, también promueven diferencias de nicho que estabilizan la dinámica de las especies que interactúan. Curiosamente, todos los efectos de los recursos y los enemigos en los mecanismos de coexistencia de plantas dependen del número de especies que interactúan.

En el segundo capítulo, utilizo datos de 8 años y 150 praderas de Alemania para investigar los efectos de la intensificación del uso del suelo en las tasas de crecimiento e interacciones de 50 especies de plantas perennes. Los datos muestran que el aumento del uso del suelo provoca la pérdida de especies al reducir

las diferencias estructurales de nicho de manera no lineal, en lugar de aumentar las diferencias de capacidad competitiva. Sin embargo, también encuentro que las diferencias de nicho juegan un papel en el mantenimiento de la coexistencia entre las especies que persisten en altas intensidades de uso del suelo.

En el tercer capítulo, evalúo el papel de diferentes componentes de redes de interacción en la determinación de las diferencias estructurales de nicho y capacidad competitiva. Para hacerlo, utilizo datos de un experimento en una pradera de Francia, donde se utilizaron cajas de tela para controlar la abundancia y combinación de seis especies de saltamontes. Primero utilizo regularización ”lasso” de grupo para distinguir y cuantificar las interacciones entre pares y de orden superior entre plantas y saltamontes que describen la dinámica temporal de las especies de plantas. Análisis detallados muestran que las interacciones entre plantas son el principal contribuyente a la coexistencia de las mismas, mientras que el resto de los tipos de interacción (de orden superior y herbivoría) juegan un papel menor. En particular, el análisis de diferentes métricas muestra que la coexistencia es más probable en comunidades multiespecie con dominancia de interacciones intraespecíficas, interacciones interespecíficas débiles y fuerte facilitación en muchos casos.

Por último, en el cuarto capítulo, aplico las últimas actualizaciones en teoría moderna de coexistencia que me permiten caracterizar el nicho y la capacidad competitiva a nivel de especie, en lugar de calcular las diferencias entre especies que interactúan. Utilizando datos de siete años de una marisma en el Parque Nacional de Doñana, en España, exploro la variación temporal del nicho de 20 plantas anuales. Mis resultados muestran que la variabilidad interanual del nicho es independiente de la identidad de la especie o de sus características funcionales, sino que está vinculada a la abundancia de las especies y a la precipitación invernal. Este hallazgo concuerda con la noción de que el nicho es un objeto multidimensional, pero al mismo tiempo desafía la idea del nicho como una propiedad puramente a nivel de especie y sugiere que puede comportarse más como una característica poblacional en respuesta al ambiente.

Por último, en el cuarto capítulo, aplico las últimas actualizaciones en teoría moderna de coexistencia que me permiten caracterizar el nicho y la capacidad competitiva a nivel de especie, en lugar de calcular las diferencias entre pares de especies que interactúan. Utilizando cinco años de datos de una marisma en el Parque Nacional de Doñana, España, exploro la variación temporal de los mecanismos de coexistencia de 20 plantas anuales. Mis resultados muestran una fuerte variabilidad interanual en el nicho y la capacidad competitiva que es independiente de la identidad de las especies o de sus características funcionales, y se relaciona en cambio con un factor ambiental como la lluvia. Este hallazgo coincide con la idea de que el nicho es un objeto multidimensional, pero al mismo tiempo desafía la idea del nicho como una propiedad puramente a nivel de especie, sugiriendo que puede comportarse más como una característica poblacional en respuesta al ambiente.

Á miña nai e ao meu pai,
polo apoio constante e por poñer, por enriba de todo, a miña educación.

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polo onte, o hoxe, e o mañá á túa beira; mesmo perto que lonxe.

Á educación pública española,
por case *todo o que son.*

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Introduction

Biodiversity maintenance: Solving the paradox of the plankton

Planet Earth is home to over 8.7 million living species of bacteria, archaea, protists, fungi, plants, and animals (Mora et al., 2011), yet only 1.2 million of them have been catalogued to this date. Such diversity of life has fascinated humanity for centuries, but it was not until the advent of ecology as a distinct discipline that we began to uncover the mechanisms by which multiple species are able to coexist.

Ecologists began by trying to explain the observed natural associations between species and environmental variation. One of the foundational concepts in community ecology is the species' niche, first introduced by Joseph Grinnell (1917) as the range of environmental conditions under which a species could survive. But this concept, far from uniform, has a long history of developments and mutations. Soon after Grinell's definition, Charles Elton (1927) independently defined the niche as the function or role that a species plays in a community, mainly through interactions with food and predators. Not much later, Georgy Gause (1934) proposed a connection between both abiotic and biotic-functional definitions of the niche. This inspired George E. Hutchinson (1957) to conceptualise the niche as a multi-dimensional space formed by all the environmental factors that a species endures, which is the base of our current understanding of this fluctuating, complex ecological concept.

In parallel with the development of the niche concept, a competitive framework for community ecology was being gestated. Together with the formulation of Alfred Lotka (1925)'s and Vito Volterra (1926)'s famous mathematical models on competition between two different species (i.e., interspecific competition), came the prediction that two species competing for a single resource may not coexist. This prediction was later experimentally demonstrated by Gause (1934), who showed that two protists growing on a single resource in controlled conditions can achieve stable populations in isolation but one of them excludes each other in combination. This phenomenon of two competing species being unable to coexist on a single resource was subsequently known as Gause's competitive exclusion principle.

With both these concepts in mind, Robert H. MacArthur and Richard Levins (MacArthur and Levins, 1967) attempted to understand the coexistence of competing species by expanding on the Hutchinsonian niche and proposing the concept of limiting similarity, which can be defined as the minimal difference in species niche requirements that allows them to coexist; or else, the maximum niche overlap. By simplifying the multidimensional space and focusing on a few critical niche axes in which competition occurs (MacArthur 1969), this approach showed a way to link the niche overlap to the processes of competitive exclusion in a quantitative manner (MacArthur, 1972), in both deterministic and fluctuating environments (May and MacArthur, 1972; May, 1973).

These ideas conceived species coexistence mainly through competition for limited resources: two species needed to display enough niche differences to reduce their interspecific competition, given that a high niche overlap would lead to the competitive exclusion of one of the species. Therefore, an ecological system could support as many species as there were available niches, i.e., n different factors or resources could accommodate n species. However, this led to a paradox, formally stated for the first time by Hutchinson (1961) as the paradox of the plankton: like the dozens of plankton species surviving on a few critical resources, we usually encounter situations in which the number of available niches an ecological system offers is not enough to explain the diversity of species found, i.e., there are more species than predicted by the niche dimensionality. Thus, the capacity of niche differentiation to structure ecological communities must be limited and other factors may be at play. From this point further, theoretical and empirical developments can be generalised as the attempt of community ecologists to solve the paradox of the plankton.

There are two main frameworks aiming to understand species diversity by using niches and competitive interactions as their core concepts: contemporary niche theory (Chase and Leibold, 2003) and modern coexistence theory (Chesson, 2000). The first one originates from MacArthur's introduction to consumer-resource models (MacArthur, 1970). These models are considered mechanistic because, by narrowing down the Hutchinsonian multidimensional niche and focusing on a few important axes (e.g., two limiting resources), they provide an explicit basis for species coexistence. This framework was first popularised by David Tilman (1982)'s prominent work on resource competition between algae, which showed that the coexistence of two species competing for two different resources requires interspecific trade-offs in the use of such resources in stable environments. Beyond that, when the competition of multiple species involves more than two resources (three or more), more complex oscillatory and chaotic dynamics arise to allow the persistence of the community (Huisman and Weissing, 1999). Jonathan Chase and Mathew Leibold are responsible for the formalisation of the theory (Chase and Leibold, 2003) and for expanding it to include a myriad of biotic and abiotic resources. However, given such an explicit treatment of coexistence mechanisms, contemporary niche models require a profound knowledge of the study system to identify the important axes of niche differentiation and sometimes lack accuracy in their predictions, presumably due to the presence of unaccounted niche axes or stochastic dynamics (Letten et al., 2017).

As opposed to these mechanistic models, modern coexistence theory uses a phenomenological approach based on Lotka-Volterra (or equivalent) models (Chesson, 2000; Adler et al., 2007). The use of such phenomenological models carries certain advantages. For example, these models need little prior understanding of the study system, which makes them more easily applicable from an empiricist's perspective; and they usually show greater accuracy in describing coexistence outcomes. However, by applying them we gain no direct understanding of the underlying mechanisms at play (e.g., relevant resources or interactions), i.e., it is not possible to directly link natural history features to differences in niche and competitive ability (Letten et al., 2017).

Modern coexistence theory

Modern coexistence theory defines two main mechanisms to explain coexistence: stabilising mechanisms that reduce niche overlap but also equalising mechanisms that reduce average fitness differences between competitor species (Chesson, 2000). The main success of this theory is that it provides the tools to quantify such mechanisms from any phenomenological model describing the population dynamics of a species, i, in terms of its inherent capacity to grow (i.e., intrinsic growth rates, r_i), its interaction with individuals of another species, j (interspecific interaction, α_{ij}), and the interactions between individuals of the same species (intraspecific interactions, α_{ii}). This framework uses invasion analyses to determine when coexistence can be achieved: starting at low density, species i need to be able to invade a community composed by species j at equilibrium density, and so has to be species j as an invader in an equilibrium-population of species i. This, referred to as the mutual invasibility criterion, implies that $\alpha_{ii} > \alpha_{ij}$ and $\alpha_{jj} > \alpha_{ji}$, i.e., coexistence can occur when intraspecific interactions are stronger than interspecific interactions (Chesson, 2000; Barabás et al., 2016). Thus, using a suitable population model to describe the growth curves of i and j, we are able to quantify their niche overlap, p, as the square root of the ratio of interspecific and interspecific interactions. From here, calculating niche differences (ND) only takes deriving the complement of the niche overlap to reach 1 $(1-p)$:

$$\mathrm{ND} = 1 - \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{ii}\alpha_{jj}}}. \tag{0.1}$$

Note that the measurement of niche differences is independent of the intrinsic growth rate of the species involved. It is called a stabilising effect because it emerges from a species' ability to limit itself more than it limits others: the lower the niche overlap, the higher the niche differentiation, making coexistence more likely.

But the invasion analysis does not only allow us to quantify niche differentiation. In the absence of interactions, a given species in low initial density can grow at a given pace, which is defined as its intrinsic growth rate (r). When invading, r is modified by intra- and interspecific interactions, shaping the species' invasion ability. The difference in the ability between a pair of species is known as their fitness differences (FD) and can be calculated as

$$\mathrm{FD} = \frac{r_i}{r_j}\sqrt{\frac{\alpha_{jj}\alpha_{ji}}{\alpha_{ii}\alpha_{ij}}}, \tag{0.2}$$

with r_i and r_j being the intrinsic growth rate of species i and j, respectively. Note that the intrinsic growth rates must be always positive, as a species with a negative growth rate can never invade and is subsequently lost from the community (eqn. 0.2). Following this, modern coexistence theory establishes that the coexistence of two species is not only determined by their niche differences but also by their differences in fitness or competitive ability, as per the inequality

$$1 - \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{ii}\alpha_{jj}}} > \frac{r_i}{r_j}\sqrt{\frac{\alpha_{jj}\alpha_{ji}}{\alpha_{ii}\alpha_{ij}}}. \tag{0.3}$$

Thus, the fate of a pairwise interaction is determined by a combination of their stabilising and equalising effects: if the equalising fitness differences can be accommodated by the stabilising niche differences, the interacting species are predicted to coexist (eqn. 0.3).

Heterogeneity and species coexistence

Despite its phenomenological nature, the framework of modern coexistence theory provided us with the tools to quantify stabilising and equalising mechanisms across a range of environments and ecological systems. This led to an explosion of studies over the last two decades, as attested by the >6,000 citations that Peter Chesson (2000)'s foundational paper has on Google Scholar as of this thesis' release date. Among such studies, we find a combination of theoretical, computational, and empirical efforts that explore and attempt to explain long-lasting questions on how heterogeneity (e.g., genetic, spatial, temporal, abiotic, or biotic) shape the ecological interactions that maintain species diversity. Below I provide some of the most relevant examples, although the diversity of topics is nearly boundless.

Adler et al. (2010) found that the stabilising mechanisms in a study of perennial plant species in Idaho (USA) were incredibly strong, far more robust than necessary to ensure coexistence. This was due to the small growth rates and even negative growth rates observed when these mechanisms were removed. Similarly, Wainwright et al. (2019) discovered that the coexistence of semi-arid annual plant communities under varying water availability was largely driven by differences in sensitivity to competition, with these differences being much greater than the stabilising niche differences. Adler et al. (2006) also found that climate variability has a stabilising effect on the coexistence of prairie grasses, with changes in both means and variances of climate variability across 30 years in a Kansas prairie. Additionally, the work of Godoy et al. (2020) showed that the coexistence of diverse plant communities is determined by a combination of niche differences and fitness differences, and that the mechanisms determining coexistence also maximise ecosystem functioning. This is demonstrated by an excess of niche differences resulting in more biomass and faster decomposition rates under drought conditions.

As found by Johnson et al. (2012), the relative abundance of tree species in eastern United States forests is heavily influenced by negative interactions between individuals of the same species. This discovery suggested a general mechanism for maintaining diversity in forests. Chesson and Kuang (2008) discovered that predation holds the same potential to promote or hinder species coexistence as competitive interactions, and thus, suggested that a multitrophic perspective considering interactions between competitors, prey and predators was needed to gain a more accurate understanding of species coexistence. Also, Stump and Chesson (2015) found that habitat partitioning, the process by which different species occupy and utilise different portions of their shared environment, can result in a phenomenon known as conspecific attraction, which is characterised by an increase in the recruitment of individuals near conspecific adults, even in the presence of distance-responsive predators, and is independent of any changes in the predatory effects on coexistence among the different species. This contradicted the Janzen-Connell hypothesis, which established that specialist predators maintain diversity by predating on seeds and seedlings near conspecific adults (Janzen, 1970; Connell, 1971). Fragata et al. (2022) found that the order in which two spider mite species arrive on tomato plants affects their competitive ability and outcome of competition, leading to spatial niche pre-emption when the inferior competitor arrives first, reducing fitness and niche differences, and resulting in a close-to-neutrality scenario for the community assembly.

According to Godoy et al. (2017), the prevalence of an indirect interaction such as intransitive competition (i.e., rock-paper-scissor dynamics in which one competitor is both winner and loser with respect to different members of the community) as a mechanism of species coexistence is less frequent than previously thought. They found that even when it does operate, pairwise niche differences are key to predicting coexistence. Matías et al. (2018) discovered that extreme climate events such as drought can result in annual plant communities with reduced niche and fitness differences in which species are less likely to coexist, even if the prevalence of intransitive interactions increases. Regarding functional traits, Kraft et al. (2015*b*) found that individual traits of plants correlate with fitness differences but do not necessarily drive the stabilising niche differences that promote coexistence among plant species. Instead, stabilising niche differences can only be described by combinations of traits. Moreover, Pérez-Ramos et al. (2019) found that functional traits, such as water- and light-use-efficiency, play a significant role in modulating plant competitive dynamics and promoting species coexistence across climatic conditions by enhancing stabilising niche differences and generating competitive trade-offs between species in annual plants. Particularly, Godoy and Levine (2014) found that phenology in annual plants promotes stabilising niche differences between exotic and native species, but that it is more strongly related to competitive ability differences, resulting in later invaders outcompeting earlier native competitors and native residents outcompeting earlier invaders. Narwani et al. (2017) found that, contrary to expectation, similarity in gene expression in phytoplankton species, rather than differentiation, was associated with weaker competition and facilitation, leading to increased coexistence among species.

The study of species coexistence in microorganisms has yielded intriguing insights into the factors that determine the ability of different species to coexist in a given environment. Violle et al. (2011) reported that species relatedness can serve as a good proxy for understanding coexistence outcomes in protists. However, other research has challenged this notion, with Narwani et al. (2013) finding that the coexistence of competing species of green freshwater algae is more strongly determined by niche differences than by fitness differences and that evolutionary relatedness (phylogenetic distance) has no effect on either coexistence or the sizes of niche and fitness differences. Shortly later, using an annual plant community, Godoy et al. (2014) found that phylogenetic distance did not link to stabilising niche differences and, in fact, increased the variability of equalising fitness differences, thereby questioning the prediction of higher coexistence between non-close relatives. Finally, in an interesting study, Aoyama et al. (2022) have recently defined a blueprint for how to use modern coexistence theory to secure restoration success, which can pave the way to the use of modern coexistence theory as a guideline for future real-life restoration efforts.

Some limitations of modern coexistence theory and alternatives

One key problem of modern coexistence theory is that its prolificacy has led to the emergence of different mathematical definitions of the stabilising and equalising mechanisms. The ten different definitions reviewed by Spaak and De Laender (2020), usually produced inconsistent outputs, making them hardly incomparable (Spaak et al., 2022). To solve this, the same authors proposed flexible, more biologically realistic definitions for the stabilising niche and equalising fitness differences that apply to any mathematical model and can be compared across any ecological system eligible to fulfil the invasion criterion (Spaak and De Laender, 2020). One particularly interesting property of these new definitions is that, even if their calculation depends on the characteristics of other species in the community, the niche and fitness differences are calculated at the species level and not at the pairwise level as in most of the previous definitions (Chesson, 2000; Spaak and De Laender, 2020; Adler et al., 2007). This allows us to easily compare species in a community and understand their persistence irrespective of their pairwise interactions with each member of the community. To date, these definitions have been used, for instance, to predict the short-lasting effects of pigment richness on phytoplankton richness (Spaak and De Laender, 2021) or to show that niche differences are behind coexisting species across ecological groups (Buche et al., 2022). Further details about these definitions can be found in Spaak and De Laender (2020) and Spaak et al. (2021), and this methodology is used in Chapter 4 of this thesis to understand the temporal niche and fitness variation in annual plant species.

Another key problem that limits the applicability of modern coexistence theory, however, is that Chesson (2000)'s definitions are limited to interactions between species pairs as its underlying algebraic equations encounter a circularity problem when extended to combinations of multiple simultaneously interacting species (Saavedra et al., 2017), making it unsuitable to explore the mechanisms of species coexistence in multispecies, diverse natural communities. Although there are some available methods (the aforementioned definitions by Spaak and De Laender (2020) are among them), one of the main developments in understanding species coexistence beyond pairwise mechanisms has been the transition from an algebraic approach to a geometric structuralist approach. To do so, Saavedra et al. (2017) have developed structural analogous to niche and fitness differences that allow us to quantify coexistence for systems of n-competing species. An introduction to these structural metrics can be found below.

Structural stability and the coexistence of multiple interacting species

Given an ecological community formed by three interacting species, i, j and k (Fig. 0.1a), we can use the structural stability approach to understand the mechanisms of coexistence between every pairwise combination in such community (i.e., ij, ik

and jk) or we can quantify the coexistence mechanisms between the three species simultaneously (i.e., the triplet ijk). To observe the conceptual parallelisms between modern coexistence theory and the structural mechanisms, we can begin at the pairwise scenario, using the pair ij as an example. As well as for modern coexistence theory, we need the coefficients that describe the interactions between the species involved (i.e., the intraspecific effects α_{ii} and α_{jj} and the interspecific effects α_{ij} and α_{ji}) together with the intrinsic growth rates, r_i and r_j, of each species. Provided this, we can plot the whole 2-species intrinsic growth rate parameter space as shown in Fig. 0.1b. Within this space, we are able to represent simultaneously the slope of the interaction effects exerted by species i (α_{ii}/α_{ji}) and species j (α_{jj}/α_{ij}), in a way that both slopes delimit a cone-shaped space of intrinsic r_j/r_i growth rates. Thus, the condition for finding a feasible equilibrium point that leads to the coexistence of the pair of species is described by the inequalities

$$\frac{\alpha_{jj}}{\alpha_{ij}} > \frac{r_j}{r_i} > \frac{\alpha_{ii}}{\alpha_{ji}}. \tag{0.4}$$

The parameter space between the two slopes is known as the feasibility domain, and is defined as the range of intrinsic growth rates, r, compatible with the occurrence of a feasible equilibrium point given the structure of species interactions, α.

From this point, we can proceed to derive the metrics of pairwise coexistence from a geometrical approach. The structural niche differences (SND) correspond to the measurement of the whole set of growth rates compatible with a feasible equilibrium, i.e., measuring the feasibility domain, which can be done by computing the normalised solid angle between the slopes of α_{ii}/α_{ji} and α_{jj}/α_{ij} as:

$$\mathrm{SND} = \frac{2}{\pi}\arcsin\left(\frac{\alpha_{ii}\alpha_{jj} - \alpha_{ij}\alpha_{ji}}{\sqrt{(\alpha_{ii}^2 + \alpha_{ji}^2)}\sqrt{(\alpha_{jj}^2 + \alpha_{ij}^2)}}\right). \tag{0.5}$$

In this 2-species structural calculation, the normalised solid angle determining SND is equal to 1 when all combinations of intrinsic growth rates lead to a feasible equilibrium and tends to zero as the feasibility domain decreases. This approach offers a probabilistic vision of species coexistence, as different growth rates can tolerate different variations without losing their feasible condition. The set of growth rates that can change the most without becoming unfeasible coincides with the centroid of the feasibility domain, r_c, and is calculated as follows:

$$r_c = \frac{1}{2}\left(\frac{1}{\sqrt{\alpha_{ii}^2 + \alpha_{ji}^2}}\begin{bmatrix}\alpha_{ii}\\ \alpha_{ji}\end{bmatrix} + \frac{1}{\sqrt{\alpha_{jj}^2 + \alpha_{ij}^2}}\begin{bmatrix}\alpha_{jj}\\ \alpha_{ij}\end{bmatrix}\right). \tag{0.6}$$

Therefore, the 2-species structural fitness differences (SFD) can be defined as the deviation of r from r_c, so the closer r is to the optimum set of intrinsic growth rates determined by the structure of species interactions, the lesser the SFD:

$$\text{SFD} = \arccos\left(\frac{r \cdot r_c}{\|r\| \cdot \|r_c\|}\right) \tag{0.7}$$

This approach can be applied to all three pairs found in our three-species community. To simplify, the feasibility domains of the three pairs of species can be visualised in a unit simplex triangle (Fig. 0.1c), which is a generalisation with unit area to n-dimensions. In this triangle, each side represents the full domain of intrinsic growth rates between the corresponding vertex species in such a way that allows us to project the feasibility domain between the pair of species involved. The pedagogical advantage of this simplex representation is that, by projecting the uni-dimensional feasibility domain of each one of the three pairs (light green cones) into the two-dimensional inner parameter space of the triangle, one can easily observe the range of 3-species intrinsic growth rate vectors compatible with different feasible equilibrium points. Importantly, the overlap of the projections of the different feasibility domains signals the common set of intrinsic growth rates compatible with feasible combinations of species, and may include a common domain in which all three domains overlap called pairwise feasibility domain (darker shade of green). As an example, in Fig. 0.1c, the 3-species intrinsic growth rates vector, r (orange dot), lies within the overlapping region of the feasibility domains of pairs ik and jk. Extending the feasibility condition in eqn. 0.4 and as seen in Fig. 0.1b, this means that there are chances for the coexistence of all three species if they interact in ik and jk pairs, but the pair ij alone would not be able to achieve a feasible equilibrium provided the current intrinsic growth rates.

But the main contribution of the structural stability approach is the ability to calculate SND and SFD for n-dimensional systems. Fig. 0.1d shows the full positive parameter space of intrinsic growth rates for our three-species community. Similarly to the 2-species case, the structure of the matrix of species interactions, α, determines a range of feasible solutions that can accommodate the differences in intrinsic growth rates, r. Calculations shown in eqn. 0.5 through eqn. 0.7 are for the specific case of a 2-species community; the structural coexistence mechanisms generalised for n number of species can be calculated as follows:

$$\text{SND} = \frac{|\det(\alpha)|}{\sqrt[n]{\pi/2}} \int \cdots \int_{R^n_{\geq 0}} e^{-x^T \alpha^T \alpha x} dx. \tag{0.8}$$

$$r_c = \frac{1}{n}\left(\frac{\mathrm{v}_i}{\|\mathrm{v}_i\|} + \frac{\mathrm{v}_j}{\left\|\mathrm{v}_j\right\|} + \cdots + \frac{\mathrm{v}_n}{\|\mathrm{v}_n\|}\right). \tag{0.9}$$

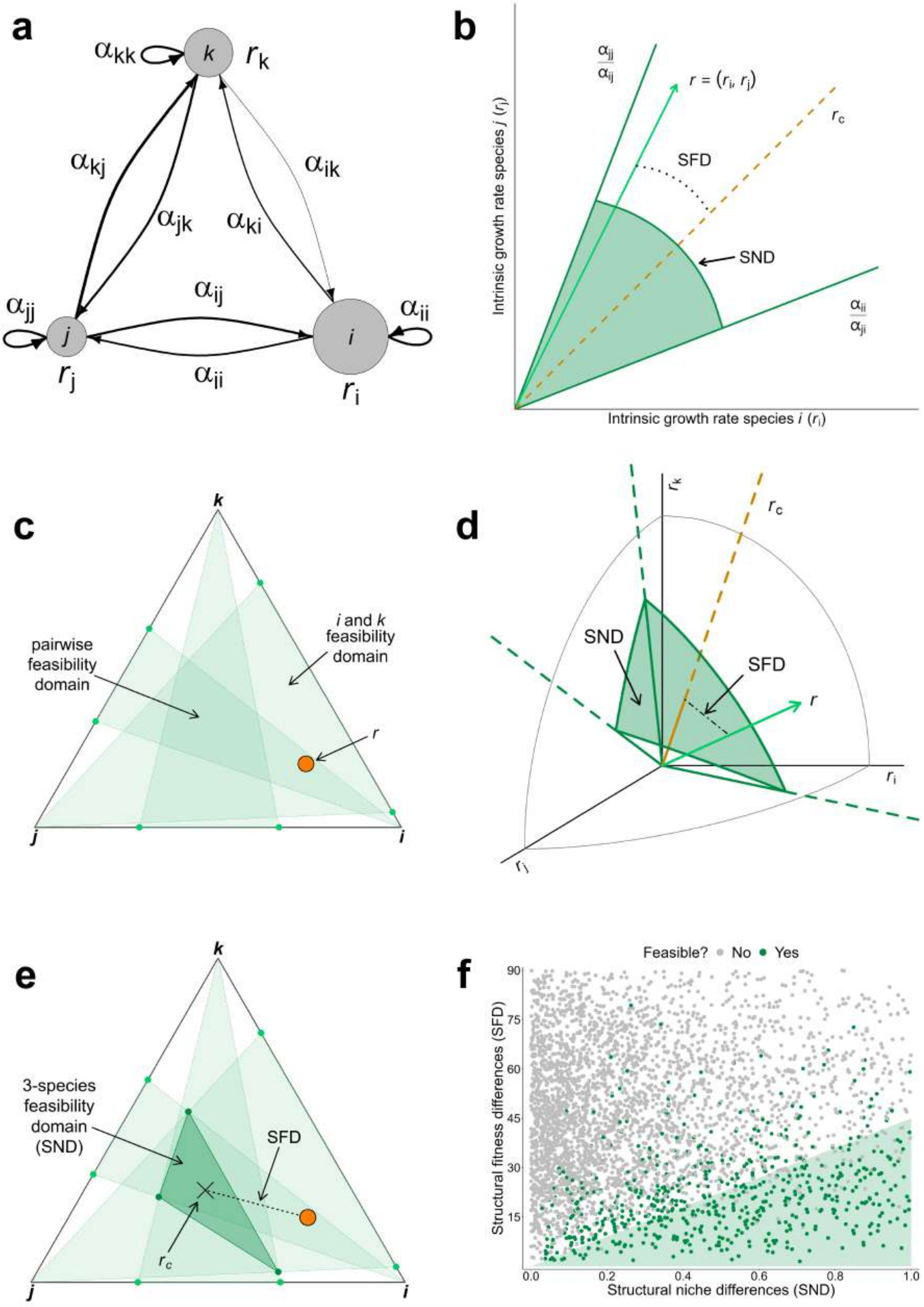

Figure 0.1: Graphical representation of the structural stability approach to multispecies coexistence. **a**, three-species (i, j, and k) interaction network, with node size representing the strength of intrinsic growth rates (r_i, r_j, and r_k) and link width representing the strength of intraspecific (α_{ii}, α_{jj}, and α_{kk}) and interspecific (α_{ji}, α_{ki}, α_{ij}, α_{kj}, α_{ik}, and α_{jk}) interaction coefficients. *(Continued.)*

Figure 0.1: *(Continued.)* **b**, graphical description of the calculation of structural niche differences (SND) and structural fitness differences (SFD) for the pair ij in panel **a**. The dark green slopes $\alpha_j j/\alpha_i j$ and $\alpha_i i/\alpha_j i$ limit the feasibility domain, which corresponds to the SND or the normalised solid angle between both slopes (dark green curved line). The SFD can be calculated as the angle between the centroid (r_c) or the feasibility domain and the vector formed by the intrinsic growth rates of both species (r). **c**, geometric projection on the unit simplex for the feasibility domains (light green triangles) between the three species in **a** (e.g., between i and k as pointed in the panel). The overlap between the three feasibility domains creates a region called pairwise feasibility domain, which indicate the range of intrinsic growth rates (r) that are compatible with the coexistence of the three pairs of species. In this example, r lies on the region where the feasibility domains of jk and ik overlap, determining the feasibility for these pairs but not for the pair ij. **d**, Expansion of the pairwise structural approach for multispecies communities. Instead of two axes, we now have a parameter space of n axes formed by the r of the n species involved (in this example, the three species in panel **a**). As seen in panel **b**, the structure of species interactions defines a multidimensional parameter space of intrinsic growth rates compatible with the feasibility of the n species, the multispecies feasibility domain (green hypervolume), the solid angle of which corresponds to the measurement of multispecies SND. SFD corresponds to the angle between the vector of intrinsic growth rates, r, and the centroid of the feasibility domain r_c. **e**, geometric projection on the unit simplex for the three-species feasibility domain, with indication of the approximation of SND and SFD in this representation. In this example, the three species are not predicted to coexist as the SFD are larger than the SND (r does not lie inside of the 3-species feasibility domain). Comparing the size and shape of the multispecies feasibility domain with the pairwise feasibility domain can be a powerful tool to understand the role of indirect interactions, which only emerge in multispecies assemblages, to promoting coexistence. Finally, **f** provides a representation of the relationship between SND and SFD in a number of random 3-species communities, together with their feasibility predictions. The green triangle represents the region of SND and SFD values for which a pairwise combination is predicted feasible. Comparing the 3-species feasibility predictions with the 2-species feasibility predictions allows us to understand the importance of indirect interactions, as the limit that determines feasibility is not as clear for 3 as it is for 2 species. Panels **b**—**e** after Saavedra et al. (2017).

$$\text{SFD} = \arccos\left(\frac{r \cdot r_c}{||r|| \cdot ||r_c||}\right). \tag{0.10}$$

Using the same simplex triangle as for the 2-species approach (Fig. 0.1c), we can now visualise the feasibility domain for the triplet (dark green; Fig. 0.1e). Note that, in this example, the multispecies feasibility domain differs with respect to the pairwise feasibility domain. The only reason this happens is the effect of indirect interactions that only emerge in multispecies systems, such as intransitivity (Saavedra et al., 2017; Godoy et al., 2017; Soliveres et al., 2015). The best example of an intransitive loop of indirect interactions is the well-known game of rock-paper-scissors, where all components lose and win against one other component of the network. Take only two of the three and one will exclude the other, but take the three of them together and they reach a feasible equilibrium. Therefore, through the structural stability approach it is possible to quantify the contribution of indirect effects to multispecies coexistence by comparing the pairwise and the multispecies feasibility domains through two different metrics.

First, by comparing the relative size of the multispecies versus the pairwise feasibility domain, we are able to understand whether indirect interactions hinder or help promote coexistence opportunities. This metric, named community-pair differential by Saavedra et al. (2017) (hereafter, differential), takes continuous values from -1 to 1, with negative values indicating less and positive values indicating more coexistence opportunities in multispecies assemblages compared to the pairwise approach. Second, by analysing the percentage of the multispecies feasibility domain that lies inside of the pairwise domain we can understand the contribution of pairwise mechanisms to explaining multispecies coexistence opportunities. This metric was named community-pair overlap (hereafter, overlap), and takes values from 0 (when there is no overlap between the two domains) to 1 (when the whole multispecies feasibility domain lies inside the pairwise domain). Note that, although both metrics are correlated (for example, the higher the differential, the lower the overlap will tent to be), they describe well differentiated mechanisms driven by indirect interactions.

Finally, Fig. 0.1f shows feasibility predictions for a number of 3-species communities, with random α and r coefficients, in relation to their calculated multispecies SND and SFD. Additionally, the green triangle in the background of the panel indicates the feasibility condition for any 2-species community (i.e., any pair of species showing SND and SFD values within this area would be predicted feasible, and vice versa). By comparing how the 2-species and the 3-species behave to determine coexistence, we observe that, unlike the hard transition from feasibility to unfeasibility seen for species pairs, the 3-species communities show a transition region in which the feasibility prediction is uncertain and seemingly independent of the structural niche and fitness differences. This phenomenon is evidence of the importance of indirect interactions on determining the feasibility of multispecies communities.

Further details on the structural stability approach can be found in Rohr et al. (2014), Saavedra et al. (2017), and Song et al. (2018). For a graphical illustration of this approach, I strongly recommend the interactive shiny app by Petry and Lepori (2022), available at the following web address as of February, 2023: https://ecodynamics.shinyapps.io/StructuralCoexistence. Importantly, the R code to calculate the multispecies mechanisms of species coexistence is available through Saavedra et al. (2017) and also through Granjel et al. (2022).

To date, most advances around the structural coexistence framework remain theoretical or apply a combination of theory and demonstrations using existing empirical datasets (Cenci and Saavedra, 2018, 2019; Song and Saavedra, 2018; Song et al., 2020*b*; Saavedra et al., 2020; Medeiros et al., 2023). For example, in their original paper, Saavedra et al. (2017) used a dataset previously analysed under modern coexistence metrics (Godoy and Levine, 2014; Godoy et al., 2014; Kraft et al., 2015*a*) to show that coexistence predictions increase under a structural perspective. Only a handful of *ad hoc* empirical studies have been carried on. One of the first results was found by Petry et al. (2018), who showed that a competition-defence trade-off, where ants preferentially consume bigger, competitive dominant seeds in an annual plant community with stabilising niche differences, did not increase diversity but reduced coexistence by disadvantaging and even excluding the superior competitor through overconsumption, particularly in multispecies assemblages. García-Callejas et al. (2021) explored the role of spatial variability in the coexistence of an annual marshland, finding that spatial heterogeneity plays little role in the accumulation of species richness with increasing spatial scale; instead, more interactions are realised and species coexist through direct rather than indirect effects. Working with microbes, Deng et al. (2021) found that non-resident species are likely to change the game rulebook of resident communities, establishing and suppressing resident species under changing environments. Also, Tabi et al. (2020) revealed that, at warmer temperatures, microbial communities show relatively more asymmetry in their feasibility domain and idiosyncrasy of responses compared with those communities at cooler temperatures. In a resolute multitrophic manipulation experiment, Bartomeus et al. (2021) showed that the persistence of plants increases when accounting for plant-pollinator interactions and that multitrophic structure manipulation results in unpredicted changes in persistence probabilities. Johnson et al. (2022), however, found that competition for pollinators destabilises interactions and plant coexistence, and competitive imbalances are expected to increase as pollinators are lost, hindering the coexistence of assemblages of 3, 4 and 5 species. Also for pollinators, recently Arroyo-Correa et al. (2023) found that communities with high plant species-level degree and low overlap among species are more feasible and that interindividual variation plays an important role in promoting feasibility by lessening the overlap in pollinator use among plant species, with communities composed of variable populations being 28% more feasible than communities comprising similar individuals.

Brief statement on knowledge gaps and research opportunities

Although hundreds of different theoretical and empirical studies emerged after modern coexistence theory was formalised with Chesson (2000), and therefore we have acquired a well-rooted understanding of how abiotic and biotic heterogeneity shapes the mechanisms of coexistence between pairs of species, the current state of the literature reveals clear gaps in our knowledge on how coexistence mechanisms operate in multispecies, more realistic species communities (Levine et al., 2017). Recent efforts have shed light on different key aspects of the ecology of natural communities, but we are lacking studies on the role of main drivers of diversity in ecological systems, such as fluctuations in resources and habitat availability, natural enemies, or temporal variability, in determining the fate of multiple interacting species. Overall, the latest theoretical developments in community ecology offer valuable tools to comprehend the mechanisms of species persistence and biodiversity maintenance in complex ecosystems, and enable us to connect these findings to the underlying abiotic and biotic heterogeneity in the face of global change.

Objectives

The general goal of this thesis is to combine the most recent developments in ecological theory with thorough empirical data to begin to understand how species interactions shape the mechanisms of species coexistence in response to different sources of biotic and abiotic environmental heterogeneity. I address fundamental questions regarding the consequences for biodiversity of resource availability, natural enemies, land use intensity, different types of interactions, and temporal variability. Specifically, I propose the following objectives:

- In **Chapter 1**, I aim to examine how changes in resources (nitrogen availability) and natural enemies (foliar pathogens) modify the mechanisms of plant diversity and composition. Specifically, I attempt to elucidate whether the mechanisms of multispecies plant coexistence differ in their response to resource availability and natural enemies from the mechanisms of coexistence between pairs of species. Also, I seek to measure the contribution of indirect interactions between plant species to explain the multispecies mechanisms of coexistence.

- In **Chapter 2**, I seek to investigate the effects of land use intensification on the mechanisms of multispecies coexistence. In particular, I intend to understand whether the pattern of species loss that generally occurs following land intensification is explained by a reduction in structural niche differences that promote coexistence through self-limitation or by an increase in the structural fitness differences that favour one species over the others.

- In **Chapter 3**, I propose to evaluate the role of different components of interaction networks in shaping structural niche and fitness differences. In detail, I aim to analyse the contribution of direct interactions between plants, higher-order interactions between plants, direct interactions between plants and herbivores such as grasshoppers, and higher-order interactions between plants and grasshoppers, to the multispecies mechanisms of plant coexistence. Moreover, I attempt to describe how different metrics of interaction configurations (sign, strength distribution, and intraspecific vs. interspecific effects) predict the observed structural niche and fitness differences between plant species.

- In **Chapter 4**, I attempt to apply the latest developments in modern coexistence theory to characterise niche and fitness at the species level, rather than calculating differences between interacting species. With this in mind, I seek to describe the temporal variation in a plant's niche and fitness and understand whether such variation can be explained by species-specific features (e.g., functional traits) or whether it responds better to environmental characteristics (e.g., rainfall).

Nitrogen enrichment and foliar fungal pathogens affect the mechanisms of multispecies plant coexistence

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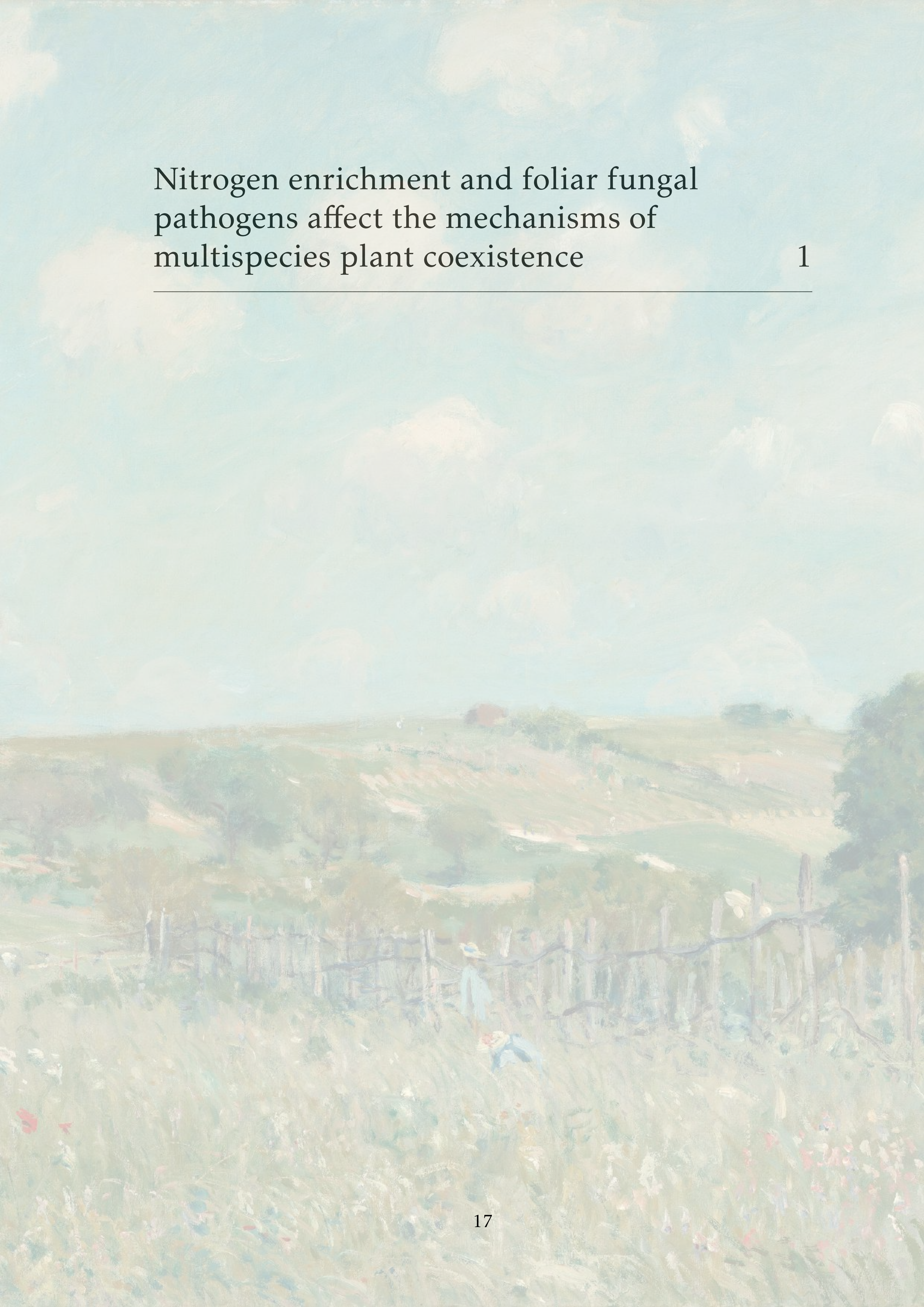

Abstract

Changes in resources (e.g., nitrogen) and enemies (e.g., foliar pathogens) are key drivers of plant diversity and composition. However, their effects have not been connected to the niche and fitness differences that determine multispecies coexistence. Here, we combined a structural theoretical approach with a detailed grassland experiment factorially applying nitrogen addition and foliar fungal pathogen suppression to evaluate the joint effect of nitrogen and pathogens on niche and fitness differences, across a gradient from two to six interacting species. Nitrogen addition and pathogen suppression modified species interaction strengths and intrinsic growth rates, leading to reduced multispecies fitness differences. However, contrary to expected, we also observed that they promote stabilising niche differences. Although these modifications did not substantially alter species richness, they predicted major changes in community composition. Indirect interactions between species explained these community changes in smaller assemblages (three and four species) but lost importance in favour of direct pairwise interactions when more species were involved (five and six). Altogether, our work shows that explicitly considering the number of interacting species is critical for better understanding the direct and indirect processes by which nitrogen enrichment and pathogen communities shape coexistence in grasslands.

Keywords

Grasslands, nitrogen enrichment, foliar fungal pathogens, multispecies coexistence, structural approach, niche differences, fitness differences, indirect effects

1.1 Introduction

Nitrogen (N) enrichment is a key global change driver (Stevens et al., 2004; Galloway et al., 2008), which often causes a reduction in plant diversity and changes in species composition (Stevens et al., 2004; Vellend et al., 2017; Midolo et al., 2019). There are several mechanisms by which N can alter plant communities. Adding N may remove a limiting resource for plant growth and thereby decrease the number of niche dimensions (Harpole et al., 2016). This would reduce stabilising niche differences between species and therefore coexistence and diversity (Cenci et al., 2018; Godoy et al., 2018; Losapio et al., 2019). The addition of N may also lead to an increase in fast-growing and tall species (often grasses) which are very competitive for light (Harpole et al., 2016; Seabloom et al., 2017). As light competition is highly asymmetric (Lamb, 2008; DeMalach et al., 2017), a shift towards light limitation is likely to increase competitive differences between species. Hence, N addition has the potential to reduce diversity and change species composition by altering the main classes of processes that maintain coexistence between species pairs. These are the stabilising niche differences that occur when intraspecific exceeds interspecific competition and the fitness differences which drive competitive exclusion by favouring one competitor over the others, regardless of their abundance. Species are predicted to coexist only when niche differences overcome fitness differences (Chesson, 2000; Saavedra et al., 2017). Changes in niche and fitness differences could also alter species composition if the competitive balance between species changes without coexistence becoming more likely. However, very few studies have empirically assessed how changes in environmental conditions modulate the species interactions that determine coexistence (Matías et al., 2018; Bimler et al., 2018; Hallett et al., 2019). Although many studies have shown that N reduces diversity, we still know little about how it alters these classes of coexistence mechanisms, leading to a large gap in our theoretical knowledge of N enrichment effects. A better understanding of how N affects coexistence mechanisms may allow us to better predict its impact on the diversity and composition of plant communities and unify global change and coexistence research.

In addition to its direct effects, N enrichment can also indirectly impact the diversity and composition of plant communities by altering the abundance of consumers, such as foliar pathogens. N addition will increase the concentration of N in plant tissues, which could benefit pathogens directly (Dordas, 2008), and a shift towards fast-growing species following N addition might also increase pathogen abundance (Cappelli et al., 2020). Changes in pathogen abundance are likely to have further consequences for plant coexistence. Pathogens can promote species coexistence through negative density-dependence, suppressing species that become common and preventing them from taking over (Petermann et al., 2008; Mordecai, 2011; Bagchi et al., 2014; Bever et al., 2015), thereby increasing stabilising niche differences. Alternatively, leaf fungal pathogens can act as an equalising rather than as a stabilising mechanism by attacking the most dominant species (Mordecai, 2011). For instance, they can reduce the abundance of dominant grasses and thereby reduce competitive asymmetries among dominant and subordinate species in grasslands (Allan et al., 2010; Seabloom et al., 2017).

However, other studies have found smaller effects of pathogens: for example, Spear and Mordecai (2018) found a weak relationship between pathogen damage and seed production, suggesting limited effects of fungal pathogens on plant populations. Uricchio et al. (2019) also found that fungal infection had weak fitness effects and was unlikely to affect coexistence. Therefore, the efficacy of pathogens as drivers of coexistence remains unclear. Pathogens could also modify the effects of N on coexistence and might offset some of the negative effects of N by reducing species dominance, as shown for insects (Allan and Crawley, 2011). Finally, if pathogen removal and N addition favour different species then this might lead to complex interactions between the two.

Traditional approaches to understanding coexistence have focused on pairs of species (modern coexistence theory (Chesson, 2000)). However, it has recently become possible to explore niche and fitness differences between multiple species using a structural approach (Saavedra et al., 2017; Song et al., 2018). These advances open now the possibility to investigate how coexistence mechanisms change as we increase the number of species in an assemblage. Theory posits that three or more species can coexist when species interactions create opportunities for coexistence (i.e., structural niche differences) that can accommodate the differences in population growth rates between the species (i.e., the ability of a species to increase its population in the absence of others), so that species vary in abundance over time but none goes extinct (Rohr et al., 2014; Saavedra et al., 2017; Godoy et al., 2018) (Fig. 1.1). Here, the structural fitness differences are a combination of differences in intrinsic growth rates among species and also differences in the position of the feasibility domain, which are determined by the degree of asymmetry in the strength of species interactions (Tabi et al., 2020). Similar to the pairwise scenario, structural niche differences in a multispecies community are partly promoted when a species limits itself more than it limits others (intraspecific competition exceeds interspecific competition) (Barabás et al., 2016) but also when indirect interactions provide further opportunities for coexistence. The best-studied of these indirect interactions is intransitivity, i.e., rock-paper-scissors dynamics in which species A beats species B, species B beats species C, and species C beats species A (Soliveres et al., 2015; Godoy et al., 2017; Gallien et al., 2017). These indirect interactions may also respond to environmental variation and there is some evidence that intransitivity might be reduced by nitrogen addition (Soliveres et al., 2018). However, there is very little information on how resources and enemies might alter indirect interactions in multispecies communities.

Adding more species to a community might increase the opportunities for indirect interactions and therefore could make coexistence more likely. Conversely, adding more species can hinder rather than promote multispecies coexistence because it increases the chances of including a species with a broad niche that overlaps with the niches of other species (Berlow, 1999; Neutel et al., 2002); and/or augment the chances of including a superior competitor that increases fitness differences (Saavedra et al., 2017). In that case, the multiple species assemblages would need stronger species self-limitation compared to the pairwise case to offset the negative effects of strong and indirect interspecific competition (Barabás et al., 2016; Godoy et al., 2017).

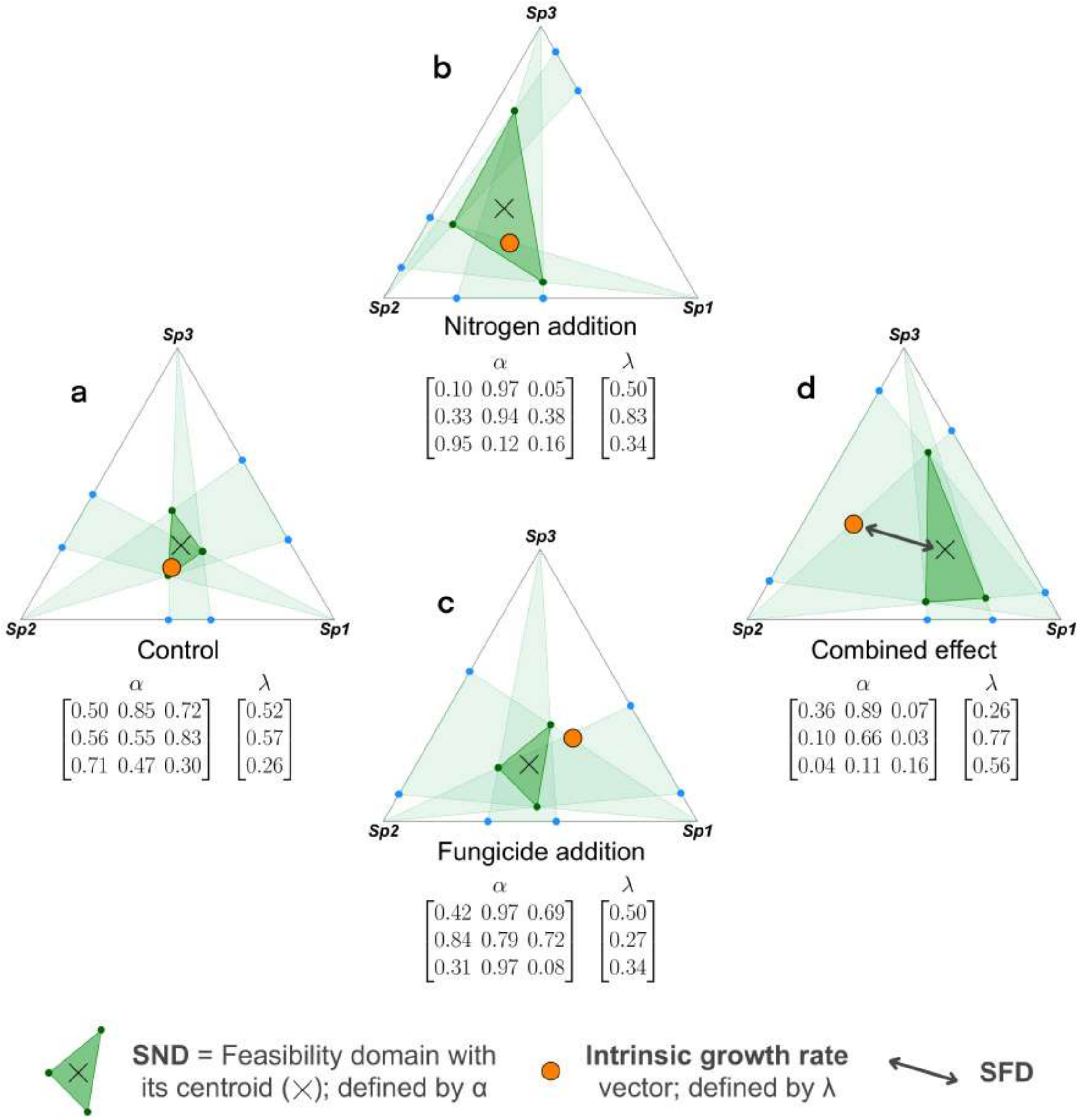

Figure 1.1: The structural approach (Saavedra et al., 2017), applied to understand how environmental changes alter the mechanisms of coexistence between multiple species. Each green triangle is defined by the structure of species interactions (α) and represents the range of intrinsic growth rates that are compatible with species coexistence (i.e., where all species have abundances > 0). Such parameter space is also called the feasibility domain and is shown here for direct pairwise species interactions (light green triangles) and for the matrix of direct and indirect interactions among three species (dark green triangles). This feasibility domain corresponds to the structural analogue of niche differences (SND). Moreover, the distance, displayed as a grey double arrow in panel D, between the centroid of the feasibility domain (×) and the vector formed by the intrinsic growth rates (λ; orange dot) of the species, corresponds to the structural analogue of fitness differences (SFD) for the triplet. Given all this, a triplet can coexist when its feasibility domain can accommodate its intrinsic growth rate vector. Here we hypothesise that nitrogen and fungicide addition could affect both structural niche and fitness differences. In this example, the control **(a)** triplet could be feasible despite having small SND if the SFD are also small (the intrinsic growth rate vector is close to the centroid of the feasibility domain). Meanwhile, nitrogen addition **(b)** is also feasible because of its larger SND, fungicide **(c)** is unfeasible because SND ¡ SFD, and the combined **(d)** effect shows large SND but also increased SFD, resulting in an unfeasible combination. We show the values of the matrix of species interactions (α matrix) and the vector of intrinsic growth rates (λ vector) that underlies each representation.

In sum, this means that nitrogen enrichment and pathogens can change the number and the identity of species that can coexist by modifying: (1) species intrinsic growth rates, (2) the strength and sign (both competition and facilitation) of direct interactions among species, and (3) the strength of indirect interactions (e.g., intransitive competition). Experiments manipulating N addition and pathogen infection are required to determine the relative importance of these three mechanisms in affecting multispecies coexistence and how they are modified by nitrogen and pathogens.

In this study, we present results from a grassland biodiversity experiment where nitrogen addition and pathogen suppression, with fungicide, were factorially applied. We quantified the two key pieces of information (species intrinsic growth rates and intra- and interspecific interaction coefficients) required to derive metrics of structural niche and fitness differences among eight perennial plant species. To do so, we measured the growth of individual plants, in terms of biomass change, as a function of the density and composition of their neighbours, in the different experimental treatments (nitrogen, fungicide, and their combination). With this information, we then parameterised a population model to predict which communities could coexist and to characterise the way in which they did so (their structural coexistence metrics). We did this for different numbers of species from two to six. This combination of ecological theory, modelling and detailed field observations allowed us to ask: (1) how do structural niche and fitness differences vary with N addition, pathogen suppression, and the interaction between them? (2) How do these effects vary if coexistence between different numbers of species is considered? (3) What is the relative importance of pairwise direct effects and indirect effects arising in multispecies assemblages for coexistence? And (4) what are the predicted consequences of N enrichment and pathogen suppression for biodiversity and species composition?

1.2 Materials and methods

1.2.1 Study system

We conducted this study in the PaNDiv biodiversity experiment, which was set up in October 2015 in Münchenbuchsee (Bern, Switzerland; 47°03'N, 7°46'E, 564 m a.s.l). The mean annual precipitation at the site is 1012.62 mm and the mean annual temperature is 9.2 °C (Federal Office of Meteorology and Climatology MeteoSwiss). The site was formerly an extensively managed grassland that had remained unfertilised since at least 2005, and the field was mown and ploughed to remove the vegetation prior to the start of the experiment. The PaNDiv experiment consists of 336 experimental plots of 2 x 2 m, where species richness (1, 4, 8 or 20 species) and plant functional composition (fast versus slow-growing species) are manipulated and crossed with applications of nitrogen and fungicide. Communities differing in species number and composition were assembled from a set of 20 common Central Europe perennial grassland species (Table S1.1). The experiment was arranged in four blocks, with each particular species combination present in each block. The composition of each plot was maintained by manually removing weeds three times a year. The field was mown twice a year, in the middle of June and in August, which corresponds to intermediate management intensity for lowland grasslands.

The nitrogen and fungicide treatments were applied every year: nitrogen addition plots were fertilised with urea in April and after the first mowing and received in total kg N ha^{-1} $year^{-1}$ (50 kg ha^{-1} each time). The fungicides Score Profi (Syngenta Agro AG, 24.8% difenoconazole) and Ortiva (Syngenta Agro GmbH, 22.8% azoxystrobin) were used to suppress foliar pathogens. They were applied four times during the growing season (0.2 ml of Score Profi and 0.4 ml of Ortiva mixed with 0.062l of water per plot and time), whereas no fungicide plots were sprayed with the same amount of water. Each particular combination of species received the four nitrogen × fungicide treatments, to allow us to separate treatment and composition effects. Data from Cappelli et al. (2020) showed that the fungicides reduce pathogen incidence by 25.33%, on average, and are particularly effective in reducing rusts and powdery mildews (which were virtually absent from sprayed plots). For further details on the experimental setup, see Cappelli et al. (2020) and Pichon et al. (2020).

1.2.2 Estimation of species interactions and intrinsic growth rates across experimental treatments

To study coexistence mechanisms and how they vary across treatments and levels of diversity, we focused on eight species that had established well at the start of the PaNDiv experiment thus avoiding environmental filtering processes (which would result in negative intrinsic growth rates). These species are *Taraxacum officinale* Weber (fast-growing), *Crepis biennis* L. (fast), and *Centaurea jacea* L. (slow-growing) (Asteraceae), *Rumex acetosa* L. (fast) (Polygonaceae), *Dactylis glomerata* L. (fast), and *Anthoxanthum odoratum* L. (slow) (Poaceae), *Salvia pratensis* L. (slow) (Lamiaceae), and *Plantago media* L. (slow) (Plantaginaceae). These are common plant species in temperate grasslands and the selection involves a mix of grasses and herbs and fast- and slow-growing species. For each species, we measured the biomass of multiple individuals in different competition neighbourhoods to calculate the strength and sign of species interactions and how they varied across nitrogen and fungicide treatments. The natural variation in density, and the experimentally imposed relative frequency of these species across plots allowed us to separate and estimate with confidence intra- and interspecific competition as well as species' intrinsic growth rates (see below). We then parameterised a model that describes the population dynamics of competing species, and their likelihood of coexisting, as a function of their network of interactions and their intrinsic growth rates in the absence of competition (see below, structural approach). With eight species, we need to estimate 64 pairwise interactions per treatment (8 intraspecific interaction coefficients and 56 interspecific) and a total of 256 pairwise interactions across the four treatments (i.e., control, nitrogen only, fungicide only, or both combined).

To account for this high dimensionality, we proceeded as follows. Prior to the growing season of 2017 (February and early March), we marked focal individuals of each species in neighbourhoods varying in density and relative frequency of co-occurring species, using a bamboo stick together with a coloured plastic tag. We defined the neighbourhood for each focal individual as a 40cm diameter circle, marked with a PVC ring during sampling. We selected neighbourhoods in the monoculture plots to estimate intraspecific interactions and we did the same in the polyculture plots to estimate interspecific interactions (Fig. 1.2). We measured eight randomly selected neighbourhoods per pairwise interaction, across the four blocks in order to produce gradients of neighbour density (8 neighbourhoods/pairwise interaction × 256 pairwise interactions in the whole experimental design = 2,048 neighbourhoods).

A minimum cover of at least 5% of either the same or another species was the criterion taken to measure a selected neighbourhood. Those neighbourhoods that did not meet this criterion (because they were dominated by some of the other 12 species in the experiment) were replaced by another one within the same plot. For example, a focal-neighbour combination could be *T. officinale* surrounded by at least 5% cover of *A. odoratum*. It is worth noting that, contrary to previous studies in which polycultures were established containing only pairs of species (e.g., Godoy et al. (2020)), we mostly measured polycultures with several different neighbour species (e.g., *T. officinale* surrounded by *C. biennis*, *C. jacea* and *R. acetosa*), as the experiment did not contain two species plots. However, we were able to estimate all pairwise interspecific interactions thanks to the modelling approach applied (see below). This approach is part of the so-called individual fitness models, which are commonly used to estimate interactions between pairs from multispecies data (Bimler et al., 2018; García-Callejas et al., 2020; Martyn et al., 2021). The effect of higher-order interactions (Mayfield and Stouffer, 2017) on coexistence was beyond the scope of this article and we therefore did not estimate them in our models but our interaction coefficients average across any higher order interactions that might occur. We visually estimated the cover of the focal species inside the rings as well as the cover of the other species. The cover of the neighbouring species was estimated individually for the eight target

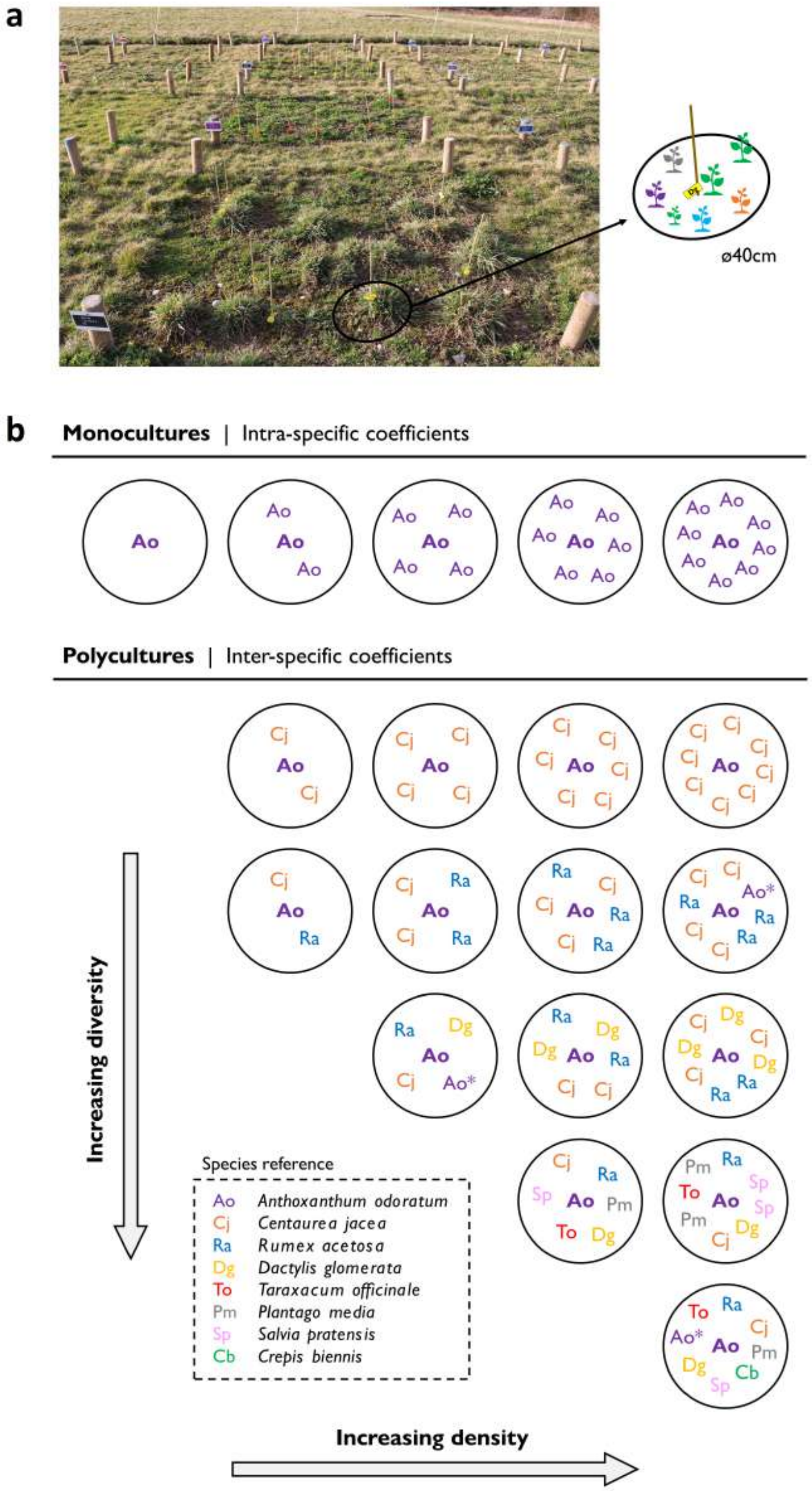

Figure 1.2: Sampling method through the PaNDiv experiment. (**a**) Picture of the field with detail of the 40 cm diameter ring used to measure the cover of the focal (marked with a stick and labelled) and neighbour plants. (**b**) Sampling design, using a natural gradient of neighbour density (x-axis) in monocultures and polycultures to estimate the intra- and inter-specific interaction coefficients, respectively. The y-axis represents a gradient of neighbour diversity for the polycultures. In this example, we have used *Anthoxanthum odoratum* (Ao) as focal (bold letters), but each one of the 8 species is sampled both as focal and neighbour. Note that asterisks indicate that the focal species may also be present as neighbour in polycultures, contributing to estimating the intra-specific coefficients together with the information from monocultures.

species we selected, and we estimated the abundance of all other species grouped into three functional groups (herbs, grasses, and legumes). We did not explore the likelihood of coexistence of these groups but we determined their competitive effects on our eight target species (see statistical models below).

We measured plant cover at the beginning of the growing season and in June, at peak biomass, before the first cut. This therefore represents the majority of aboveground growth for each plant in that year. We measured plant cover for logistical reasons, but we converted the cover values to biomass for the modelling. To do so, we harvested (and oven-dried, at 60 °C for two days) the aboveground biomass of eight focal plants per species and treatment, to estimate a relationship between cover and the biomass of each focal species. We obtained reasonably good fits for all and each of the individual species ($R^2 = 0.6 \pm 0.2$, mean ± SD) (Fig. S1.1). Finally, we repeated the whole procedure in 2018 on newly-selected focal neighbourhoods, which meant we measured, across the two years, the cover and species composition of 4,096 neighbourhoods (2,048 each year). Once we obtained data from the experiment, we visually checked for outliers. We took the decision to remove the 2018 data from one species, *Rumex acetosa*, from further analyses because it declined strongly in biomass from February to June during this year (Fig. S1.2), most likely because conditions were very dry during 2018 and *R. acetosa* had already begun to senesce by June. Modelling this strong decline would indicate that this particular species is not able to grow at the experimental site (i.e., environmental filtering), which is not the case in general.

We then modelled the interactions between all pairs of species. We used a modified Beverton-Holt function to estimate model parameters (Beverton and Holt, 1957). This modification was applied to model population size as biomass and not as numbers of individuals because the clonal growth of several of these perennial plant species makes it impossible to distinguish separate individuals in the field. However, the model retains the mathematical behaviour of prior work that has shown this function can be applied to both annual and perennial plant species (Lanuza et al., 2018; Cardinaux et al., 2018), and is also suited to apply a structural approach (see details in Appendix 4 in Saavedra et al. (2017)). Since we marked individuals already growing in the field (we did not plant out individuals), we needed to account for the initial biomass of each focal. Focal individuals with greater initial biomass might experience less competition and grow more, as bigger individuals are less sensitive to competition (Rees, 2013). Therefore, we modelled the biomass response (N) of the focal species i in the year $t+1$ as follows:

$$\frac{N_{i,t+1}}{N_{i,t}} = \frac{\lambda_i(1+\theta_i N_{i,t})}{1+\sum_{j=1}^{n}(\alpha_{ij}+N_{i,t})N_{j,t}} \tag{1.1}$$

where λ_i is the per capita growth rate measured as biomass of i in the absence of interactions and α_{ij} represents the effect that each neighbour species has on i. The parameters θ_i and Ω_i reflect the effect of the initial size of species i on its intrinsic growth rate (λ_i) and its interactions with neighbour species ($\alpha_{ij}\alpha_{ij}$), respectively. We fitted eqn. 1.1 to empirical observations using maximum likelihood methods (`optimx` function within the “optimx” package (v. 2022-4.30, (Nash and Varadhan, 2011); method = “Nelder-Mead”). We estimated λ_i (bounded to be positive as negative intrinsic growth rates for species growing alone do not make sense given that all species can persist at the site) and α_{ij} for each treatment separately (control, N addition, fungicide, and combined). α_{ij} is not bounded and can be negative (competition) or positive (facilitation) because the structural approach can compute niche differences when both types of interactions are combined. We fitted three different models for each species to deal with initial biomass effects. We considered that intial biomass (Ω) had: 1) no effect on the other model parameters (i.e., the effect of size is negligible), 2) a common size effect across all species, or 3) a species-specific size effect. To identify the best model for each species and treatment, we used AIC (Burnham and Anderson, 2002). All the details about the three different specifications of the function, and their AIC estimates for each species, are included in Table S1.2. Note that this modelling procedure was also done separately for the years 2017 and 2018. To better understand the confidence of our results, we calculated the Hessian matrix with the `ghgen` function within the “optimx” package and transformed its diagonal to obtain the standard error for all model parameter coefficients (95% confidence), assuming that the gradient is well approximated by a quadratic function. Mean model estimates and standard errors for the α_{ij} and λ_i parameters under each treatment are provided in Table S1.3—S1.6.

1.2.3 Predicting the effects of N enrichment on species coexistence with the structural approach

With the field parametrisation of the dynamic model describing the population dynamics of interacting species, we applied the structural approach to explore how coexistence mechanisms varied across the experimental treatments. We followed previous definitions of Saavedra et al. (2017) to compute metrics of structural niche differences (SND), structural fitness differences (SFD), and feasibility (i.e., an equilibrium point which is assumed to be globally stable following previous approaches (Zhao and Luo, 2010; Saavedra et al., 2017); thus, here we consider a feasible combination to be one in which all species coexist). We did this for species pairs and for combinations of three to six species. The great advantage of the structural approach is that we can evaluate the probability of coexistence for any number of species at the same time if we have information on their intrinsic growth rates and the full matrix of pairwise interactions. For all species combinations, coexistence conditions are the same: a species combination can coexist when SND are greater than SFD 1.1. Importantly, we can compare the variation in SND and SFD across treatments, but we cannot compare them across combinations of different numbers of species, i.e., SND in a three species combination (triplet) cannot be compared with the SND in a four-species combination. This is because SND and SFD inherently vary as more species are included in the combination, as indirect interactions between species contribute to SND and these vary with the number of species included. We also evaluated the importance of indirect interactions (e.g., intransitivity and other types of species interaction chains) in allowing species to coexist using the metric called community-pair differential (Saavedra et al., 2017). Community-pair differential varies between −1 and 1. A positive value indicates that indirect interactions promote coexistence, i.e., there are greater opportunities for coexistence in the multispecies combination than in the individual pairs that make up the combination. For example, in the case of an intransitive triplet, no individual pair could coexist, but the triplet could coexist. Negative values of the differential indicate the opposite, i.e., fewer opportunities for coexistence in the multispecies combination. These computations were performed for the mean model parameters, the lower and upper tails of the 95% confidence intervals, and for the years 2017 and 2018 separately.

We finally used generalised linear models (GLMs) to evaluate the effect of N addition and fungicide application on these coexistence metrics, for each number of species in the combination. To determine what distribution to use for the residuals in the analysis of SND, SFD, community-pair differential, and feasibility metrics, we used the function `descdist` within the package "fitdistrplus" (Delignette-Muller and Dutang, 2015), which led us to fit a GLM with gamma errors (link = "identity") for SND, SFD, and community-pair differential metrics, and binomial errors for the feasibility metric. A summary of the results from the different models for all metrics is available in Table S1.7—S1.26. All computational analyses were performed in R version 4.2.1 (R Core Team, 2022).

1.3 Results

1.3.1 Determinants of feasibility across treatments

Our experimental treatments resulted in communities with a wide range of structural niche differences (SND) and structural fitness differences (SFD). Nevertheless, the variation was higher for SFD than for SND regardless of the number of species considered. Accordingly, most communities were predicted to be unfeasible and not able to coexist (Fig. 21.3). We did not observe a clear threshold between feasible and unfeasible assemblages in multispecies communities mainly because indirect interactions promoted species persistence for some species combinations and hindered it for others. For instance, feasible communities were those in which we found 1) intra-specific strongly exceeded inter-specific interactions, 2) quasineutral-like dynamics in which all interactions and growth rates were equivalent but intraspecific interactions were slightly stronger than inter-specific ones, and 3) intransitive dynamics. Conversely, examples of predicted unfeasible communities were those in which inter-specific interactions were stronger than intra-specific interactions (i.e., priority effects), and 2) high variation in intrinsic growth rates among species destabilised the effect of niche differences.

1.3.2 Effects of nitrogen enrichment and fungal pathogen suppression on structural niche and fitness differences

The effect of nitrogen (N) addition, pathogen suppression, and their interaction on changing SND compared to the control varied depending on the number of interacting species (Fig. 1.4a). Within species pairs, N addition and pathogen suppression increased SND when combined but did not differ from control conditions when applied alone. N addition slightly decreased SND in three-species combinations; however, the combination of N addition and pathogen suppression increased SND. For assemblages of four, five, and six species the pattern was more consistent: N addition, pathogen suppression, and their combination tend to increase SND compared to control conditions. Separating each year individually, we observed in 2017 that N addition, pathogen suppression, and their combination decreased SND. This result differs from both years combined and such differences are more pronounced with a higher number of interacting species (Fig. S1.3a). Analysing 2018 alone showed similar results to both years combined (Fig. S1.3b). We also explored how our experimental treatments modified SND at the lower and upper limits (95% CI), which rendered smaller and less variable SND values than the mean SND for both years (Fig. S1.4).

On the other hand, SFD did not vary between treatments and control conditions for combinations of two species whereas for assemblages of three species only the combination of N addition and pathogen suppression slightly increased SFD. Similar to the case of SND, we observed parallel trends from four to six-species assemblages. Specifically, N addition, pathogen suppression, and their combination strongly decreased SFD (Fig. 1.4b). The separated analyses of SFD in 2017 and 2018 showed contrasting results. In 2017, the reduction of SFD compared to control conditions is repeated for assemblages of four, five, and six species (Fig.

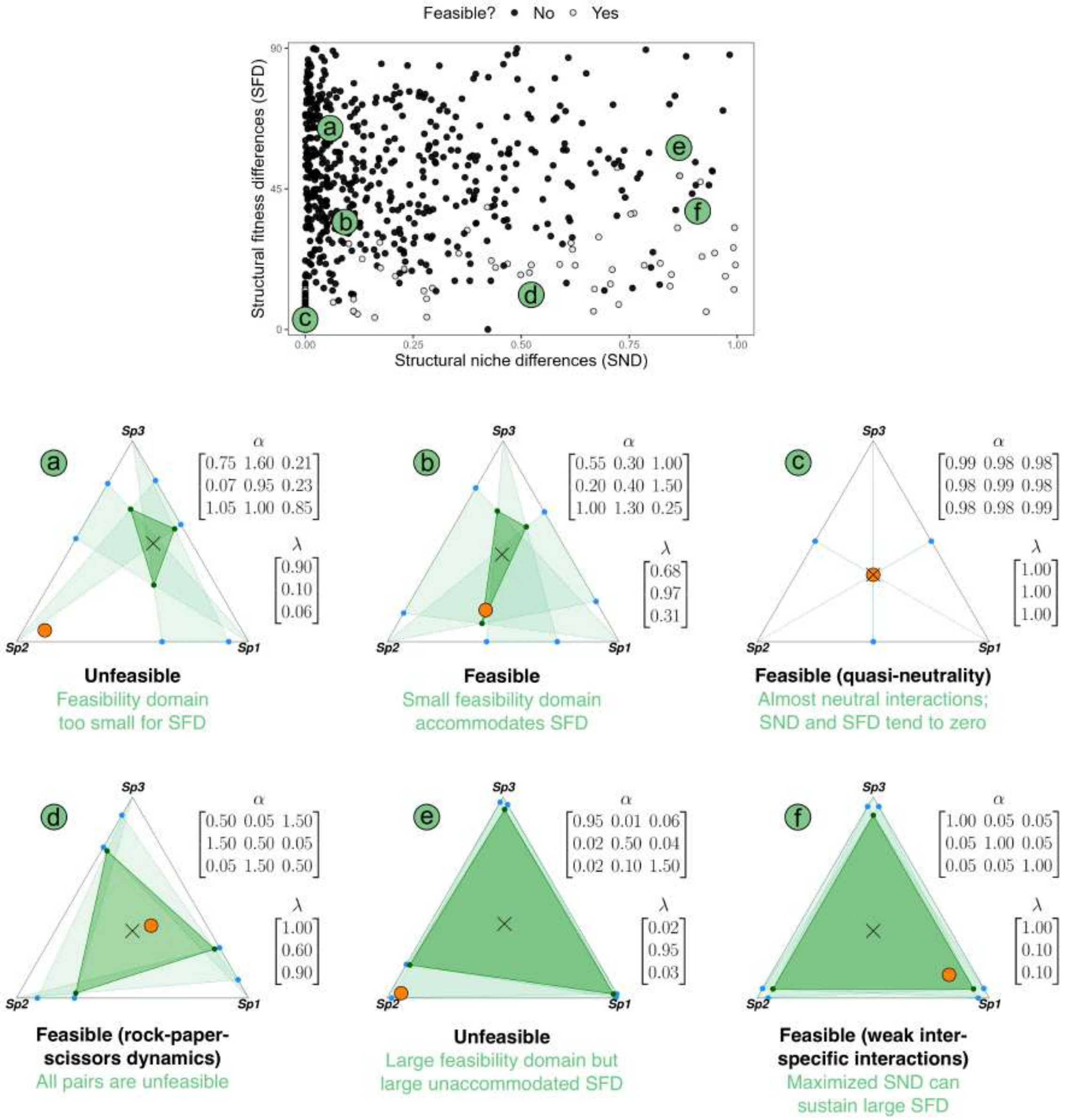

Figure 1.3: Diagram describing the relationship between the structural niche differences (SND) and the structural fitness differences (SFD) for all three-species combinations across treatments in our study, together with some examples of how these mechanisms relate to the underlying network of interaction coefficients. Data points in black and white represent unfeasible and feasible triplets of species, respectively. The triangles visually represent the structural approach (Saavedra et al., 2017) as thoroughly described in Fig. 1.1 and the main text. As a reminder, coexistence is achieved when the feasibility domain or SND (green triangles, defined by the structure of species interactions (α)) are capable of accommodating the vector of intrinsic growth rates (λ; orange dot). The distance between λ and the centroid of the feasibility domain (×) visually approximates the SFD. Low values of SND usually generate unfeasible combinations (**a**), although even some small feasibility domains can have shapes that may accommodate intermediate values of SFD (**b**) due to the structure of the interaction network. Moreover, quasi-neutrality (**c**) is a particular example of a feasible combination where both SND and SFD tend to zero. Intransitive dynamics such as rock-paper-scissors (**d**) may also occur, in which the multispecies combination is feasible but all the pairwise interactions are unfeasible. Finally, even assemblages with high niche dimensionality can be unfeasible in combination with specific intrinsic growth rates (**e**), although the most likely scenario is that these intrinsic growth rates can be accommodated and a feasible assemblage produced (**f**). We show the values of the matrix of species interactions (α matrix) and the vector of intrinsic growth rates (λ vector) that underlies each representation.

S1.5a). However, we observed the opposite pattern in 2018; namely, all treatments increased SFD in assemblages of four-, five- and six-species combinations (Fig. S1.5b). Despite the contrasting results, analysing both years individually confirms the general pattern that variation of SFD differences between treatments was highly dependent on the number of interacting species. Finally, the exploration of how our experimental treatments modified the lower and upper limits (95% CI) of SFD showed smaller and more variable values but comparatively consistent with the mean values for both years, particularly in the upper limit (Fig. S1.6).

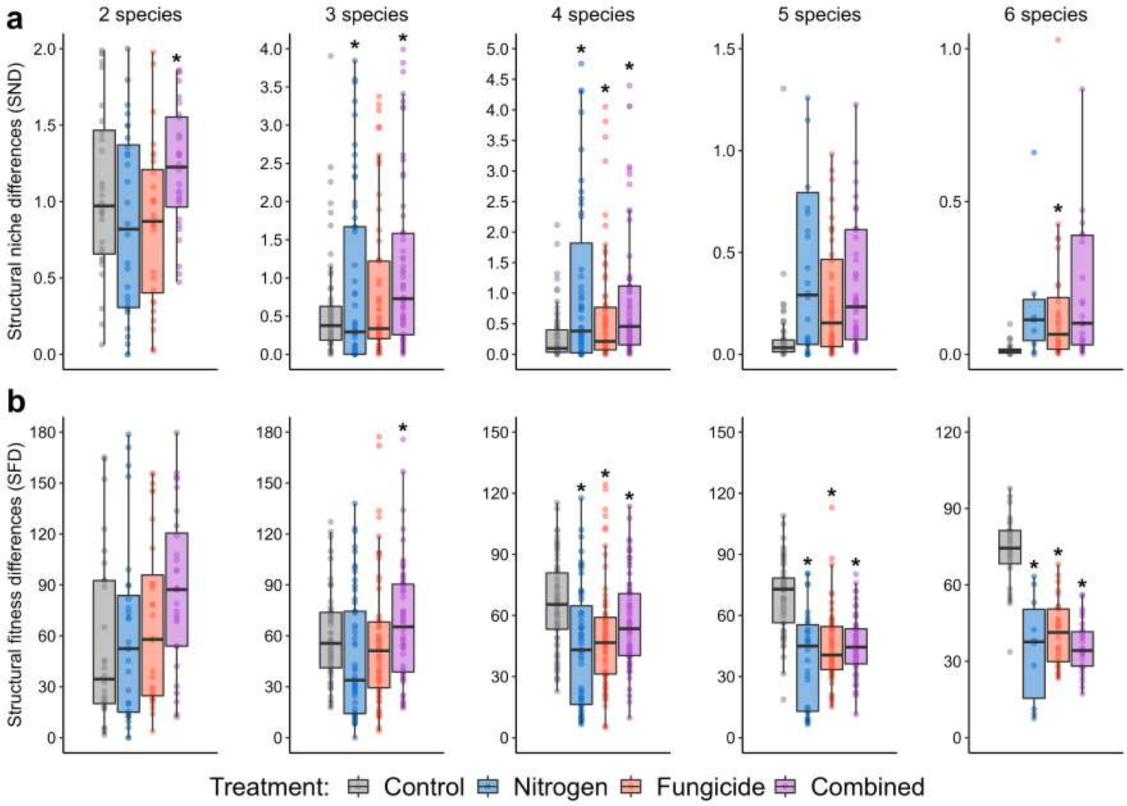

Figure 1.4: Structural niche (**a**) and fitness (**b**) differences for all combinations of two to six species, in the four experimental treatments (control, nitrogen, fungicide, and combined). Each point represents a value of SND or SFD of a specific species combination under a particular treatment. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.7—S1.8). Note that the axis scales differ between panels because results can be compared across treatments within each species combination but not between species combinations (see text). Results represented correspond to mean estimates of interaction coefficients and intrinsic growth rates when data from years 2017 and 2018 were combined. For mean values of each year separately, the same representation and statistical analyses can be found in Fig. S1.3 and Table S1.9—S1.10 (SND) and S1.5 and Table S1.11—S1.12 (SFD). For the lower and upper limits (95% CI) of both years combined, the same representation and statistical analyses can be seen in Fig. S1.4 and Table S1.13—S1.14 (lower limit) and in Fig. S1.6 and Table S1.15—S1.16 (upper limit).

1.3.3 The role of indirect effects

Both direct pairwise interactions and multispecies indirect interactions can affect the structural niche differences (SND), and the relative contribution of each interaction type can be evaluated by the community-pair differential. Overall, we observed small values of community-pair differential (i.e., on average across treatments, only 15% of the SND were due to indirect interactions, for three species combinations), and these community-pair differential values tended to zero with increasing species richness. Contrary to our initial expectation, this result suggests that indirect interactions have a minor role in promoting SND as more species are added (Fig. 1.5). Equally surprising is that we did not observe differences when specific treatments were compared. When decomposing the main results into 2017 and 2018, we observed the same overall decay in the importance of indirect effects with the number of interacting species and experimental treatments had no effect (Fig. S1.7). The exploration of the lower and upper limits (95% CI) of community-pair differential values corroborated the low importance of indirect interactions on promoting species coexistence because all values tended to zero in the lower limit regardless of the number of species and treatment considered (Fig. S1.8a), and upper limits reproduced the decaying importance of indirect interactions for promoting SND as the number of species increased (Fig. S1.8b).

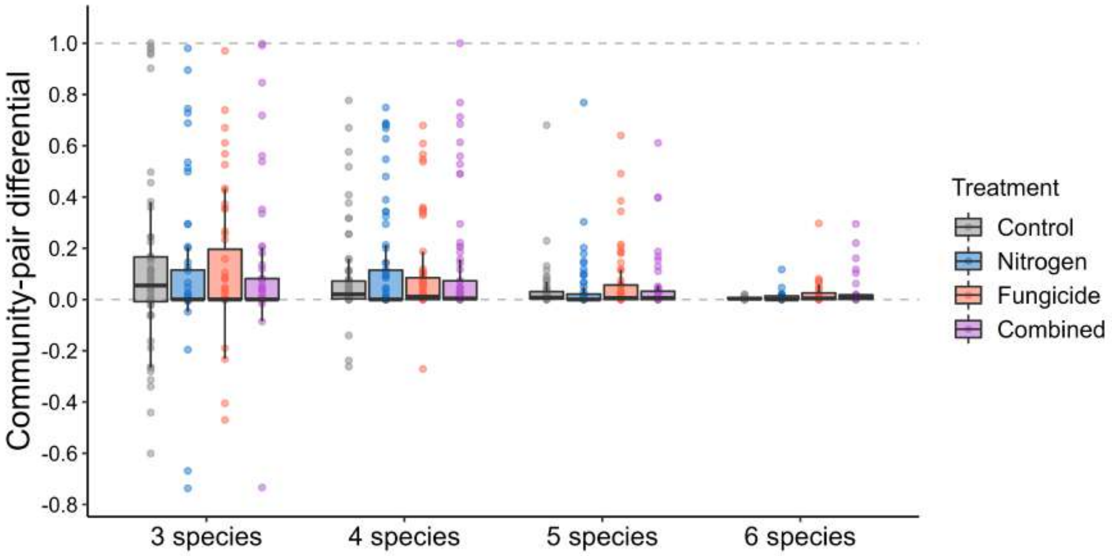

FIGURE 1.5: Community-pair differential for combinations from three to six species simultaneously, in the four experimental treatments (control, nitrogen, fungicide, and combined). Each point represents a value of community-pair differential of a specific species combination under a particular treatment. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.17). Results represented correspond to mean estimates of interaction coefficients and intrinsic growth rates when data from years 2017 and 2018 were combined. For mean values of each year separately, the same representation and statistical analyses can be found in Fig. S1.7 and Table S1.18—S1.19. For the lower and upper limits (95% CI) of both years combined, the same representation and statistical analyses can be seen in Fig. S1.8 and Table S1.20—S1.21.

1.3.4 Consequences for diversity maintenance and changes in species composition

The combination of SND and SFD resulted in a reduced proportion of feasible species combinations as the number of interacting species increased (Fig. 1.6). This result is particularly apparent for the control treatment, which does not sustain any feasible combination of six species. However, the nitrogen and fungicide treatments increased the number of feasible combinations when four or more species were considered. When we separated both years, we observed a more abrupt decay in the proportion of feasible combinations with the number of interacting species, particularly in 2018 when there are no feasible combinations of five or six species (Fig. S1.9). In 2017, the fungicide treatment seems to support the most feasible combinations, especially in combinations of three and four species. The exploration of the lower and upper limits (95% CI) in both years combined offered a substantial decrease in feasibility with almost no predictions of feasible combinations, especially for multispecies assemblages (Fig. S1.10).

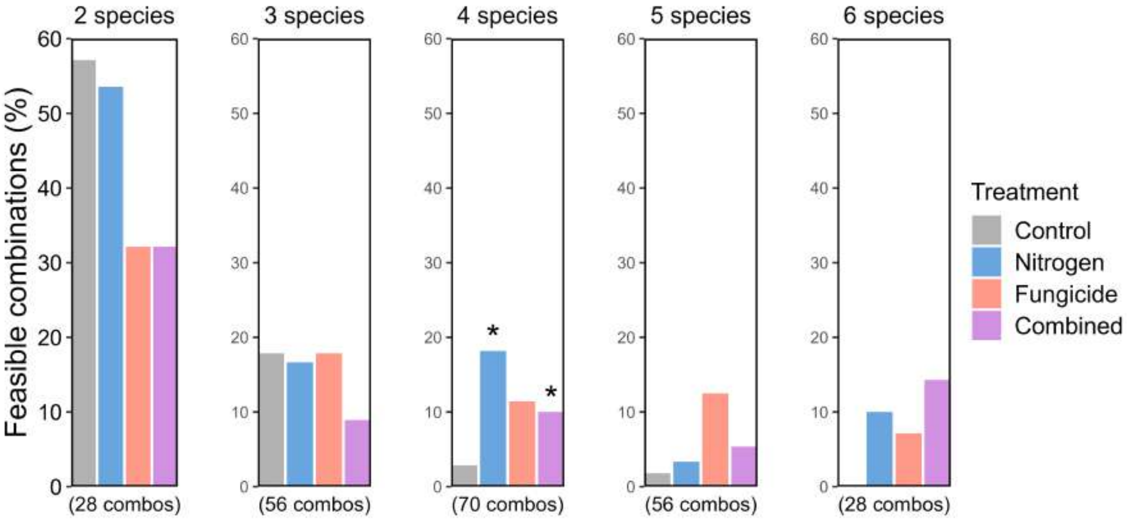

Figure 1.6: Feasible combinations (% of the total) of two to six species, in the four experimental treatments (control, nitrogen, fungicide, and combined). A feasible combination is one in which all species are predicted to coexist. Asterisks indicate significant differences ($p < 0.05$) between the treatments and the control (Table S1.22). The total number of possible combinations (combos) of two-six species is shown at the bottom. Results represented correspond to mean estimates of interaction coefficients and intrinsic growth rates when data from years 2017 and 2018 were combined. For mean values of each year separately, the same representation and statistical analyses can be found in Fig. S1.9 and Table S1.23—S1.24. For the lower and upper limits (95% CI) of both years combined, the same representation and statistical analyses can be seen in Fig. S1.10 and Table S1.25—S1.26.

Together with the impact on the overall numbers of species predicted to coexist, our treatments did predict strong compositional changes. For instance, only five species were present in all species combinations under control conditions, with *T. officinale* and *A. odoratum* being unable to coexist in five- and six-species combinations and *P. media* in six-species combinations (Fig. 1.7a). Conversely, most species were evenly distributed across all feasible species combinations under the nitrogen addition treatment, with the exception of *A. odoratum* which was unable to coexist in any assemblages of six species (Fig. 1.7b). Pathogen suppression also had large effects on composition with some species such as *C. jacea* struggling to coexist with others in the fungicide treatment and others such as *D. glomerata* expected to coexist more frequently, especially in assemblages of three or more species (Fig. 1.7c). Finally, the combined treatment of nitrogen addition and pathogen suppression had mixed effects: some species such as *D. glomerata* and *S. pratensis* were unable to coexist in some cases but it was the only treatment in which all eight species had opportunities for coexistence in six species assemblages. Altogether, we observed that different species benefited from different treatments and that these outcomes were highly dependent on the number of interacting species in a given combination.

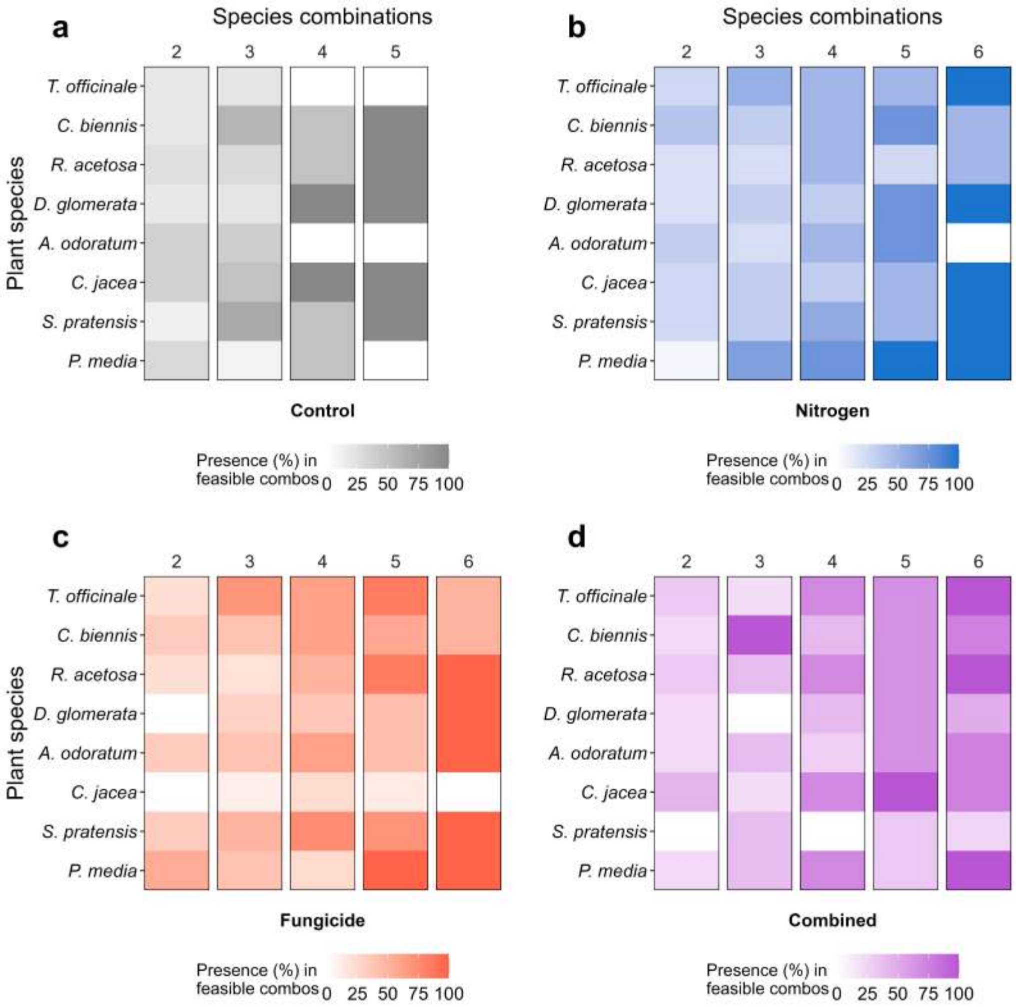

Figure 1.7: Probability (%) of each of the eight plant species to stably coexist with their neighbours in combinations from two to six species simultaneously. Results are shown for the four treatments: (**a**) control, (**b**) nitrogen, (**c**) fungicide, and (**d**) combined. The 6-species column has been removed from the control panel because no combinations were predicted to be feasible. Bear in mind that a particular species may not coexist either because it is competitively dominant or subordinate. Results represented correspond to mean estimates of interaction coefficients and intrinsic growth rates when data from years 2017 and 2018 were combined.

1.4 Discussion

Nitrogen enrichment is a major driver of biodiversity declines; however, it is not clear whether it does so by altering niche or fitness differences between species, or by changing the nature of indirect interactions emerging in multispecies assemblages. We used recent advances in ecological theory, and parameterised models describing the dynamics of competing species with data from a controlled field experiment to mechanistically explore how N addition altered coexistence mechanisms in assemblages of different numbers of species. We observed that experimental applications of N tended to increase the strength of stabilising niche differences between plant species. This finding aligns with a recent global scale study by Band et al. (2022) that did not find much evidence that N reduced niche dimensionality. Although the addition of N is expected to reduce the number of niche dimensions and opportunities for trade-offs in the uptake of different nutrients to promote coexistence (Tilman, 1982; Harpole et al., 2016), plant responses to a higher N availability depend not only on the quantity of N but also the chemical form (Bobbink et al., 2010). The mineralisation of urea (CH_4N_2O) in the soil produces different N forms at varying rates that may be assimilated differently depending on the plant species (Kahmen et al., 2006; Harrison et al., 2007). Particularly, ammonium ($NH_4{}^+$) and nitrate (NO_3) are most frequently used (Hachiya and Sakakibara, 2017), but ammonia (NH_3) (Miflin and Lea, 1976) and even urea itself may be uptaken under certain conditions (Witte, 2011). Therefore, increases in different N sources may promote stabilisation (e.g., McKane et al. (2002) and Miller and Bowman (2002)), especially if these N forms were not limiting in the initial control conditions of the experiment (Harpole et al., 2016).

Our study also finds that N enrichment decreases fitness differences between species. This result contradicts a large body of literature that has described how higher levels of N result in species loss by increasing plant productivity and selecting for fast-growing, light competitive species (Suding et al., 2005; Clark and Tilman, 2008; Bobbink et al., 2010; Storkey et al., 2015; DeMalach et al., 2017; Stevens et al., 2018). A possible explanation could be that N addition increased the competitive ability of smaller, nutrient-limited species relative to those which are bigger or not limited by nutrients (Schwinning and Weiner, 1998; Goldberg et al., 2017), and thereby equalised intrinsic growth rates and competitive ability across plant species. Additionally, if plants remained limited by resources such as water or phosphorus, we may not have seen a large increase in asymmetric light competition following N addition because plants still competed strongly for these resources. Overall, this study suggests that N enrichment may be able to promote niche differentiation via modifications in the structure of direct and indirect interactions. This allows imbalances in intrinsic growth rates (i.e., the ability of species to grow in the absence of competition) to be better accommodated and therefore promotes coexistence.

In addition, our experiment showed that natural enemies such as leaf pathogens have contrasting effects on natural communities. We found that the suppression of pathogen communities produced the same outputs as observed for N enrichment. That is, increases in structural niche differences and decreases in fitness differences. One possibility is that fungicide changed the composition of pathogen communities by reducing rusts and mildews but not leaf spots (Cappelli et al., 2020). These changes in leaf pathogen communities could have selected more aggressive and generalist pathogens that equalise fitness differences between plants and promote niche differences via negative density-dependence. In addition, reducing fungal pathogen abundance promoted the fast growing plant species (Cappelli et al., 2020) and this may have equalised their competitive ability relative to the slow species which are otherwise dominant at our site.

By applying a structural approach (Saavedra et al., 2017) to mechanistically understand species coexistence we found two novel results. First, the modifications in niche and fitness differences caused by N addition, pathogen suppression, and their combination differed between pairs of species and multispecies assemblages (three to six species). This finding shows that the effects of global change drivers on coexistence are contingent upon the number of interacting species, and therefore, suggests that changes in coexistence mechanisms may be complex as diversity is lost progressively from communities. In addition, increasing N or suppressing pathogens may have different effects depending on the initial diversity of the community. Second, comparing the importance of direct pairwise interactions against indirect interactions suggests that indirect interactions provide new opportunities for multispecies coexistence in perennial grasslands. For assemblages of three species, we observed a wide variety of indirect interactions including both intransitive competition (i.e., rock-paper-scissors dynamics) and indirect facilitation (i.e. a superior competitor harms an intermediate competitor more than a weak competitor and indirectly promotes the persistence of the weakest competitor) (Soliveres et al., 2015; Godoy et al., 2017). However, indirect interactions lost importance for promoting coexistence as the number of interacting species increased. With five or six species, indirect interactions almost did not increase the opportunities for coexistence compared to direct interactions (i.e., did not increase structural niche differences). This result may arise due to a sampling effect: when more species are considered, the effect of intra-specific competition on promoting coexistence is stronger than the effect of indirect interactions. This result suggests that processes such as extinction cascades are less likely to destabilise multispecies communities but could accelerate diversity loss once it has already started to decline to lower levels. Overall, both novel results indicate a strong synergy between environmental drivers and the number of interacting species. Further research on this synergy can give more insights into how direct and indirect interactions shaped by complex combinations of biotic and abiotic factors can modulate the effect of global change drivers on biodiversity maintenance.

When looking at predictions of biodiversity maintenance across our experimental treatments, the main pattern we observed was a reduction in the number of feasible combinations as the number of interacting species in the community increased. This pattern appeared because larger assemblages were unable to accommodate the differences in intrinsic growth rates between species, i.e., they were more likely to include a dominant competitor which would drive other species to extinction. Control conditions maintained the highest proportion of species pairs (57% feasible pairs) compared to N addition, pathogen suppression and their combination (40% feasible pairs). However, we did not predict any feasible combination under control conditions for six-species assemblages whereas the treatments were predicted to maintain 10% of six-species combinations. Altogether, this means that our global change treatments reduced diversity as expected for assemblages with low numbers of interacting species but reversed the pattern for larger assemblages. Apart from these predicted declines in species richness, we found support for changes in species composition under each treatment. Some particular species illustrate these compositional changes. For instance, *T. officinale* could coexist in most combinations under N addition because its growth rate was reduced in comparison to control conditions, as previously observed (Murray et al., 1983). Also, *C. jacea* could not coexist under pathogen suppression perhaps because it was one of the species that most strongly increased its pathogen infection in mixed communities, presumably due to spillovers from other species (Cappelli et al., 2022). Finally, *D. glomerata* under N addition and *P. media* under pathogen suppression did not coexist in species pairs but could coexist in multispecies assemblages, indicating that the compositional consequences of N addition, pathogen suppression, and their combination are also dependent on the number of interacting species.

It is worth recalling that these predictions assume that environmental conditions, either N enrichment or pathogen suppression, do not vary in space or time. However, environmental variation is pervasive in ecological communities and can promote species coexistence (Adler et al., 2006; Sears and Chesson, 2007). For example, the combination of lower dispersal rates and more patchy landscapes with high environmental heterogeneity help increase coexistence in larger areas by partitioning space (i.e., increase of niche differences) and overcoming competitive imbalances between species (Hart et al., 2003; García-Callejas et al., 2021). Similarly, temporal variation can help species to partition the environment and increase coexistence opportunities (Hallett et al., 2019). In fact, thanks to our two-year sampling effort we were able to capture how temporal variation affects the mechanism of biodiversity maintenance. For instance, N addition, pathogen suppression, and their combination tended to decrease fitness differences in 2017 but increase them in 2018 (Fig. S1.5). Future research therefore needs to span the spatial and temporal scales to study which component, either niche or fitness differences, is more variable. Another important point not covered in the present study is that we have assumed that interactions only occur between adult plants. This means that our study ignores the contribution of other life-history stages such as germination, recruitment, or seedling competition, which influence establishment success in perennial grasslands (Peart, 1989; Tilman, 1997; Jakobsson and Eriksson, 2000) and thus, the ability of species to persist and coexist with others.

With increasing evidence of the challenges that global change poses for biodiversity, it is of the utmost importance to gain a mechanistic understanding of how global change drivers such as nitrogen enrichment shape species interactions and coexistence processes (Tylianakis et al., 2008). In this study, we show that N enrichment reduces species coexistence at the pairwise level but may promote coexistence at the multispecies level by increasing niche and decreasing fitness differences between plants. We also document contrasting effects of fungal pathogens on direct and indirect interactions, with pathogens hindering coexistence by reducing niche and increasing fitness differentiation, suggesting that an increase in pathogen abundance following N enrichment would have complex effects on diversity change. The novel finding that the more interacting species there are in a community, the more likely biodiversity is maintained under a combination of N enrichment and pathogen suppression (thanks to increasing niche differences and decreasing fitness differences) suggests that changes in resources and enemies may have complex effects on coexistence. Altogether, these results highlight the importance of mechanistically understanding how N enrichment, directly and indirectly, alters biodiversity via modifications in the architecture of multispecies interactions.

Associated publication

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Data and code used to generate the results of this manuscript are publicly available in Zenodo (www.doi.org/10.5281/zenodo.7343389) (Granjel et al., 2022).

Author contributions

E.A. set up the PaNDiv experiment, R.R.G., E.A. and O.G. designed the research and the statistical analyses, R.R.G. performed the research, analysed the data, and built the figures, and R.R.G. wrote the manuscript with input from O.G. and further revisions from all authors.

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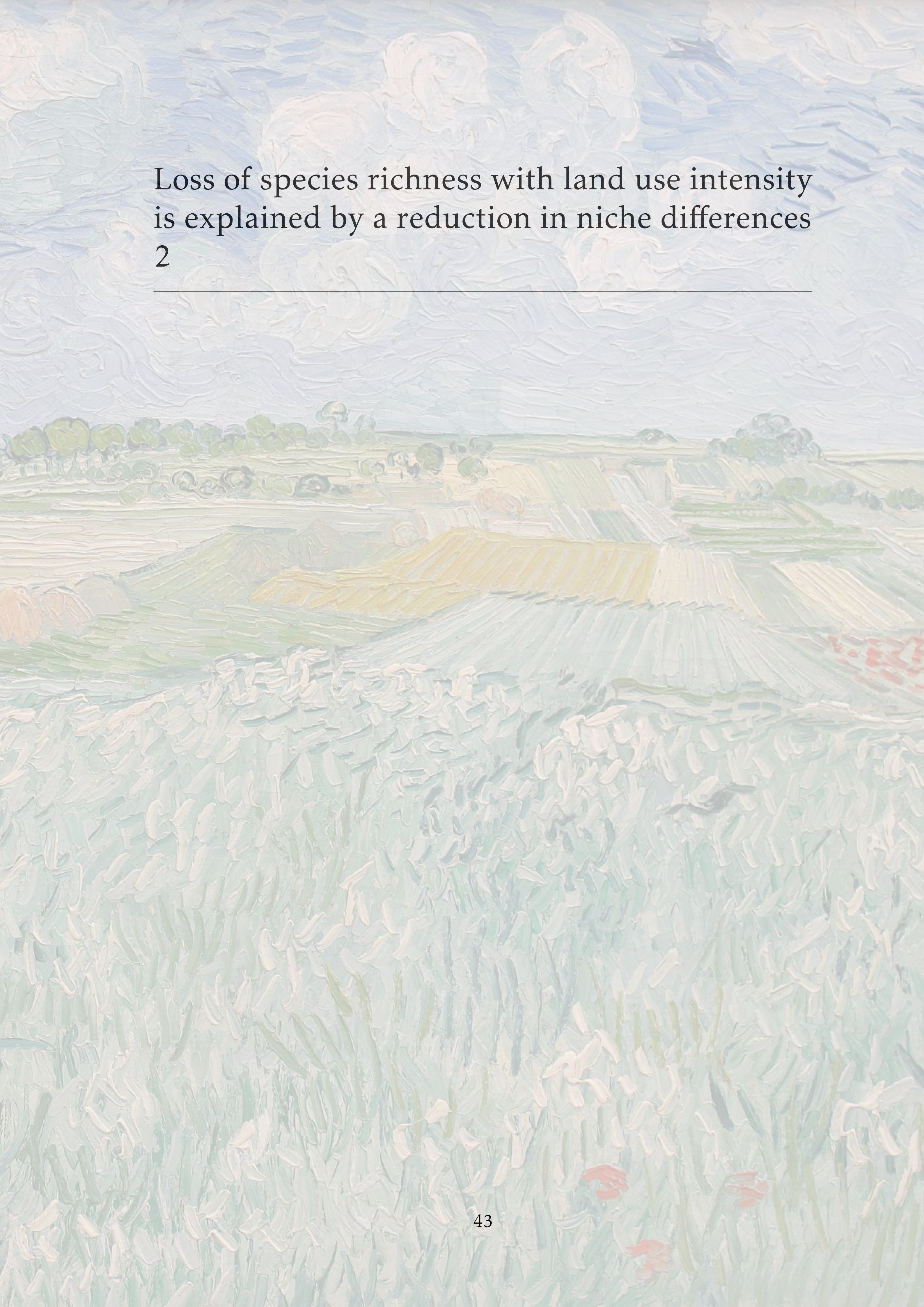
Supplementary material

Supplementary figures and tables related to this chapter are available in Appendix 1.



Loss of species richness with land use intensity is explained by a reduction in niche differences

2

Abstract

Increases in land use intensity (LUI) reduce species richness. However, we have a poor understanding of how underlying coexistence mechanisms are altered by land use and whether diversity loss occurs due to changes in plant-plant interactions (competition and facilitation) or in species intrinsic growth rates. We expect that LUI could reduce stabilising niche differences and the indirect interactions that promote coexistence (e.g., intransitivity), while increasing competitive inequalities between species. To test the importance of these different processes, we use 8-yr time series from 150 grasslands differing in LUI to evaluate the role of direct and indirect interactions in promoting coexistence between 50 plant species. We show that LUI reduces the number of coexisting species mostly by causing a non-linear reduction in niche differences, rather than by enhancing competitive inequalities. However, surprisingly, niche differences remained important in stabilising coexistence between those species remaining at high LUI. Indirect interactions were generally less important than direct ones, and played a moderate role in promoting coexistence in smaller assemblages of species at intermediate LUI. Our models could accurately reproduce the decline in diversity seen with LUI, indicating that our time series approach captures the important interactions between species. By analyzing land use effects through recent advances in structural stability applied to community ecology we provide a more mechanistic understanding of its effects. Our results highlight the importance of identifying the niche differences that are lost with increasing LUI, to better predict and manage effects of land use on biodiversity.

Keywords

Global change, land use intensity, biodiversity loss, multispecies coexistence, structural stability

2.1 Introduction

Changes in land use intensity (LUI), for instance increases in fertilisation, mowing (e.g., early and frequent cuts), or grazing intensity, are often a major threat to biodiversity (Rockström et al., 2009). Although many studies have shown that plant diversity declines (Newbold et al., 2015; Allan et al., 2014; Crawley et al., 2005) and species composition changes (Pakeman, 2011; Dormann et al., 2007) as land use is intensified, we have a poor understanding of the mechanisms responsible, which hampers efforts to predict, conserve, and manage land use effects. Several studies have tried to understand mechanisms of community assembly from changes in functional diversity and have shown that functional diversity declines with land use intensification as communities at high land use intensity become dominated by functionally similar species (Pakeman, 2011; Mayfield et al., 2010; Laliberté and Tylianakis, 2012). For instance, the reduction of plant canopy height, leaf dry matter content, and seed mass associated with land use intensification has been interpreted as an environmental filtering process (Pakeman, 2011). However, it can be challenging to infer precise coexistence mechanisms from functional trait distributions (Mayfield and Levine, 2010). An alternative perspective is to take a demographic approach in which changes in population abundance over time are analysed to estimate the role of species interactions and environmental conditions in determining species coexistence, i.e., the probability that interacting species can persist over time (Hallett et al., 2019; Song et al., 2020*b*; Wainwright et al., 2018). In general, land use intensification could alter species intrinsic growth rates, and the strength of competitive and facilitative interactions (Olsen et al., 2016; Adler et al., 2018; HilleRisLambers et al., 2012) between them, by changing the biotic and abiotic environment. If the altered environmental conditions in intensively managed grasslands result in negative intrinsic growth rates for some species, theory predicts that they will become extinct, with rare species more likely to be lost by chance (Pakeman, 2011; Hallett et al., 2019; Selwood et al., 2015). However, land use intensification can cause further diversity loss by altering species interactions and the balance of intrinsic growth rates between them. Land use intensification can therefore disrupt two main mechanisms of coexistence (Saavedra et al., 2017): 1) stabilising mechanisms, defined as niche differences, stabilise the population dynamics of interacting species, by causing negative frequency-dependent growth; and 2) equalising mechanisms, defined as fitness differences, drive competitive similarity among species when differences in intrinsic growth rates are reduced.

Various aspects of land use intensification may alter niche and fitness differences. Some well documented cases are when fertilisation favors fast-growing species, and thereby increases competitive inequalities, by increasing the intrinsic growth of good light competitors at the expense of other species (i.e., LUI could increase fitness differences) (DeMalach et al., 2017). These increases in competitive asymmetry make it harder for species to coexist, unless superior competitors suffer self-limitation, which occurs when intraspecific exceeds interspecific competition (i.e., niche differences stabilise competition between species). All processes that cause intraspecific competition to exceed interspecific (e.g., specialised resource uptake, natural enemies, etc.) contribute to these niche differences. Such self-limiting niche differences could also be reduced in intensively-managed grasslands: for instance, fertilising grasslands could prevent trade-offs in nutrient use from stabilising coexistence, by reducing the number of resources, such as N, P, and K, that plants compete for (Wilson and Tilman, 1993). These changes would lead to smaller niche differences between plant species, and therefore, less stable coexistence between them (Adler et al., 2018; Saavedra et al., 2017). On the other hand, diversity loss can lead to an increase in specialist pests and pathogens (Bagchi et al., 2014), which might stabilise coexistence between remaining species. Many other changes in coexistence mechanisms are possible and land use intensification could therefore lead to complex changes in multiple mechanisms stabilising the dynamics of interacting species. However, this range of different mechanisms can be collectively explored by quantifying overall niche differences along gradients of land use intensity.

In multispecies communities, species can also coexist through two types of indirect interactions. The first possibility is intransitive competition, e.g., the rock-paper-scissors game in which species A excludes B, species B excludes C, and species C excludes A, resulting in no universally superior competitor (Soliveres et al., 2015). Weaker forms of intransitivity are also possible and could promote coexistence in combination with niche differences (Gallien et al., 2017; Godoy et al., 2017). The second possibility is that a superior competitor differentially harms two inferior competitors and thereby indirectly facilitates one of them (Miller, 1994). For instance, species B and C cannot coexist together alone but if species A harms species B more than C, then it can indirectly facilitate species C and allow the triplet to coexist (Miller, 1994). Land use may reduce the importance of these indirect interactions for coexistence (Soliveres et al., 2015; Laird and Schamp, 2006): for example, land use intensification can lead to more hierarchical competitive networks (DeMalach et al., 2017; Wilson and Tilman, 1993), in which a few species dominate and exclude the others, because it leads to greater differences in individual size and therefore a greater imbalance in competitive effects (Rees, 2013). In addition, land use could disrupt other types of indirect interactions because it reduces facilitative interactions between species (Mayfield et al., 2010; Olsen et al., 2016).

In sum, land use intensification is expected to reduce coexistence via three main mechanisms: 1) enhancing competitive inequalities between species, 2) destabilising coexistence by reducing niche differences, and/or 3) reducing the opportunity for indirect interactions to promote species coexistence. No studies have been able to determine the relative importance of these different mechanisms in driving the species loss (i.e., reduction in number of species) frequently observed with land use intensification. A key objective is therefore to better understand how coexistence mechanisms emerging at the pairwise and at the multispecies level vary simultaneously along a LUI gradient and how this contributes to species loss in managed grasslands.

Here, we address this gap by applying a structural stability approach (Saavedra et al., 2017; Rohr et al., 2014) to a unique dataset from the German Biodiversity Exploratories. This includes data on changes in abundance/cover for 50 perennial plant species, across 8 years, in 150 grasslands differing in their degree of land-use intensification (Blüthgen et al., 2012; Fischer et al., 2010). In these grasslands, we observe strong declines in species richness with increases in LUI (Newbold et al., 2015; Flynn et al., 2009) (Fig. S2.1), similar to the decline in richness seen in a long term fertilisation (Crawley et al., 2005). The structural approach allows us to estimate the three types of mechanism that contribute to coexistence, for assemblages of any number of species (see Fig. 0.1 for a detailed description). Briefly, both niche differences and indirect interactions jointly determine the size of the coexistence region (i.e., the feasible domain), and the larger the region, the more likely species are to coexist. At the same time larger differences in the ability of a species to increase its population (i.e., differences in intrinsic growth rate) make coexistence more difficult (Saavedra et al., 2017; Godoy et al., 2018). We first use the changes in species abundances (i.e., proportion of cover) over time to estimate species intrinsic growth rates, and all pairwise interactions between species, and to assess how LUI changes these growth rates and pairwise interactions. We then couple a population model with the structural stability approach to estimate how land use changes coexistence mechanisms. We do this by calculating the expected structural niche differences, structural fitness differences and indirect interactions for all sets of two and three species, at multiple points along the LUI gradient. To validate our models and to show the importance of these modelling tools for practitioners and conservation biologists, we use them to test whether the predicted number of species that is expected to coexist along the land use intensity gradient is similar to the observed pattern in the decline of number of species with LUI (Newbold et al., 2015; Flynn et al., 2009) (Fig. S2.1).

2.2 Materials and methods

2.2.1 Study system

The Biodiversity Exploratories (www.biodiversity-exploratories.de) project (Fischer et al., 2010) is a research initiative that has established 150 permanent grasslands plots of 50 × 50 m in three different regions across Germany: the UNESCO Biosphere Area Schwäbische Alb (south-west), Hainich National Park (central) and the UNESCO Biosphere Reserve Schorfheide-Chorin (north-east). All three regions have a similar climate (a range of 3 °C in mean annual temperature, and 500 to 1000 mm annual precipitation—more details provided in Fischer et al. (2010)). These 150 plots have been managed as grasslands for at least 20 years prior to the start of the project. Farmers and landowners provide information on the intensity of land management activities, including fertilisation, mowing, and grazing. These sites, which range from seminatural to intensively managed grasslands, are either mown or grazed. Grazed plots have cattle, horses, or sheep, with varying numbers of animals and duration of grazing. Grazing intensity was quantified as the number of livestock units ha^{-1} year^{-1}. Mown plots are cut one to three times per year. Some grasslands are also fertilised and the intensity is quantified as the amount of organic and inorganic nitrogen added to the grassland (Blüthgen et al., 2012).

With this detailed land use information, prior work has quantified a compound index of land use intensity (LUI), which integrates the intensity of fertilisation (F), the mowing frequency (M), and the intensity of grazing (G) for each grassland plot (Blüthgen et al., 2012; Fischer et al., 2010). For each plot, an individual LUI component (F, M, or G) was standardised relative to its mean across all three regions and all years considered. The compound LUI is the sum of the three standardised components (more details are provided in Allan et al. (2014)). The main advantage of this index is to summarise different, correlated aspects of land use into a single metric. We use this index to estimate the effect of human mediated actions on species interaction coefficients and intrinsic growth rates (see next section, estimation of species interactions). The minimum LUI of 0.5 could be produced by mowing every 2 y, fertilising at the rate of 6 kg of N ha^{-1} y^{-1}, or grazing one cow (>2 y old) per hectare for 30 d (or one sheep per hectare for the whole year). An intermediate LUI of 1.5 would equate to around two cuts per year, the addition of 60 kg of N ha^{-1} y^{-1}, or grazing one cow per hectare for most of the year (300 d). A high LUI of 3.0 could be produced by grazing by three cows per hectare for most of the year (300 d) and fertilising at the rate of 50 kg of N ha^{-1} y^{-1} or by cutting three times and fertilising with 130 kg of N ha^{-1} y^{-1}. For more details see Allan et al. (2014); Fischer et al. (2010). Within the period of 8 years analysed, LUI varied in our study between 0.5 to 3.0 across plots (Fig. S2.1).

2.2.2 Estimation of species interactions

The main aim of our study was to investigate how coexistence mechanisms, at local scales, vary along the LUI gradient. To achieve that aim, we need to estimate species intrinsic growth rates, the matrix of species interactions, including intra-and inter-specific interactions, and the effects of LUI on both species interactions and intrinsic growth rates. By doing a space for time substitution, we analysed a time-series of changes in the proportional cover of the 50 most common species (i.e., the most frequently observed species across all plots) between 2008 and 2015. Percentage cover (converted to proportions) was measured for each species and plot in 4m × 4m subplots at peak biomass by (Socher et al., 2012). To obtain estimates of 1) species intrinsic growth rates, 2) pairwise species interaction coefficients, and 3) the effect of LUI on these parameters, we fitted generalised linear mixed models (GLMM). Using a similar approach to (Tredennick et al., 2017; Clark et al., 2020; Grenfell et al., 1998), we analysed the log ratio of cover at time $t+1$ and cover at time t for each focal species (as the dependent variable). The log ratio of cover was modelled as a linear function of LUI, together with the cover of each species in the community (including itself) at time t, and we further included the interaction between LUI and cover of all species. The model also included plot nested within region as a random effect and accounted for temporal autocorrelation using an ARMA structure $corAR1$ $(t+1)$. We also tested for autoregressive components with greater time lags ($t+2$ and $t+3$) but they resulted in a worse statistical fit. Models were fitted using the function `lme` in the R package ”nlme” (Pinheiro, 2011). The general form of the statistical model fitted is as follows:

$$\ln \frac{Cover_{i,t+1}}{Cover_{i,t}} \sim r_{i,LUI} + \sum_{j=1}^{n} \alpha_{ij,LUI} \times Cover_{j,t}, random = plot/region + corAR1(year) \tag{2.1}$$

In this statistical model, the intercept ($r_{i,LUI}$) was interpreted as the intrinsic growth rate because it estimates the maximum ability of a species to change in cover between two years ($t+1/t$), at different LUIs, in the absence of any neighbours in the previous year (t), i.e., at intra- and inter-specific cover of 0. Because we fitted a separate model for each species i, values of the intercept can vary across species and LUI. In turn, the alpha coefficients ($\alpha_{ij,LUI}$) describe how positive (facilitation) or negative (competition) per-capita interactions with conspecific and heterospecific neighbours change with plant cover over time. Again, because our model is fitted for each species i independently, it includes the possibility that LUI differently modifies each pairwise interaction coefficient and therefore changes the whole network of species interactions at the community level. Values of species' intrinsic growth rates at different LUI values ($r_{i,LUI}$) are inferred from the regression and lack of data did not allow us to corroborate them empirically. However, we did test the sensitivity of our results to uncertainty in the parameter estimation by calculating coexistence mechanisms (i.e., structural niche and fitness differences) using parameter values at the 2.5% and 97.5% confidence intervals around each parameter (Figs. S2.5 and S2.6 and Table S2.2 and S2.3). We observed that LUI linearly changed intrinsic growth rates for all species. In fact, some intrinsic growth rates were negative at certain LUI values indicating that LUI can act as an environmental filter excluding certain species (Kraft et al., 2015*a*). Finally, we did not explicitly model dispersal in our study because there is no information available to assess the influence of propagule pressure on species local abundances (at the plot level). However, previous work in our study system has shown that seed addition does not increase the abundance of the established species, suggesting that seed limitation does not determine population growth rates in these perennial species (Klaus et al., 2016).

2.2.3 Using a structural approach for modelling species coexistence

Following the estimation of species intrinsic growth rates, the matrix of interactions, and the linear effects of LUI on these parameters, we explored how the structural coexistence mechanisms vary along the LUI gradient, using the following discrete time Lotka-Volterra population model (Saavedra et al., 2017). We could do so because there is a direct correspondence between the structure of the statistical (eqn. 2.1) and the population model (eqn. 2.2).

$$\ln \frac{N_{i,t+1}}{N_{i,t}} = r_i - \sum_{j=1}^{n} \alpha_{ij} N_{j,t} \tag{2.2}$$

Here, N_i and N_j are population sizes of interacting species (measured as proportion of cover), and r_i and α_{ij} are species' intrinsic growth rates and interaction coefficients respectively. Because LUI modified both species' intrinsic growth rates and their pairwise interaction coefficients, we parameterised this model eleven times. Each time we used a different LUI value from 0.5 to 3.0, with a 0.25 LUI increment between parameterisations. This means that we computed structural coexistence mechanisms for each LUI value independently.

The structural approach is a well-established approach in engineering and mathematical sciences to study whether the qualitative behavior of a dynamical system is unaffected by small perturbations. In ecology, it has been recently applied to understand how species within ecological communities persist despite showing variable population dynamics (Tabi et al., 2020; Song et al., 2020*a*; Bartomeus et al., 2021) but it has not been extended to understand how global change drivers such as land use intensity alter structural coexistence mechanisms. Using this approach has two main advantages over prior work addressing the importance of species interactions for coexistence (Adler et al., 2007): firstly, it allows us to determine how competition, facilitation, and indirect effects jointly combine to maintain biodiversity and second it allows us to explore these ecological mechanisms in a multispecies context. The structural stability approach computes metrics analogous to the pairwise niche differences, which stabilise coexistence between competitors, and the average fitness differences that drive competitive dominance (Chesson, 2000; Godoy and Levine, 2014). As in the pairwise case, coexistence is possible when structural niche differences exceed structural fitness differences (Saavedra et al., 2017; Rohr et al., 2014). The disadvantage of the approach is that it can be highly demanding of computer power, particularly when considering large numbers of species. For instance, computing all of the coexistence metrics used here, for all possible combinations of the 50 most common species, would take approximately 220 years using 36 CPUs/cores computing in parallel. Given these time limitations, we decided to use the initial pool of

50 species to compute structural niche and fitness differences for all possible combinations of 2 and 3 species only. We then explored the effect of indirect interactions in larger combinations of species, where indirect interactions might be expected to play a larger role. However, we only calculated the measures of indirect interactions (community-pair differential and community-pair overlap) with the 26 most common species (which saves a significant amount of time because there are 4,835 times fewer potential species combinations for 26 than for 50 species). Within the subset of 26 species, the maximum number of species considered in combinations was 11, corresponding to the maximum number of these most common species observed together in a plot.

We first calculated the structural niche differences (SND) and structural fitness differences (SFD) for all combinations of species pairs and triplets and predicted which species combinations were, or were not, stable and feasible (i.e., all species within a combination can coexist because they have abundances greater than 0). Greater SND indicate that species are more likely to coexist, whereas greater SFD indicate the opposite (Saavedra et al., 2017; Rohr et al., 2014). We then fitted a null model to test whether observed values and outcomes differ from a random expectation. We designed the null model to keep the range and shape of the original distribution of species interactions obtained from analysing the data, while modifying the location of the interaction coefficients within the matrix of species interactions. In other words, we maintained the overall variation in interaction strengths from positive (facilitation) to negative (competition) interactions, because we know this strongly modulates stability conditions (Neutel et al., 2007; McCann et al., 1998), but we randomised the strength of interaction coefficients between given species pairs. To do so, we simply reshuffled 100 times the row and column names of the interaction matrix obtained for each LUI value. In this approach species' intrinsic growth rates were not randomised.

We then performed nonlinear quantile regression using the function `nrlq` in the R package "quantreg" (Koenker and Hallock, 2001) to test the relationships between niche and fitness differences and LUI, both for empirical observations and the null models, and for all combinations of two and three species. Briefly, quantile regressions allow us to explore statistical relationships across the distribution of the response variable (i.e., across quantiles). We took this approach because, after inspecting the scatter plots of SND, SFD and LUI, we expected different relationships for the median than for the extremes of the distribution of niche and fitness differences. We used a polynomial regression of the following form: $y \sim a * LUI2 + b * LUI + c$, where y was either SND or SFD. Results obtained from shuffling interaction coefficients in the randomisations showed different patterns from the observed results (compare observed results in Fig. 2.1 versus results from randomisation in Fig. S2.3). In particular, in the randomised data we observed no change in SND with LUI but a clear increase of SFD as well as much greater variation in SFD along the LUI gradient. These changes in niche and fitness differences could not explain the decline in species richness with LUI. Importantly, we did not compare niche and fitness differences between combinations of two and three species because these metrics inherently change with increasing species richness in the community.

With the structural analogues of stabilising niche and fitness differences, we can evaluate the importance of direct pairwise interactions for species coexistence. However, the structural stability approach also allows us to test the importance of indirect interactions for multispecies coexistence. To evaluate whether indirect effects increase or reduce species coexistence compared to the pairwise case, we computed a metric called the community-pair differential. Following Saavedra et al. (2017), we computed the difference in the size of the feasibility domain for combinations of 3, 5, 7, and 11 species and the feasibility domain for all pairs of species. The community-pair differential ranges between −1 and 1. A positive value indicates greater opportunities for coexistence in the full community (i.e., larger feasibility domain for all species together than for the pairs of species) whereas negative values indicate the opposite. This metric tells us whether indirect interactions create greater opportunities for coexistence by enlarging the feasibility domain. However, it tells us little about whether species actually can coexist thanks to indirect interactions, or if coexistence is achieved by pairwise interactions only (i.e., if the feasibility domain created by pairwise interaction is already sufficient for all species to coexist). Therefore, to evaluate the degree to which indirect, versus pairwise, mechanisms actually explain the coexistence of combinations of 3, 5, 7, and 11 species, we computed a related metric called community-pair overlap. This metric involves calculating the proportion of the feasibility domain for the entire community that lies inside the feasibility domain for all pairs. A value of community-pair overlap close to zero indicates a stronger importance of indirect interactions for species coexistence, whereas a value close to one indicates the opposite (stronger importance of pairwise interactions for coexistence).

After obtaining empirical estimates of all these mechanisms of species coexistence, arising from pairwise and indirect interactions, we finally tested whether our coexistence predictions based on direct interactions between pairs of species are able to reproduce the observed pattern of species diversity decline along the LUI gradient. To make this comparison possible, we calculated the average and standard deviation of the number of species found in each plot versus the maximum number of species predicted to coexist. These predictions were done based on the number of pairs predicted to coexist, with each pair treated independently. For instance, if species A coexists with B, B excludes C, but species C coexists separately with D, we assume that all four species can coexist. All analyses were conducted in R version 4.2.1 (R Core Team, 2022).

2.3 Results and Discussion

Changes in the intensity of grazing, mowing, and fertilisation, summarised in our LUI index (Blüthgen et al., 2012), altered the strength of intra and interspecific competition in opposite ways. Intraspecific competition remained high across the LUI gradient, which should result in the preservation of strong SND and a large coexistence region. However, we also observed complex changes in interspecific competition, which was stronger and more variable at low and particularly at high LUI (Fig. S2.2). These combined shifts in intra- and inter-specific competition resulted in a nonlinear decline in SND with LUI, as well as in more variation in SND between sets of species (pairs, triplets) at low and high LUI (Fig. 2.1). In particular, there were more assemblages with very low SND at high LUI (e.g., 10% quantile SND ~ 0.5 at LUI = 0.5 and 3 while SND ~ 0.75 at LUI = 1.75). The observed change in SND was different to that expected by chance: a null model in which we shuffled competition coefficients between species showed no variation in the mean trend of SND but a marked increase in variance across the LUI gradient (Fig. S2.3). Against our expectations, SFD, which arise from differences in intrinsic growth rates between species, did not vary on average across the LUI gradient (Fig. 2.1). This lack of variation across the LUI gradient was due to the fact that species with higher intrinsic growth rates also showed more sensitivity to interspecific interactions (Fig. S2.4). We also speculate that this lack of variation could be explained by the fact that fertilisation increases fitness differences (DeMalach et al., 2017) but at the same time mowing acts as an equalising mechanism, and grazing reduces fitness differences between fast-growing but palatable versus slow-growing but non-palatable species (Borer et al., 2014). However, it is challenging to separate these effects with our dataset as mowing and fertilisation are strongly correlated with each other (DeMalach et al., 2017). Interestingly, however, SFD were key drivers of whether assemblages of two and three species could coexist or not, and this effect was consistent along the LUI gradient (Fig. 2.1). Large differences in competitive ability therefore are the main factor restricting coexistence in general but as they do not change with LUI, changes in competitive ability do not contribute to reduce diversity in intensively managed grasslands. The observed SFD also differed from that expected by random. Using our null model, average SFD, and the variation between them, increased along the LUI gradient. Because we only randomised interactions coefficients but not intrinsic growth rates, the increase of SFD with LUI was due to the fact that we broke the positive relationship between intrinsic growth rate and sensitivity to interspecific interactions (Fig. S2.4), so species that grow more are no longer those that suffer more at the same time from interspecific interactions. These overall changes in SND and SFD were also observed when we accounted for uncertainty in the estimates of intrinsic growth rates and interaction coefficients (Fig. S2.5 and S2.6 and Table S2.2 and S2.3). Taken together, our results show that LUI reduces SND more strongly than it increases SFD, and this reduction occurs nonlinearly. Such a nonlinear relationship was not predicted by theory, but it implies that coexistence between the 50 most abundant species is actually more likely at low and at intermediate LUI (Fig. 2.1a and 2.1c); however, when LUI is increased beyond a certain threshold, coexistence is strongly reduced.

It is also worth noting that changes in SND and SFD were more pronounced for combinations of three species. In particular, the nonlinear decrease in SND with LUI was more pronounced for three (Fig. 2.1b) than for two species (Fig. 2.1a). This might suggest that land use intensification does reduce SND but this reduction is higher when the assemblage includes a dominant species at high LUI. If dominant species are included this will increase the strength of interspecific effects (this was observed for instance for *Poa trivialis* and *Trifolium pratense*), and therefore, make it more likely to detect a decrease in SND. Similarly, we observed an increase in the variance of SFD with LUI for sets of three species (Fig. 2.1d) compared to pairs of species (Fig. 2.1c). Again, this might imply a division between a group of particular competitor species and a group of subordinates. These results underline the importance of considering coexistence not only between pairs of species but also between multispecies assemblages.

Although SND declined with land use intensification, they remained surprisingly high across the LUI gradient and were often large enough to overcome the

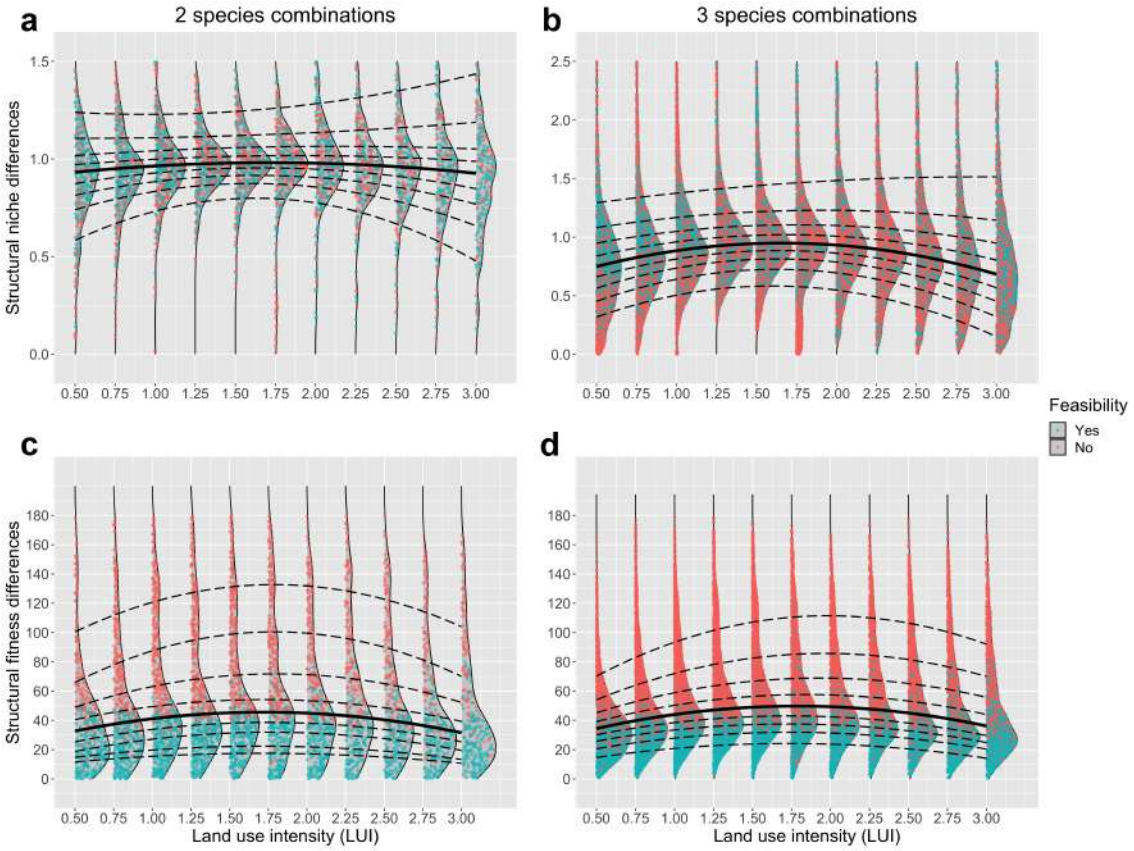


Figure 2.1: Distribution of structural niche (panels **a** and **b**) and structural fitness differences (panels **c** and **d**) for two (**a** and **c**) and three (**b** and **d**) species combinations, across the LUI gradient. Each point corresponds to a species combination and its color denotes whether this combination is predicted to be feasible (blue; all species can coexist) or not (red). The lines across the graph correspond to nonlinear quantile regressions evaluating whether LUI changes structural niche and fitness differences for combinations of two and three species. We performed 9 nonlinear quantile regressions (using a polynomial form $y \sim a * LUI2 + b * LUI + c$) including the median (thicker solid line) for each panel. Statistical significance is provided in Table S2.1.

observed SFD, at least for pairs of species (Fig. 2.1). This result suggests that regardless of how grasslands are managed, the species present are not maintained by quasi neutral dynamics, in which intra- and interspecific interactions are weak and very similar (Matías et al., 2018), and in fact coexistence is maintained by highly structured processes of niche differentiation. We cannot identify the processes that underlie such niche differentiation, but it is likely that in some cases the same processes might maintain coexistence in grasslands differing in land use intensity. For instance, it has been shown that mycorrhizal diversity and abundance do not decline with LUI in these perennial grasslands (Gossner et al., 2016), and mycorrhizae might therefore contribute to coexistence across a range of grasslands (Hart et al., 2003). In other cases, the mechanisms underlying SND might differ between high and low LUI grasslands. For example, foliar pathogen infection and soil fungal pathogen diversity both increase with LUI (Harpole et al., 2016) and specialist fungal pathogens might therefore play a stronger role in driving SND in low diversity, high LUI communities. Specialist pathogens could have driven the strong negative frequency dependent population growth seen in *Poa pratensis*, a dominant grass at high LUI. In contrast, at low LUI, we might expect trade-offs in nutrient use to play a greater role in driving SND (Harpole et al., 2016). More work is needed to identify the mechanisms underlying the niche differences we find, but our analysis highlights the key importance of niche differences in maintaining coexistence and explaining declines in diversity following land use intensification.

We also found that positive facilitative interactions were common at all land use intensity levels (Fig. S2.2). In some cases, they even allowed species to persist in grasslands where they would otherwise have been excluded because the prevailing land use acted as an environmental filter (i.e., intrinsic growth rates below 0) in agreement with suggestions from studies on functional trait distributions (Pakeman, 2011). For example, the intrinsic growth rate of *Holcus lanatus* became negative from intermediate to high LUI (range -0.17 to -0.35) but the species persisted and our results suggest this could be due to facilitation from several species, including *Lolium perenne* (grass), *Trifolium repens* (legume), and *Plantago lanceolata* (forb). These results showing facilitative interactions in managed grasslands suggest that facilitation is not restricted to harsh and stressful environments, such as drylands or alpine systems (Cavieres, 2021; Liancourt and Dolezal, 2021; Hart and Marshall, 2013; Le Bagousse-Pinguet et al., 2014) and that it may be widespread in many situations. Both niche differentiation and interspecific facilitation have recently been identified as the main drivers of species coexistence in natural ecosystems globally (Adler et al., 2018; Losapio et al., 2021) and our results also show that they are the main drivers of species coexistence in heavily human-modified ecosystems. Detailed research is needed to better understand how facilitation operates in intensively managed systems, but our results suggest it could be an important process.

The structural niche differences describe the net effect of both direct and indirect interactions on coexistence. The reduction in niche differences with LUI that we observed for sets of three species (compared to pairs) could arise if LUI more strongly modified indirect interactions than direct pairwise ones. To evaluate the relative importance of indirect interactions in creating (or reducing) novel opportunities for species coexistence, we computed a pair of related metrics for sets of three species (Saavedra et al., 2017; Song et al., 2018). These metrics are community-pair differential and community-pair overlap. Briefly, community-pair differential is a metric ranging between -1 and 1 that quantifies whether the coexistence region increases or decreases with indirect interactions, i.e., whether indirect interactions promote or reduce coexistence. Community-pair overlap is a metric that quantifies the proportion of the coexistence region for a triplet that is similar to its constituent pairs. It ranges between 0 when triplets and pairs are completely different in their coexistence region, i.e., they coexist in different ways, and 1 when regions are exactly the same.

Neither of the metrics quantifying the effect of indirect interactions on coexistence varied along the LUI gradient in sets of three species, indicating no change in the importance of indirect interactions. Community-pair differential values (which quantify whether indirect interactions decrease or increase the coexistence region) were close to zero (Fig. 2.2a), indicating that indirect interactions do not enhance coexistence. In addition, community-pair overlap values (the proportion of the coexistence region for a triplet that is similar to its constituent pairs) ranged from 0.5 to 0.8 (Fig. 2.2b), indicating that the majority of three species combinations can coexist because each of the three pairs that compose the triplet can also do so. These results contrast with prior work done in this system, which suggested a major role for indirect interactions, and particularly intransitivity (Soliveres et al., 2015), in promoting species richness. One potential explanation for these contrasting results is that different indirect interactions (e.g., intransitivity against indirect facilitation) have contrasting effects on coexistence and cancel out to reveal no overall effect of indirect interactions in this analysis. Another possibility could be that the current analysis included subdominant species, while the previous analysis (Soliveres et al., 2015) focused only on intransitivity between dominant species. As intransitive competition has been shown to be stronger between dominant species (Soliveres et al., 2015) this might explain the low importance of indirect interactions in the current study. Further work is needed to explore a wider range of indirect interactions and to determine how they change with land use intensification.

It is also possible that indirect interactions become more important for coexistence as we consider larger sets of species. This is because the likelihood of finding a configuration of species interactions that maintains biodiversity is higher in larger sets of species. We therefore also computed community-pair differential and community-pair overlap metrics for assemblages of 5, 7, and 11 species, but only focused on the 26 most abundant species in this analysis to save computing power. We selected these numbers because it has been shown that indirect interactions promote coexistence when there is an odd number of species in the community, under the assumption of a random tournament (Gallien et al., 2017; Allesina and Levine, 2011), and 11 species is on average the maximum number of species found per plot (from the set of 26 species analysed). We found that community-pair differential remained low in combinations of 5, 7, and 11 species. This suggests that indirect interactions did not increase or decrease the opportunities for coexistence amongst larger sets of species (Fig. 2.2a). However, indirect interactions did change the shape of the feasibility domain, particularly

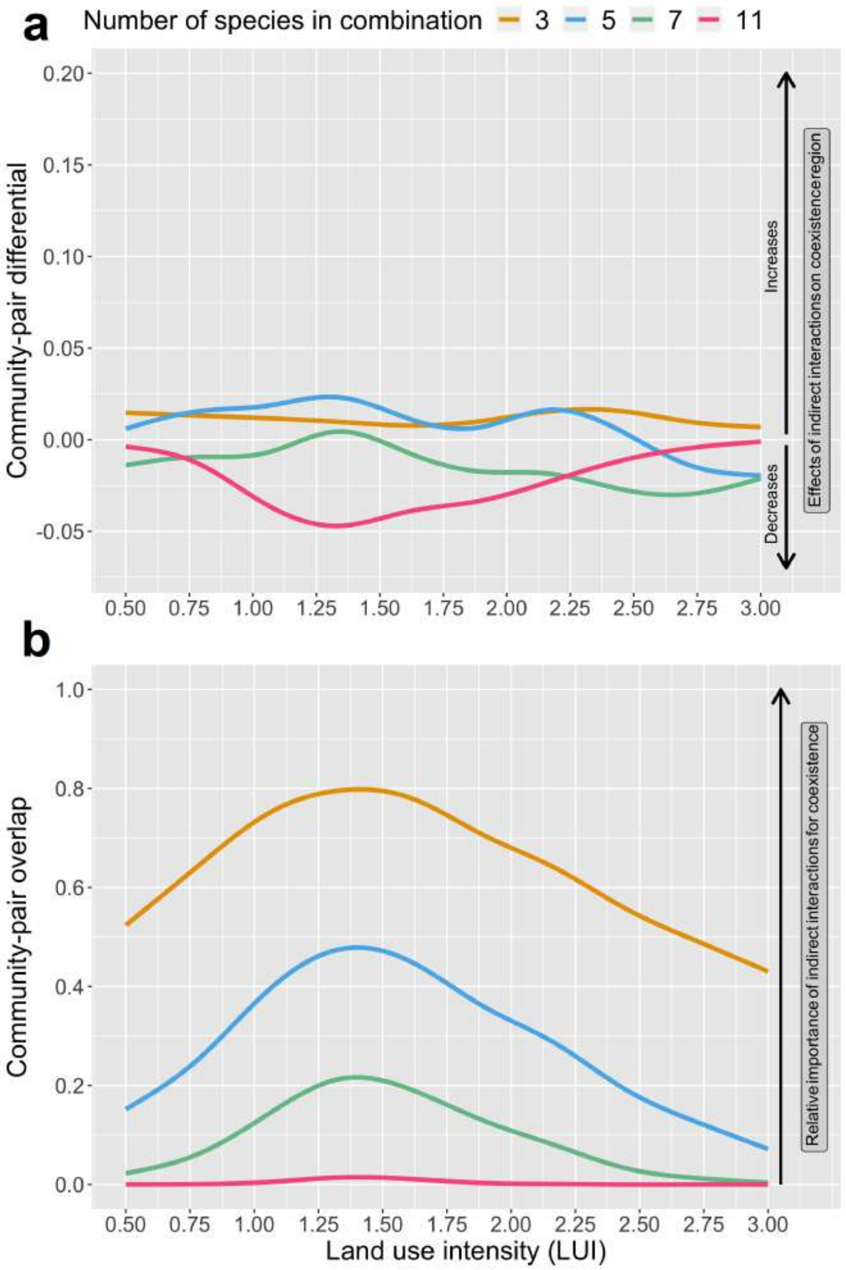


Figure 2.2: Community-pair differential (i.e., the importance of indirect effects for coexistence, panel **a**) and community pair overlap (i.e., the importance of pairwise only interactions against indirect effects for coexistence, panel **b**) across the LUI gradient for assemblages of 3, 5, 7, and 11 species. The colors show species assemblages of different sizes. Values correspond to the mean for each number of species. Standard errors are not provided to facilitate visualisation.

for larger assemblages at intermediate LUI (community-pair overlap at LUI = 1.5 was ∼ 0.20 and 0.05 for 7 and 11 species respectively, i.e., 80% and 95% of the coexistence region due to indirect interactions do not overlap with the coexistence region promoted by direct interactions) (Fig. 2.2b). This indicates that indirect interactions that occur in multispecies assemblages change the way in which species coexist but they do not lead to more or less coexistence, perhaps because different indirect interactions have opposing effects on coexistence. Overall, these results show that direct pairwise interactions are the main mechanism promoting coexistence across assemblages; however, the opportunities for coexistence that indirect interactions create are completely different compared to those created by direct interactions, particularly at intermediate LUI.

We then analysed whether we could reproduce the observed decline in species richness with LUI, based on our estimates of how species interactions varied. This allows us to validate our estimates of species interactions by using them to predict diversity. As we found that pairwise interactions were most important for coexistence overall, we focused on the coexistence predicted between species pairs (see the slope of the decline of species richness with LUI; Fig. S2.1). We computed the cumulative number of species predicted to coexist in any pairwise combination.

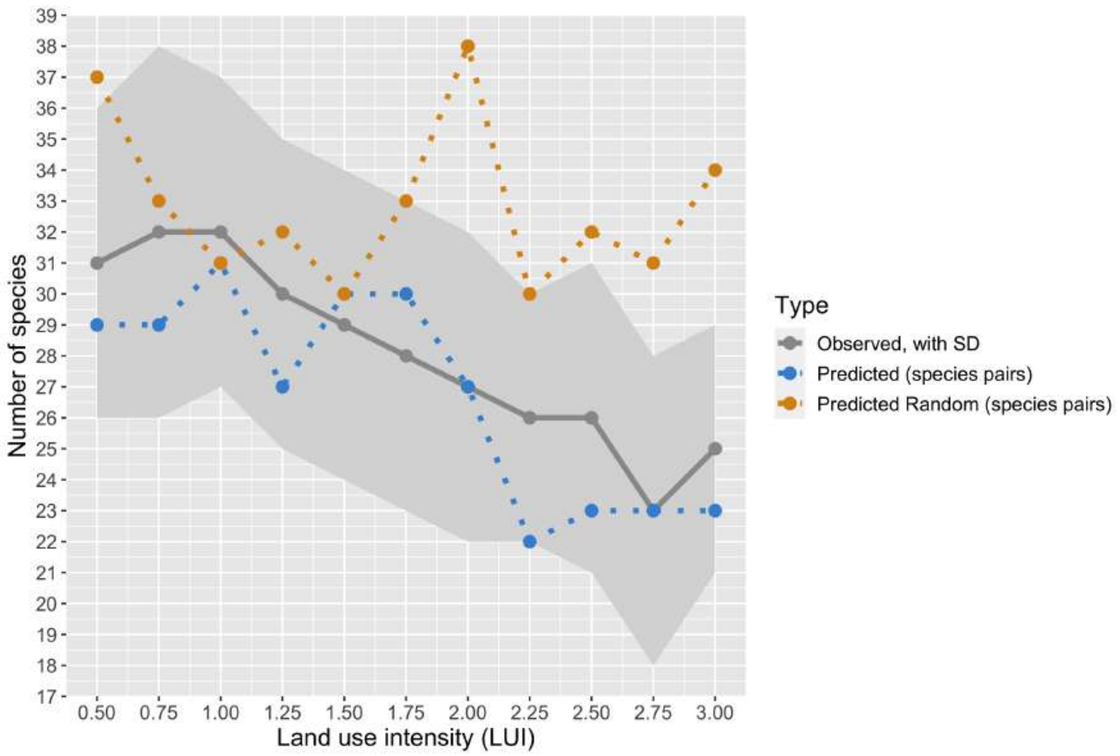


Figure 2.3: The average number of species (grey line) (± standard deviation) observed to co-occur in a plot, the maximum number of species predicted to coexist according to the structural stability approach (in blue), and the maximum number of species predicted to coexist according to null expectations by randomising species interactions (in orange). Predictions from the models parameterising species interactions fit well with the observed decline in species richness with LUI. However, randomising interactions between species leads to much higher coexistence than observed. See methods for details on how the predictions were made.

For instance, if at LUI = 0.5, species A coexists with B, B excludes C, but species C coexists separately with D, we assume that all four species can coexist within the grassland. We then repeated this procedure along the LUI gradient (from 0.5 to 3.0). This approach has been recently shown to reproduce the increase of species richness with area in spatially variable environments because it implicitly allows different species pairs to coexist in different patches (García-Callejas et al., 2021). Despite the simplicity of the model, we were able to reproduce the decline in species richness with LUI (Fig. 2.3). Note that we only calculated species richness based on the 50 most common species analysed in our study, which resulted in an initial slight increase in observed species richness with LUI, followed by a strong decline. However, including all species results in an exponential decline in species richness with LUI because many rare species are only present at the lowest LUI (Allan et al., 2014). It was not possible to include these rare species in our analysis as we lacked data to estimate changes in their abundance. Further experimental work would be extremely valuable in identifying mechanisms of coexistence between rare species. Overall, we found that the cumulative number of species predicted to coexist across the LUI gradient was close to the observed species richness, and different from randomisation predictions which did not capture the reduction in species richness with LUI. Instead the random expectation would be for variation in diversity but no net change with LUI. The only deviation was that we predicted slightly fewer coexisting species than observed at intermediate to high LUI values (LUI = 2.25—2.50) (Fig. 2.3). This difference is most likely due to the reduction in intraspecific competition at intermediate LUI (Fig. S2.2). Although the comparison between predicted and observed diversity suggests that we might have slightly underestimated the strength of niche differences at intermediate to high LUI, our overall ability to describe the decline in species richness with LUI provides strong evidence that the theoretical approach used here can well describe the observed patterns.

By using time series data from 150 plant communities, we were able to estimate how land use intensification causes declines in plant diversity. Our findings provide strong evidence that a decline in niche differences with LUI explains the loss of diversity and, surprisingly, that changes in competitive ability play a relatively minor role. Determining what niche differences are lost as we intensify grassland management is a crucial next step that could enable us to offset the negative effects of land use on diversity. We also find that niche differences and interspecific facilitation maintain coexistence even in heavily modified ecosystems. Very little work has been done on how species coexist at high land use intensity, but our results show that, although diversity is reduced, coexistence between the remaining species is highly stable. Uncovering the processes that drive niche differences at high LUI might allow us to better understand and even optimise the ecosystem functioning of intensively managed grasslands. Our coexistence models were able to reproduce the observed diversity declines suggesting our results are robust. The approach of using time series to estimate species interactions and coexistence mechanisms could therefore be widely applied to derive a more mechanistic understanding of global change effects on biodiversity.

Associated publication

- Godoy O, Granjel RR, van der Plas F, Soliveres S, Penone C, Saiz H, Hölzel N, Prati D, Fischer M, Allan E. 2022. Loss of species richness with land use intensity is explained by a reduction in niche differences. *bioRxiv*: 2022.12.15.520291.

Open science

- R code: www.github.com/oscargodoy/LUI_coexistence
- Plant cover data: www.bexis.uni-jena.de/ddm/data/Showdata/27386
- LUI data: Vogt et al. (2019)

Author contributions

O.G., F.v.P., S.S., and E.A. conceptualised and formalised the idea. O.G., R.R.G., and E.A. designed the methodology and carried out the investigation. O.G. and R.R.G. computed the analyses and prepared the visualisation of results. O.G. and E.A. wrote the original draft, with subsequent review and editing from R.R.G. and all authors.

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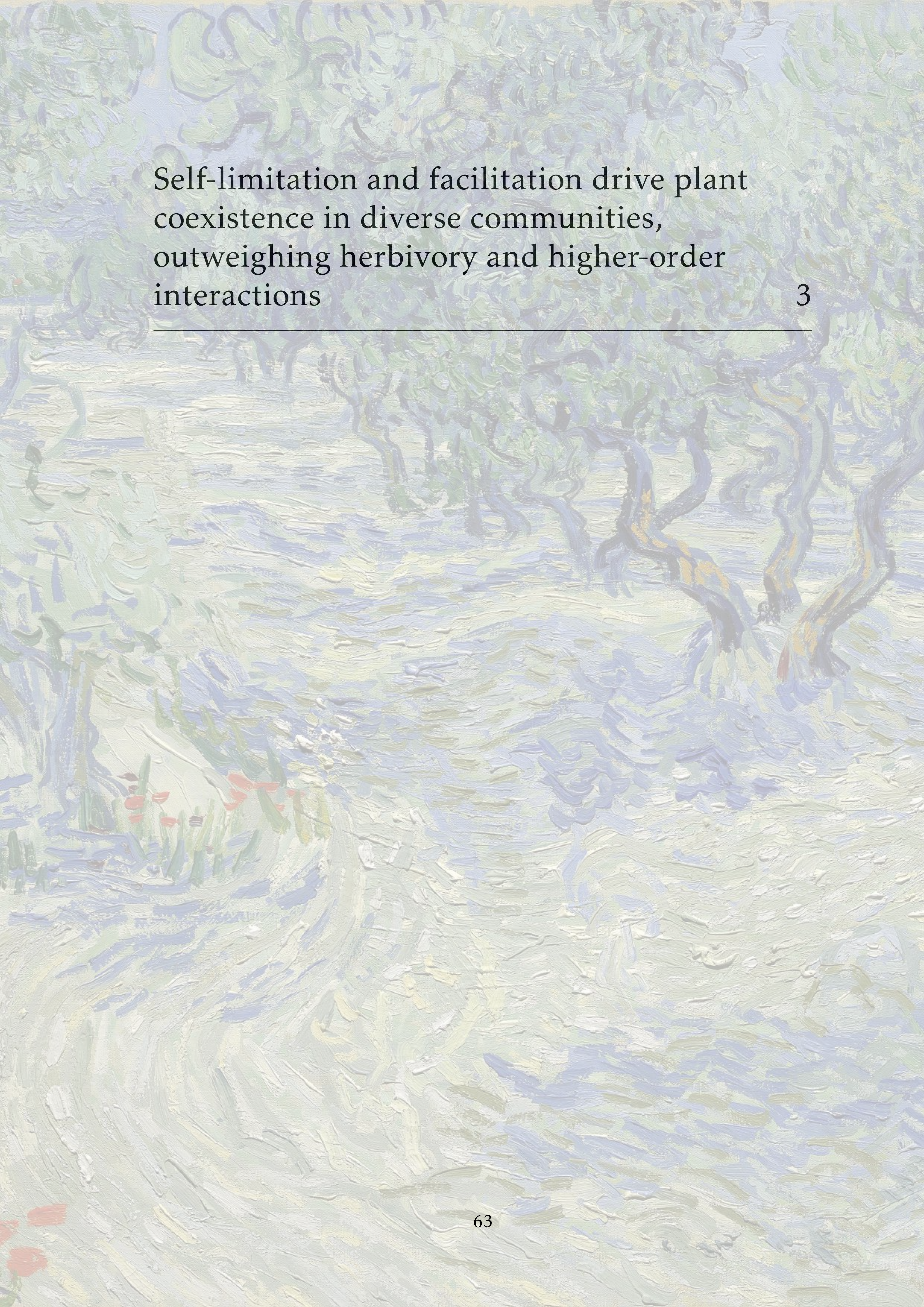
Supplementary material

Supplementary figures and tables related to this chapter are available in Appendix 2.



Self-limitation and facilitation drive plant coexistence in diverse communities, outweighing herbivory and higher-order interactions

3

Abstract

Highly-diverse ecological communities, such as plant-herbivore systems, involve a wide range of interactions that vary in sign, strength, and species. Due to the complexity of describing these interactions, it is difficult to understand which specific pairwise (plant-plant and plant-grasshopper) and higher-order interactions are critical in determining the long-term viability of plants (feasibility). In this study, we applied a regularisation approach to a detailed field experiment to identify the strength and sign of specific interactions among 36 plant species and 6 grasshoppers, which describe the spatiotemporal variation in plant cover. We then used this information along with theoretically informed metrics (structural niche and fitness differences) to understand what type and structure of species interactions drive the determinants of community feasibility. We found that only a subset of interactions across all types was selected, and among them, plant-plant interactions played the predominant role in promoting the feasibility of modules of three and four plant species in the community. Within this key plant interactions compartment, the opportunities for species to coexist increased with facilitation and stronger intraspecific effects. Our results suggest that the specific structure of plant-plant interactions is key in promoting the feasibility of diverse systems with plant-herbivore interactions.

Keywords

Group lasso regularisation, grasshoppers, interaction metrics, multispecies coexistence, perennial plants, species modules, structural stability

3.1 Introduction

Understanding the role of ecological interactions in promoting species persistence (i.e., long-term positive abundance) is a longstanding research topic in ecology (Chesson, 2000; Saavedra et al., 2017). Theory predicts that coexistence between two competing species can be achieved when they limit their peers more than their competitors (i.e., intraspecific competition is greater than intraspecific competition) (Chesson, 2000; Barabás et al., 2016). However, natural ecological systems are not composed of two but of multiple species belonging to different trophic levels, forming complex interaction structures that challenge our ability to interpret the mechanisms of coexistence (May, 1972; Mougi and Kondoh, 2012; Levine et al., 2017). Ecologists have addressed this challenge through three main perspectives. The first one aims to analyse the prevalence of positive, neutral, or negative interactions in ecological communities and the effect of the strength of such interactions on species' growth rates (e.g., McCann et al. (1998); Neutel et al. (2002); Losapio et al. (2021)). The second perspective aims to compare whether interactions that occur horizontally within species of the same trophic level (e.g., direct and indirect competition) are more relevant to maintain species diversity than vertical interactions that occur between species of different trophic levels (e.g., herbivory) (e.g., Godoy et al. (2018); Song et al. (2018); Bartomeus et al. (2021)). Finally, the last perspective examines the configuration of species interactions within modules across whole networks (i.e., a combination of n-species that interact within a more complex, m-species network, with n being any natural number from 2 to m) (e.g., Melián et al. (2009); Stouffer and Bascompte (2010)). Although these three approaches have been critical in providing us with important insights into the drivers of species persistence, there are still several main challenges that need careful consideration.

It is a common practice to define the sign of interactions aprioristically, based on preconceived notions, which can lead to incorrect assumptions about the nature of such interactions (Bastolla et al., 2009; Rohr et al., 2014). For example, it is commonly assumed that pollinators and herbivores exert either positive or negative effects on plant fitness, but this is not always the case. Pollinators can also hinder plant fitness if their densities are too high (Magrach et al., 2017), whereas herbivores can increase, rather than decrease, per capita growth rates of perennial plants by pruning leaves and stems, potentially boosting biomass production (Karban and Strauss, 1993; Meyer, 1998; Kim et al., 2013). Therefore, a more accurate and nuanced understanding of the role of the interaction sign in species persistence can be obtained by empirical estimations rather than by theoretical assumptions (Godoy et al., 2018). In this regard, a recent study by Losapio et al. (2021) showed that alpine plant communities harbour more biodiversity when common modules including both positive and negative interactions occur. Nonetheless, the distribution of interaction strengths within each module together with their sign is key to predicting species' persistence (May, 1972; Barabás, 2021). Previous knowledge, mostly from food webs, has shown that the persistence of diverse systems arises when many weak and only a few strong interactions occur (McCann et al., 1998; Berlow, 1999; Neutel et al., 2002). Complementary, diverse systems are also found to be stable if their interactions show low variability irrespective of the magnitude, i.e., most of the species interact with similar strength (Kokkoris et al., 2002). Consequently, to assess the role of interactions in stabilising ecological systems we need network metrics that account for the sign and the structure of interaction strengths within species modules (de Ruiter et al., 1995; Rossberg et al., 2011).

Another key consideration is that direct pairwise species interactions, (i.e., interactions involving two species that affect each other due to shared resources or natural enemies) (Barabás et al., 2016), have been the basis of most ecological efforts to investigate the mechanisms of species coexistence (Chesson, 2000; Barabás et al., 2018). However, species can also interact indirectly through interaction chains that retain a pairwise nature (Levine et al., 2017), such as intransitive dynamics exemplified by the well-known game of rock-paper-scissors (Laird and Schamp, 2006; Soliveres et al., 2015; Godoy et al., 2017; Alcántara et al., 2017; Gallien et al., 2017; Matías et al., 2018; Stouffer et al., 2018). On top of that, these direct and indirect interaction chains can be modified in the presence of a different species, generating higher-order interactions (HOIs) which are common in ecological systems and well-documented in the literature (Billick and Case, 1994; Bairey et al., 2016; Mayfield and Stouffer, 2017; Grilli et al., 2017; Kleinhesselink et al., 2022). Recent studies addressing complex communities suggest that it is likely to expect a combination of these interactions within trophic levels (e.g., pairwise plant-plant interactions and plant HOIs) as well as across trophic levels (e.g., pairwise herbivore-plant interactions and HOIs modifying plant-herbivore interactions due to the presence of a third plant species) (Chesson and Kuang, 2008; Melián et al., 2009; Fontaine et al., 2009; Thébault and Fontaine, 2010; Mougi and Kondoh, 2012; Godoy et al., 2018; García-Callejas et al., 2018). Yet, the consequences of this combination of interaction types for the ability of particular modules within communities to persist remain virtually unexplored (but see Buche et al. (2021) for a recent effort).

The lack of studies integrating all these main elements and characteristics of the structure of interactions within an ecological community is preventing us from identifying which are the key mechanisms driving species coexistence in local natural systems. Here, we combine a detailed experiment of 36 perennial plant species and 6 grasshoppers species with a strong modelling approach to understand how the multitrophic structure of interactions determines plant coexistence by promoting niche and fitness differences using a structural stability approach (Rohr et al., 2014; Saavedra et al., 2017; Song et al., 2018). According to theory, structural niche differences determine the opportunities for species to coexist and the larger the niche differences, the more variation in intrinsic growth rates the community can withstand without losing species. These niche differences occur, for instance, when intraspecific interactions exceed interspecific interactions (Barabás et al., 2016) although indirect interactions can also promote niche differences (Saavedra et al., 2017). On the other hand, structural fitness differences determine the dominance of species within an ecological community and the species with the highest fitness (i.e., the species with the highest intrinsic growth rate will exclude the rest of the species in the absence of niche differences) (Saavedra et al., 2017).

A clear problem given the high dimensionality of our approach (estimation of pairwise interactions within plants, the effect of herbivores on plant species, as well as HOIs) is to identify which particular interactions are meaningful versus which ones can be discarded because they contribute little to plant species growth rates. We address this challenge by conducting first regularisation techniques (Lim and Hastie, 2015), which consequently leads us to hypothesise that only a subset of pairwise interactions and HOIs will be statistically selected. Next, we document the configuration of the strength (weak versus strong), sign (positive versus negative), and type (pairwise plant-plant and plant-grasshopper versus HOIs plant-plant-grasshopper and plant-grasshopper-grasshopper) of those interactions selected for modules of three species. We hypothesise that all these characteristics are not randomly distributed but they will differ in their relative contribution to promote the structural niche and fitness differences that determine multispecies coexistence. In sum, our final goal is to investigate whether we can identify particular structures of interactions within the complexity of ecological communities that are fundamental to promoting species persistence, which would help avoid the daunting and often impossible task of estimating interaction types for all co-occurring species.

3.2 Materials and methods

3.2.1 Study system

The experiment was set up for two full years (from July 2012 to May 2014) in a species-rich calcareous grassland (76 plant species at the field scale, 65 recorded in our experiment) located in the long-term ecological research (LTER) site ”Zone Atelier Plaine et Val de Sèvre” (46°110 N, 0°280 W) in Central-Western France. This grassland was established at least 20 years ago on shallow soil and managed using extensive options (no fertilisation, cutting frequency once or twice a year), which resulted in a prairie dominated by grasses (average cover of 38.5%), forbs (30.5%), and legumes (7.5%). A full list of plant species can be found in Table S1 and a broader description of the field site can be found in Deraison et al. (2015*a*).

3.2.2 Grasshoppers and experimental design

The experiment was performed using a randomised block design (Hurlbert, 1984) with five blocks and 14 treatments for a total of 70 cages (Fig. S3.1). Each cage corresponded to a 1 m^3 enclosure made from transparent insect-proof netting (PE 2230, 920 x 920 μ; DIATEX, Saint Genis Laval, France). All treatments were randomly assigned within each block. The grasshopper diversity treatments had the following four levels: (1) a control treatment with no grasshoppers; (2) six single-species treatments; (3) six three-species treatments and (4) one six-species treatment. Six grasshopper species were selected: *Chorthippus biguttulus* L. (hereafter, Cb), *Chorthippus dorsatus* Zetterstedt (Cd), *Calliptamus italicus* L. (Ci), *Euchorthippus elegantulus* Zeuner (Ee), *Pezotettix giornae* Rossi (Pg) and *Pseudochorthippus parallelus* Zetterstedt (Pp) because they are numerically dominant in the study area, representing 90% of grasshopper total abundance, and present a great functional variation (e.g., different mandible strength) (Deraison et al., 2015*b*). Grasshopper density was fixed at 24 individuals per m² with a constant sex ratio of 1:1, following previous observations in the study area (Scherber et al., 2010; Deraison et al., 2015*b*).

3.2.3 Experimental procedure

A botanical survey was conducted in June 2012 to assess the initial state of the plant community in all 70 cages. This was done by visually estimating individuals and their cover in nine 10 × 10 cm quadrats that were evenly spaced within each cage. 1,560 young individual adults of each grasshopper species were collected from neighbouring grasslands, sexed, and then randomly assigned a treatment. Then, a vacuum cleaner was used to remove aboveground invertebrates from all cages and the experiment was initiated by placing the grasshoppers in their corresponding cages. During the experiment, checks of grasshopper density were performed and the presence of other aboveground invertebrates (e.g., spiders) was inspected, removing them by hand if present without disturbing the vegetation patch. Dead grasshoppers were replaced to keep species density constant from the beginning of the experiment until the end of August 2012, when grasshopper replacement ceased. In September 2012, all grasshoppers were removed by hand from each cage. A final botanical survey was conducted in May 2013 to measure plant cover at the end of the growing season. The experiment was repeated starting in June 2013 and finishing in May 2014 following the same procedures as described.

3.2.4 Estimation of interaction coefficients for the full network and structural stability

The experimental design described above in which plant species were either growing without grasshoppers, with just one grasshopper species or with multiple grasshopper species, allows us to estimate the five different elements describing the dynamics of interaction effects in our grassland community. These parameters are, according to a Lotka-Volterra population model (eqn. 1), the vector of plant intrinsic growth rates (r), representing how much plants could grow in the absence of species interactions; the plant-plant interaction matrix (α), representing both the intra- and interspecific pairwise effects within the plant community; herbivory (γ), i.e, the direct effect of grasshoppers on each plant species; and two types of higher-order interactions (β), such as the effect of a plant on plant-plant interactions (β_α) and the effect of a grasshopper on plant-plant interactions (β_γ). The model is

$$C_{i,t} = r_i \left(1 + \sum_{z=1}^{m} \gamma_{iz} N_{z,t} \right) + \sum_{j=1}^{n} \alpha_{ij} C_{j,t} + \underbrace{\sum_{k=1}^{n} \beta_{ijk} C_{j,t} C_{k,t}}_{\beta_\alpha} + \underbrace{\sum_{z=1}^{m} \beta_{ijz} C_{j,t} N_{z,t}}_{\beta_\gamma}, \qquad (3.1)$$

where $C_{i,t}$ is the cover of plant species i, $C_{j,t}$ and $C_{k,t}$ are the cover of interacting plant species i and k, $N_{z,t}$ is the number of individuals of grasshopper species z, r_i is the intrinsic growth rate of i, α_{ij} is the interspecific effect of j on i, β_{ijk} is the higher-order modification caused by plant k on the effect of j on i, and β_{ijz} is the higher-order modification caused by grasshopper z on the effect of j on i.

Given the high dimensionality of the approach, we used group lasso (least absolute shrinkage and selection operator) regularisation techniques (Tibshirani, 1996; Bakin, 1999; Yuan and Lin, 2006) through the function `glinternet.cv` within the package "glinternet" (Lim and Hastie, 2015), to reduce the number of variables selected by the statistical model. Briefly, a lasso regression works by adding a penalty term to the objective function of the model, producing sparsity and shrinking the coefficients of less important variables to zero. The amount of sparsity added can be controlled by a tuning parameter, λ. When $\lambda = 0$ no sparsity is added and all coefficients are selected, whereas when $\lambda = \infty$ all coefficients are set to zero. It is worth mentioning that group lasso is a hierarchical regularisation, therefore an interaction can only be selected if its component direct effects are also selected. Thus, group lasso regularisation can be used to improve the interpretability of a statistical model by automatically selecting the most important variables and quantifying the relevant direct and indirect interaction coefficients in any complex system, such as our multispecies plant-herbivore community. For further details, Appendix 3 provides a more thorough description of the regularisation technique used.

For each of the 36 plant species, we fitted a statistical model with the same notation as the Lotka-Volterra model including a lasso regularisation approach in which the response variable was plant cover of a given species, and the predictors were the neighbour plant cover (within each quadrat) and the abundance of each species of grasshopper present in the cage. We fitted the statistical model with the information from the botanical surveys conducted in June 2012, May 2013 and May 2014. To select the appropriate value for the tuning parameter λ, we performed cross-validation with `glinternet.cv`, fitting 50 different models with increasing values of the tuning parameter λ (from 0.05 to 0.5) and then selected the model with the λ that minimised the cross-validation error for each focal species (Fig. S3.2). This allowed us to quantify the coefficients of the intrinsic growth rate vector (r) and the interaction matrices α, β_α, γ, and β_γ, that describe the interaction network in our study system. Importantly, six species (*Elytrigia repens*, *Geranium rotondifolium*, *Picris hieracoides*, *Lolium perenne*, *Sonchus* sp., and *Verbena officinalis*) were removed from further analysis because the λ selected was high enough to shrink all variables to zero (e.g., Fig. S3.2d), leaving us with 30 plant species remaining. These six plant species correspond to the least common species in our communities, representing less than 5% of the total plant cover.

Once the strength and sign of those interactions selected by the regularisation approach were identified, then, they were used to estimate the structural niche and fitness differences (SND and SFD, respectively) that determine whether a community or a module of multiple species within a community is feasible or not (i.e., species can persist in the long-term; see Introduction and Fig. 0.1 for a thorough explanation of the approach). We computed, for all possible modules of three and four plant species, structural niche and fitness differences. Results of three species modules are presented in the main text and for modules of four species in Appendix 3 (Fig. S3.3-S3.5 and Table S3.4-S3.5). It is worth noting that we opted to use the structural approach to understand the effect of biotic interactions on species persistence compared to other approaches such as modern coexistence theory (Chesson, 2000), because it allows us to estimate niche differences for an arbitrary number of species with positive and negative interactions stemming from multiple trophic levels (Rohr et al., 2014; Saavedra et al., 2017; Song et al., 2018).

3.2.5 Assessing the importance of each interaction layer for the mechanisms of species persistence

To understand the contribution to the structural mechanisms of coexistence of each interaction layer in our empirical plant-grasshopper community, we created five different in silico scenarios (Fig. 3.1). Each scenario accounted for different layers of species interactions. The first scenario is the baseline for our study and accounts only for the pairwise interactions between plants, the α layer. The second

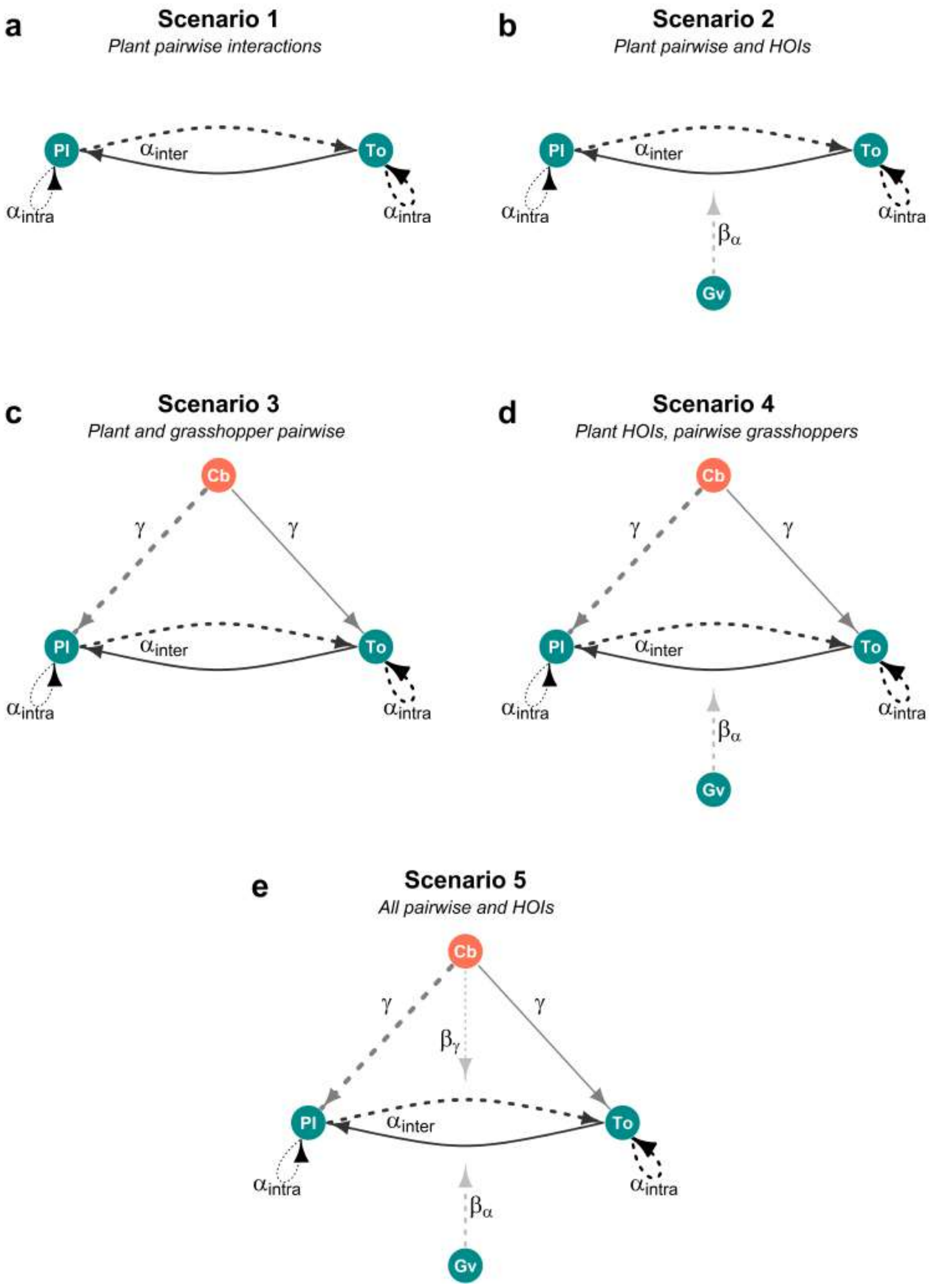


FIGURE 3.1: Five scenarios are proposed to analyse the contribution of each interaction layer to the mechanisms of plant coexistence in a plant-grasshopper interaction network. Scenario 1 (**a**) is the baseline of the community and accounts only for the plant-plant interaction network (α), with both interspecific (α_{inter}) and intraspecific (α_{intra}) interactions. In scenario 2 (**b**), the effect of the higher order interactions (HOIs) between plants (β_{α}) is considered. Scenario 3 (**c**) accounts for both plant and grasshopper (γ) pairwise interactions without HOIs. Scenario 4 (**d**) incorporates plant HOIs to the direct plant and grasshopper effects. Finally, scenario 5 (**e**) represents the full community, with direct plant and grasshopper interactions together with both plant and grasshopper (β_{γ}) HOIs. Cyan circles represent plant species (Pl, *Plantago lanceolata*; Gv, *Galium verum*; To, *Taraxacum officinale*) and coral circles stand for grasshopper species (Cb, *Chorthippus biguttulus*). Dotted arrow lines indicate negative interaction effects and solid arrow lines indicate positive interaction effects.

scenario accounts only for plant interactions but included both pairwise and those selected higher-order interactions between plant species, the β_α layer. For the third scenario, we focus only on pairwise interactions but combine plant-plant interactions together with grasshopper-plant interactions, the γ layer. In the fourth scenario, we test a combination of multitrophic pairwise effects (plant-plant and plant-grasshopper interactions) together with higher-order interactions among plants. Finally, in the fifth scenario, we investigate the full empirical community by incorporating into the fourth scenario the higher-order interactions caused by grasshoppers, the β_γ layer. Note that these scenarios range from simplicity to complexity hierarchically (i.e., β_γ cannot take place without γ interactions, but vice versa).

3.2.6 Metrics for understanding the configuration of species interactions within modules

In the last step of the analyses, we explored how the configuration of species interactions drives SND and SFD, the structural mechanisms of plant coexistence. To do so, we computed four different metrics for each three-species interaction module. To describe the shape of the distribution of interactions within a module, we first calculated its skewness and kurtosis. On the one hand, skewness is a measure of the symmetry of a distribution. It describes the extent to which a distribution is asymmetrical or skewed to one side or the other. Positive skewness indicates that the distribution has a higher concentration of values on the left side, with fewer values on the right side, whereas negative skewness indicates the opposite. On the other hand, kurtosis is a measure of the peakedness or flatness of a distribution. It describes the extent to which a distribution is more (positive or excess kurtosis) or less (negative or platykurtic kurtosis) peaked than a normal distribution, meaning that values in the distribution are respectively more or less concentrated near the mean. Together, they can be used to interpret the prevalence of weak and strong effects in distributions of interaction coefficients.

Provided that theory predicts that coexistence requires interaction modules in which intraspecific effects are greater than interspecific effects, we measured in addition as the third metric the strength of intra- versus interspecific interactions using an index called diagonal dominance (dom), which can be formulated as follows:

$$\mathrm{dom} = \log_{10}\left(\frac{\frac{\sum_{i=1}^{n}|A_{i,i}|}{n}}{\frac{\sum_{i=1}^{n}\sum_{j=1}^{n}|A_{i,j}|-\sum_{i=1}^{n}|A_{i,i}|}{n(n-1)}}\right), \tag{3.2}$$

where A is any given square matrix of coefficients describing a species interaction module and n is the number of columns in such a matrix. This index takes the mean of all diagonal coefficients, in absolute value, and divides it by the mean of non-diagonal coefficients, in absolute value. Therefore, positive values of diagonal dominance indicate stronger intraspecific than interspecific interactions and vice versa, with most of the values comprehended between -3 and 3 in our system.

Finally, the fourth and last metric computed was the positive-negative ratio (PNR), which describes the relative strength of positive versus negative effects within an interaction matrix and can be formulated as follows:

$$\mathrm{PNR} = \log_{10}\left(\frac{\sum_{i=1}^{n}[A_i|A_i > 0]}{\sum_{j=1}^{m}\left|\left[A_j|A_j < 0\right]\right|}\right). \tag{3.3}$$

Here, negative values of PNR indicate greater negative effects and positive values indicate greater positive effects in the matrix. In our system, most values of PNR range from -3 to 3. Thus, this metric can be used to assess the contribution of mutualisms and antagonisms across multispecies interaction modules in our empirical network.

These four metrics were computed for all modules of three and four species under the full species interaction network (scenario 5). We then assessed with generalised linear models (GLMs) their individual and joint contributions to promoting SND and SFD. To determine the distribution of the residuals we used the function `descdist` within the package "fitdistrplus" (Delignette-Muller and Dutang, 2015), leading us to fit a GLM with gamma errors (link = "log") for both SND and SFD. A summary of the results of these analyses is available in Table S3.2-S3.3, and specific results for modules of four species are available in Table S3.4-S3.5. All computational analyses were performed in R version 4.2.1 (R Core Team, 2022).

3.3 Results

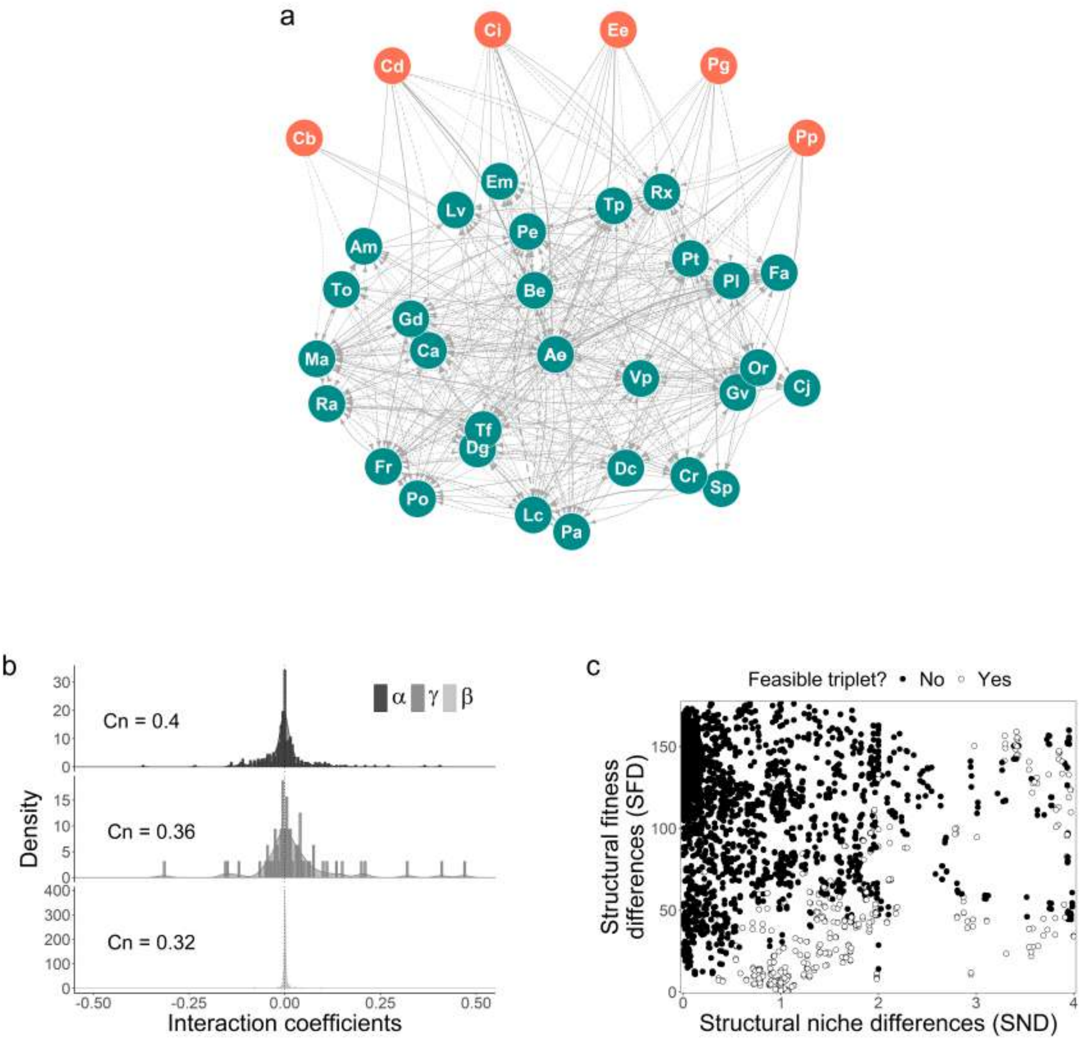


FIGURE 3.2: Components of our empirical system and their associated coexistence outcomes. (**a**) Representation of the inferred empirical interaction network for the full community. Cyan circles represent the set of 35 plant species and coral circles stand for the six grasshopper species. Notice that interactions between species can be either positive (solid links) or negative (dashed links) and that the width of such links is directly proportional to the strength of the effect. (**b**) Diagram showing all the different interaction types occurring within our empirical network: a plant-plant interaction α layer composed of intra-specific (α_{intra}) and inter-specific (α_{inter}) interactions, a γ layer accounting for the effects of grasshoppers on plants, and a β layer corresponding to the higher-order effects on plant-plant interactions. (**c**) Histograms show the density of positive and negative interactions for each of the aforementioned interaction layers. As zero values have been removed from the visualisation, we show a connectance value (Cn) for each layer that indicates the proportion of values different from zero. (**d**) Mechanisms of coexistence (structural niche and fitness differences) and predicted coexistence outcomes (feasibility) for the full community, i.e., including all interaction layers, calculated for all plant triplets (i.e., combinations of three plant species).

The regularisation approach allowed us to identify the structure of biotic interactions that characterise the complex network of relationships between the plants and grasshoppers in our experimental system (Fig. 3.2a). This characterisation connects the selected biotic interactions with the growth and population dynamics of plant species. Across types of interactions, including plant-plant pairwise interactions or α, pairwise grasshoppers effects on plants or γ, and the aggregated higher-order interactions (HOIs) of plants and grasshoppers or β; we observe similar levels of connectance (i.e., percentage of interactions with a net effect different from zero). For instance, the connectance value of the α layer is Cn = 0.4, which means that 60% of interactions had a net effect of zero) (Fig. 3.2b). However, it is worth noting that the connectance showed for β is achieved by the joint effect of 36 different interactions matrices (30 matrices of plant HOIs and 6 matrices of grasshopper HOIs), which individually show very low connectance (Cn = 0.043 ± 0.014).

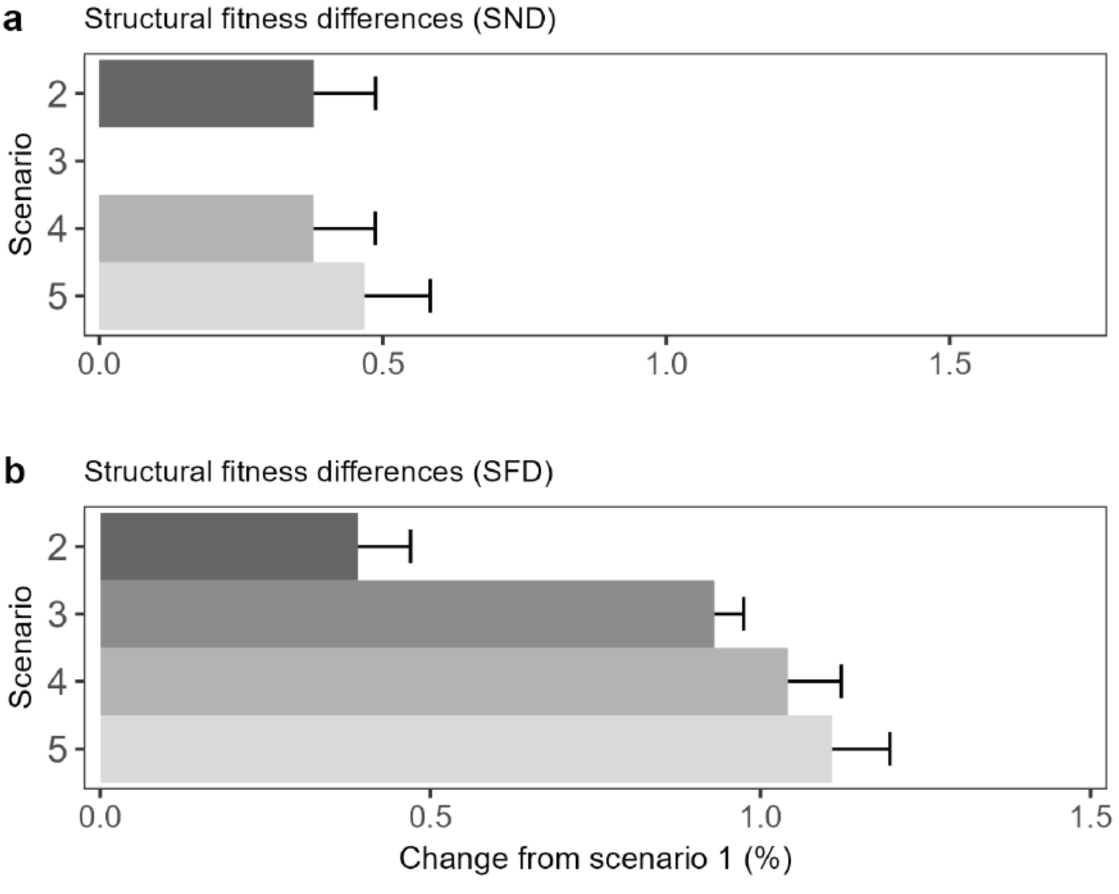


FIGURE 3.3: Contribution to the coexistence mechanisms ((**a**) structural niche differences and (**b**) structural fitness differences) of the effects of grasshoppers on plants (γ) and the additive higher-order effects of plants on plant-plant interactions (β). This contribution is measured as the change (%) in the coexistence mechanisms compared to a community with only plant-plant interactions (α). Whiskers represent the standard error for each bar.

Regardless of the interaction type, all of them displayed both positive and negative signs but their distribution of strengths varied. While we observed a wider spread for plant-plant (α) (range from -0.57 to 0.59) and grasshopper-plant interactions (γ) (range from -0.32 to 0.47), the distribution of HOIs (β) was concentrated around zero, meaning that the majority of effects were very weak. With this parameterisation, we explored how the different types of interactions drive the structural niche and fitness differences (SND and SFD) and determine the feasibility of three plant species modules (i.e., triplets). Following theoretical expectations, we observed that the majority of triplets predicted to be feasible followed the condition that SND overcomes SFD (Fig. 3.2c). In addition some triplets were predicted to be feasible despite showing low SND and high SFD. This was likely due to the positive effect of indirect interactions such as intransitivity (rock-paper-scissors dynamics) on promoting feasible triplets. Finally, it is worth

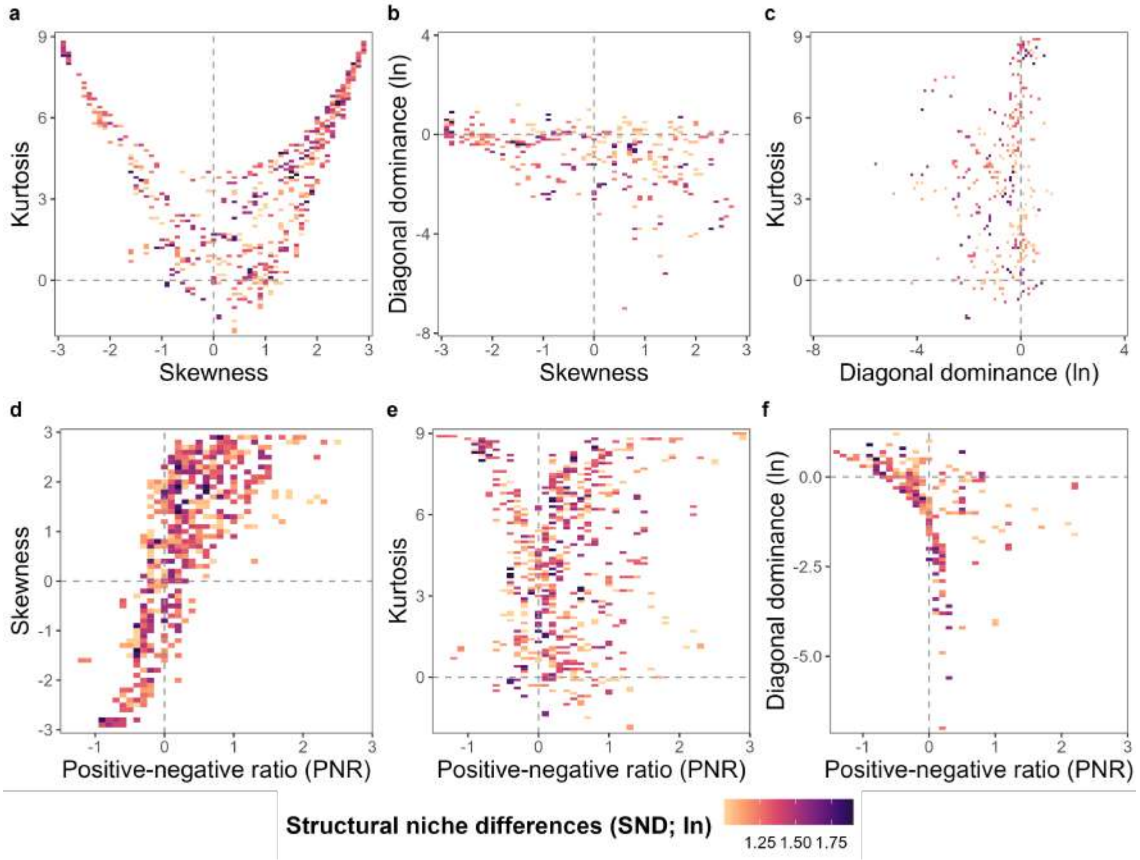


FIGURE 3.4: Structural niche differences (**a—f**) as a function of four metrics (skewness, kurtosis, diagonal dominance, and PNR) measuring the configuration of interaction strengths within each three-species module in the community. Skewness describes how much a distribution is shifted to the left (stronger negative interactions) or right (stronger positive interactions) of its centre. Kurtosis describes the peak and tails of a distribution: higher values indicate a higher peak and thinner tails (more weak interactions) and lower values indicate a lower peak and thicker tails (more strong interactions). Diagonal dominance is an index describing the relative difference in the strength of the diagonal of the interaction matrix (intraspecific interactions) compared to its non-diagonal elements (interspecific interactions), with logarithmic values below zero indicating higher intra- than interspecific effects and vice versa. Positive-negative ratio (PNR) is an index describing the preeminence of positive (PNR > 0) or negative (PNR < 0) interactions within each module. See methods for a description of each metric.

noting that we observed many triplets not predicted to be feasible and these correspond to a range of situations in which low SND do not overcome low SFD as well as high SND do not overcome SFD (Fig. 3.2c).

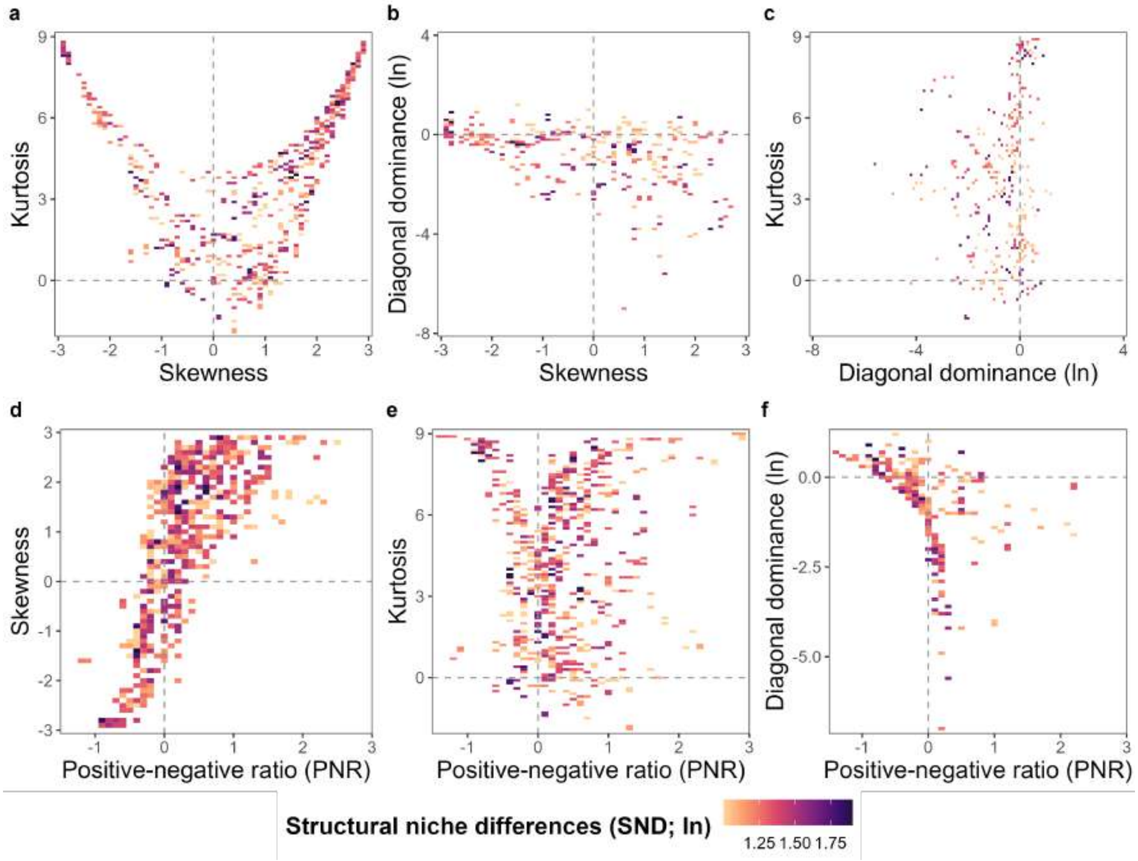


Figure 3.5: Structural fitness differences (**a—f**) as a function of four metrics (skewness, kurtosis, diagonal dominance, and PNR) measuring the configuration of interaction strengths within each three-species module in the community. Skewness describes how much a distribution is shifted to the left (stronger negative interactions) or right (stronger positive interactions) of its centre. Kurtosis describes the peak and tails of a distribution: higher values indicate a higher peak and thinner tails (more weak interactions) and lower values indicate a lower peak and thicker tails (more strong interactions). Diagonal dominance is an index describing the relative difference in the strength of the diagonal of the interaction matrix (intraspecific interactions) compared to its non-diagonal elements (interspecific interactions), with logarithmic values below zero indicating higher intra- than interspecific effects and vice versa. Positive-negative ratio (PNR) is an index describing the preeminence of positive (PNR > 0) or negative (PNR < 0) interactions within each module. See methods for a description of each metric.

We initially hypothesised that not all interaction types would contribute equally to the maintenance of species coexistence. Accordingly, we observed that adding the effect of different types of interactions to the baseline of plant-plant interactions (scenario 1; Fig. 3.1) did not further modify the structural niche and fitness differences (SND and SFD) that determine the triplets' feasibility (Fig. 3.3). Analyses for modules of four species (i.e., quadruplets) rendered similar results (Fig. S3.3). For instance, incorporating the full complexity of interactions, which include the direct effects of grasshoppers on plant species plus the plant-plant and plant-herbivore HOIs (scenario five), produced a slight and positive increase (0.5—1%) of SND and SFD. These results identify the structure of plant-plant interactions (α matrix) as the key element that modulates the structural mechanisms of multispecies plant coexistence and diminishes the importance of multitrophic interactions.

Given the previous result, we selected the matrix of plant interactions to analyse what particular types of network configurations explain the variation observed in SND and SFD. Two particular metrics, diagonal dominance and the positive-negative ratio (PNR) usefully described SND variation (Fig. 3.4, Table S3.2). In particular, an increase in SND was observed in modules with higher PNR (stronger positive than negative values of interactions) and higher diagonal dominance (stronger strength of intraspecific interactions compared to interspecific ones). Additionally, we observed similar results in modules composed of four species, but for those, we also found significant interactive effects of both PNR and diagonal dominance on describing the observed variation in SND (Fig. S3.4 and Table S3.4). No other relevant relationships were found between SND and any other investigated metric in triplets and quadruplets.

On the other hand, several network metrics and their interactions explained the observed variation of SFD across triplets and quadruplets. Regarding the PNR, we observed that stronger positive than negative interactions notably decrease SFD. Also, higher kurtosis is associated with lower SFD, which indicates that weaker interactions decreased SFD. Nevertheless, the dominance of the diagonal (ratio of intra- versus interspecific effects) was not observed to have an influence on SFD (Fig. 3.5). For the case of interactions between network metrics, we observed that all two-metric combinations (except the interaction between diagonal dominance and skewness) were relevant to describe SFD variation. Finally, triple interactions (diagonal dominance—skewness—PNR and diagonal dominance—kurtosis—PNR) slightly decreased and increased SFD, respectively (Table S3.3). Very similar results were observed for modules of four species, except for the case that diagonal dominance did positively contribute to explaining SFD variation in quadruplets (Fig. S3.5, Table S3.5). Overall, these results suggest that the SFD that drive competitive dominance can be promoted by multiple network configurations of species interactions whereas fewer particular network characteristics can explain the SND that drive the opportunities for species to coexist.

3.4 Discussion

Ecologists have long sought to understand the role of species interaction in promoting the maintenance of biodiversity. Traditionally, this aim has been focused on systems of low diversity where estimating the strength and sign of biotic interaction that describe population dynamics was challenging but not impossible. However, the goal of recent work is to extend these efforts to highly diverse and multitrophic systems in which multiple interactions are simultaneously occurring (Levine et al., 2017; Godoy et al., 2018). It is clear that these new opportunities bring new challenges and it is meaningless to consider relevant all biotic interactions occurring between species of the same and different trophic levels, and between pairs or multiple species. Consequently, we first explored which subset of species interactions describes the spatiotemporal distribution of plant cover in our study by means of a regularisation approach. Similar methodologies have been followed in other studies working with annual plant species (Weiss-Lehman et al., 2022) and with bacteria (Coyte et al., 2021) to address this challenge. We found statistical support for all types of interactions including pairwise plant-plant and plant-grasshoppers interactions as well as HOIs, but it is also important to highlight that we found a strong pruning in the proportion of interactions retained. For instance, more than half of all potential interactions within plant species were considered to be negligible, and the proportion went up to 2.00±0.89% for complex HOIs describing how the interaction between a pair of plant species is modified by the presence of a third (plant or grasshopper) species.

With such information at hand, we could in principle conclude that all interaction types are relevant and what matters is maintaining a combination of simpler and more complex interactions among a subset of species. Yet, the effort we did in our study to connect this subset of interactions with the determinants of multispecies coexistence gives further insights. Specifically, we found that not all interaction types selected by the regularisation approach contributed similarly to the mechanisms determining the feasibility of the community. In particular, the plant-plant network of interactions had a predominant role in promoting the structural niche and fitness differences that drive multispecies coexistence compared to the plant-herbivore interactions and to the HOIs. The underlying explanation for this finding is that the variation in the strength and sign of interaction coefficients among plant species was larger compared to the rest of the interaction types. For instance, while for plant-plant interactions the strength varied from -0.40 to 0.45, the rest of the interactions were weaker and peaked more towards zero. Our results, therefore, agree with previous findings (Rossberg et al., 2011) and discussions (Barabás, 2021) that the variation in interaction strength plays a key role in driving the feasibility of ecological systems and suggest that the wider the variation in interaction strength in a particular trophic level, the greater its importance for modulating the diversity of the system.

Together with this main result, we add the novelty that different structures of interactions also promote different coexistence mechanisms. On the one hand, we observed for modules of three and four species that the opportunities for species to coexist (i.e., structural niche differences) increased when intraspecific exceeds interspecific plant competition, and when positive interactions were more prevalent than negative ones. That self-limitation processes coming from using particular soil resources or specific plant enemies are an important driver of diversity had been well acknowledged before (Chesson, 2000; Chesson and Kuang, 2008; Barabás et al., 2016), but less clear is the fact that facilitation can be as important as strong intraspecific competition for multispecies coexistence (see Losapio et al. (2021) for a suggestion without including interaction strength). On the other hand, our results show that the variation in interaction strength that determines plant competition dominance (i.e., structural fitness differences) can be structured in many different ways. For instance, modules with stronger plant interactions, represented by a small kurtosis, are likely to find a stronger competitor that reduces the ability of the system to coexist. In addition, our results indicate that this small kurtosis interacts with the positive-negative ratio (PNR) suggesting that higher dominance occurs when interactions are biased towards competition but not facilitation. Taking this into an applied context, our results highlight that we can be certain in the specific ways by which we can promote or maintain diversity, that is strong self-limitation and equally important facilitation, yet competitive dominance is harder to control or predict because there are many different ways by which some plant species can be more competitive than others.

Regardless of the interaction types (pairwise versus HOIs) and trophic position (plant versus grasshoppers), our regularisation approximation indicates that species are simultaneously involved in negative and positive interactions. This is an important finding for discussing how we should approximate the study of the complexity of interactions in multitrophic systems. As we introduced, the traditional approach has been to assign a priori the sign of interactions (e.g., competition and herbivory with negative effects while pollination with positive effects) (Chesson and Kuang, 2008; Bastolla et al., 2009; Rohr et al., 2014). However, these decisions have been made due to theoretical reasons and knowledge limitations, but have no empirical justifications. If we want to accurately describe a network of multispecies interactions in multitrophic systems that connect with the spatiotemporal dynamics, we advocate that we should expect positive and negative interactions across ecological communities. In other words, there are no specific types of interactions exclusively negative or positive. The most evident case in our study system comes from the layer of interactions describing the grasshopper effect of plant cover. Herbivory is generally considered to have a negative effect on plant growth, yet we found that while some grasshoppers strongly reduced plant cover in some plant species (e.g., *Chorthippus dorsatus* had a strong negative effect on *Bromus erectus*) others did the contrary (e.g., *Pezotettix giornae* had a strong positive effect on *Galium verum*). We could not test the ultimate mechanisms by which these opposing effects are occurring but according to previous studies in the same system, the sign of these effects could be related to the feeding traits of grasshoppers. It has been shown that our six grasshopper species differ in their feeding niche as a function of their incisive strength and two simple plant traits (C/N ratio and leaf dry matter content) (Deraison et al., 2015*a*). Another possibility is that the negative or positive herbivory effects could be related to an interactive effect between grasshopper traits and plant abundance. *Bromus erectus* is a dominant plant species in the system and therefore it is an abundant resource for many grasshopper species that have enough incisive strength to eat these leaves with a high C/N ratio. These linkages suggest that this is a suitable system to study the role of plant and grasshopper traits in mediating the role of species interactions in the maintenance of plant diversity.

The most important findings of our study rely on the regularisation approach we used to infer the structure of interactions. This approach was fitted with information of three botanical surveys and with grasshopper abundance that was held constant across the experiment. Despite the fact that this experimental effort represents a unique example in the literature, we do not certainly know whether the vast amount of data collected is enough to represent the dynamics of the system. For instance, the estimation of interaction strength and sign obtained could be different if the experiment was conducted during a longer period with more frequency of drier and wetter events. That is, we did not consider the role of environmental variation but this is something needed for future work as many studies have highlighted, for example, the importance of precipitation variability in maintaining plant diversity (Hallett et al., 2019). Another limitation of our approach is that we could study the feasibility of the plant species but not the combination of plant and grasshopper species altogether. This was due to the fact that the number of grasshoppers was held constant across the entire duration of the experiment, and therefore we could not know how plant species affect changes in grasshopper abundance across time. This information is harder to obtain from experiments conducted under natural conditions but it could be more easily done in experimental approximations like ours in which insects are kept in large cages (see Bartomeus et al. (2021) for an example with pollinators). This discrepancy between what was done and what could have been done to obtain the maximum profit from the extensive experimental effort also calls for a better dialogue between empiricists and theoreticians.

Although our study presents a complex approximation to study the mechanisms based on species interactions that determine the dynamics of ecological communities, we reach a straightforward conclusion. Pairwise plant-plant interactions and the prevalence of strong plant self-limitation and facilitation are the key components of the feasibility of our system. Despite studying a plant-herbivore community, herbivores play a minor role in plant dynamics, and the plants can be seen as the substrate over which they can display their niches according to their feeding strategies. We do not know numerically whether these asymmetries in the importance of interactions across trophic levels are common in nature, as this topic has been rarely explored in the literature. Therefore, that is a clear knowledge gap that needs further exploration. Another important conclusion is that, in order to realistically address the challenge of understanding how diversity is maintained, we need to explore multispecies, multitrophic systems, and for this exploration is fundamental a combination of empirical data and statistical approaches that allow distinguishing important interactions to reproduce the spatiotemporal patterns observed. For this study, we have used state-of-the-art statistical techniques via hierarchical group lasso regularisation (Lim and Hastie, 2015) but this is an area of rapid development, which will likely benefit studies addressing the complexity of ecological communities. Taken together, our results show that simple processes of ecological interactions (self-limitation and facilitation) structure the dynamics of complex ecological communities, in our case, a plant-herbivore multispecies community.

Associated publication

- Granjel RR, Gross N, Godoy O. Strong plant self-limitation and facilitation matter more than herbivory and complex high-order interactions to describe coexistence in a highly diverse plant-herbivore network. *In preparation.*

Open science

Data and code available through www.github.com/Granjel/FG.

Author contributions

N.G. designed and conducted the original experiment. All authors contributed to the original idea of the manuscript. R.R.G. and O.G. designed the computational analysis. R.R.G. analysed the data, built the figures and wrote the manuscript draft with input from O.G. and revisions from all authors.

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Supplementary material

Supplementary figures and tables related to this chapter are available in Appendix 3.

Precipitation, not species functional traits, explains the interannual variability in plant niche

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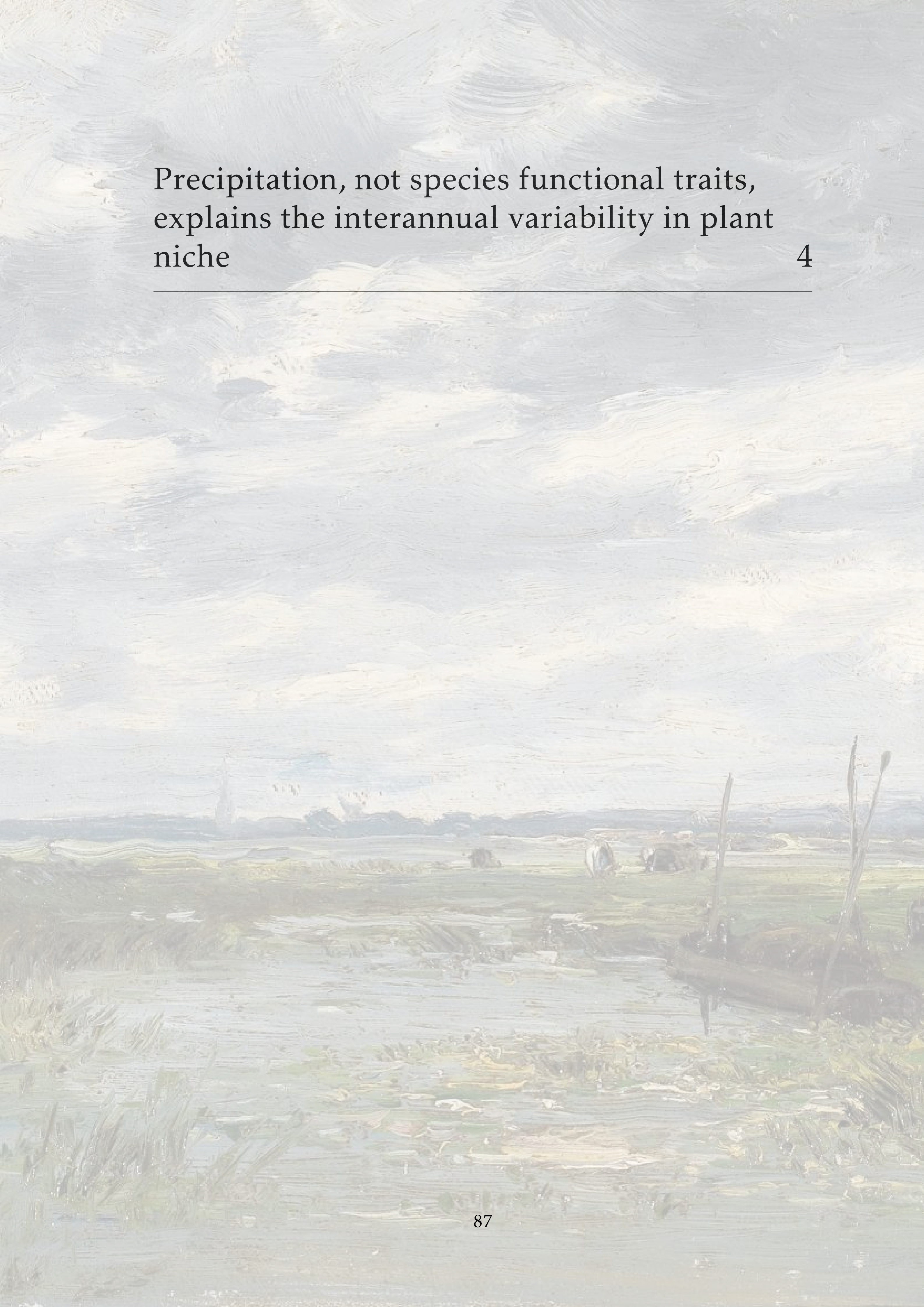

Abstract

The niche is a central tenet in ecology that conceptualises the multiple dimensions determining the ability of species to thrive in complex ecological communities under environmental heterogeneity. Attempts to define the ecological niche of species have not been successful in explaining their population dynamics, limiting our understanding of the importance of the niche for species persistence. Even when this connection has been made, it has only been possible to characterise niche differences between pairs of species and not at the species level. Recent theoretical developments, however, provide us with a series of tools to solve previous limitations, bringing the unprecedented opportunity to rigorously measure a species' niche and its population consequences. But more importantly, this possibility also allows us to ask whether the niche is a property of the species as it has been assumed during the last century. Here, we conducted a detailed field survey on 18 annual plant species in a Mediterranean grassland for five consecutive years. With this survey, we empirically parameterised population models that allow estimating the niche and fitness of species, which jointly determine the outcome of biotic interactions. Contrary to expectations, we found that climatic conditions, particularly precipitation, explained more variation in niche and fitness than species identity or their functional characteristics associated with leaf, root and whole-plant traits that are key for competition for shared resources and tolerance to natural enemies. As a result, the niche that species explored each of the five years was different because precipitation was also different. Taken together, our results challenge our understanding of the niche as a mere species property and instead suggest its environmental dependency.

Keywords

Niche, fitness, coexistence mechanisms, interannual variability, precipitation, functional traits, annual plants

4.1 Introduction

The concept of the niche is a central pillar in ecology. Although the definition of the term has been strongly debated over the last century, ecologists agree on the idea it is a species property that defines the range of environmental conditions a species could endure (Grinnellian niche) (Grinnell, 1917), their role within food webs (Eltonian niche) (Elton, 1927), and more generally, the multiple abiotic and biotic dimensions that allow species to develop, reproduce and persist (Hutchinsonian niche) (Hutchinson, 1957). During most of the 20th century, the definition of the niche was used to explain how biodiversity can be maintained within ecological communities. It was formulated that the biodiversity we observe is the result of evolutionary processes that have promoted enough niche differences between species to avoid competitive exclusion (Gause, 1934; MacArthur and Levins, 1967). The main prediction was that a community can accommodate as many species as limiting resources unique to each species exist. That is, if two species compete for similar resources but one is more limited by carbon (C) and the other species more limited by nitrogen (N), both could coexist without excluding each other. However, the first attempts to test these predictions showed that niche differentiation for limiting resources was not able to accurately explain the co-occurrence of multiple competing species. Some of these contradictory results between theoretical expectation and empirical findings, formulated as the paradox of the plankton, demonstrated that it is possible to observe high diversity of planktonic organisms despite being assembled in environments with few limiting nutrients, and indeed, fewer resources than the number of competing species (Hutchinson, 1961).

These discrepancies stimulated a plethora of further studies highlighting that, as important as the number of limiting resources is, there are also other critical aspects of niche differentiation between species that promote coexistence such as natural enemies, rates of resource uptake and their spatial and temporal variation (Holt, 1977; Chesson and Warner, 1981; Chesson, 1985; Tilman, 1990, 1993). The advances in mechanistically understanding the multiple abiotic and biotic sources of niche differences reinforced the idea that the niche has a multidimensional nature (Chase and Leibold, 2003). Yet, these advancements were still not useful to predict if species could coexist, or even if they coexist, whether species are rare or abundant or whether their abundances could change with time. The link between the species' demography with their niche differences was done by P. Chesson during the decades of the 80s, and 90s. His contributions, summarised in Chesson (2000), posit that because knowing all sources of niche differences between species is almost impossible, we can instead focus on the demographic signature of niche differences. This demographic signature allows for maintaining the diversity of species by limiting their abundance when they become common but also by buffering them from extinction when they become rare.

Nevertheless, this framework, currently named modern coexistence theory (MCT), brought another lesson: niche differences alone do not determine whether species can coexist, they only stabilise the dynamics of interacting species. There is another source of species differences. These other differences, named fitness differences, arise from the combination of the intrinsic ability of species to produce offspring and their tolerance to competition, promote competitive dominance, and in the absence of niche differences indicate which species is the superior competitor (Godoy and Levine, 2014). With both species differences acting simultaneously, MCT predicts that species can coexist when niche differences "overcome" fitness differences (Chesson, 2000; Barabás et al., 2018).

Applications of MCT during the last two decades have triggered an unprecedented advancement of our mechanistic understanding of the role of competition (Allesina and Levine, 2011; Hart et al., 2016, 2018; Adler et al., 2018), shared natural enemies (Chesson and Kuang, 2008; Mora et al., 2011), mutualisms (Lanuza et al., 2018; Johnson et al., 2022), and environmental variability (Matías et al., 2018; Hallett et al., 2019) on maintaining biodiversity through promoting niche differences. Yet, mathematical and methodological tools associated with MCT cannot measure the niche of a species but only differences between pairs of species. Fortunately, recent progress by Spaak and De Laender (2020) has provided an intuitive and broadly applicable definition of the niche and the fitness that can be evaluated at the species level.

These new advances allow us now to answer questions such as which is the position of one species with respect to the rest of the community (Spaak and De Laender, 2020), but rather more importantly, it provides the tools for asking, for the first time, whether the niche is an intrinsic property of the species. If that is the case, then niches could be differentiated by analysing differences among species and differences also in their functional characteristics that determine the constraints of the niche. Alternatively, it could be also possible that the niche is an ecological property more variable than previously thought, and the variation in niches we find within ecological communities can be better explained by environmental variation or by species abundances rather than by their identity. This is for instance, the case of the species' fitness, that has been shown to operate as a mutable property determined by environmental drivers (Matías et al., 2018; Wainwright et al., 2019; Van Dyke et al., 2022) and species abundances (Hart et al., 2016), which result in competitive rankings among species to be susceptible to change across space and time (Stouffer et al., 2021).

Here, we challenge the central idea in ecology that the niche is a species' property. To do so, we applied the most recent, species-level definitions of Spaak and De Laender (2020) to quantify the niche and the fitness of 18 annual plant species in Mediterranean grasslands for five consecutive years. This temporal variation allows us to test whether the niche position, and therefore niche differences between species were consistently maintained among species across time, or conversely, the niche position of species we observe does not carry taxonomic signals. Following current theory, we would expect greater variability in species fitness than species niche, with the fitness varying greatly between years depending on environmental conditions and the niche behaving in a more conservative way, showing less variation and being mainly explained by the traits of species that allow them to thrive under a limited range of environmental conditions.

4.2 Materials and methods

4.2.1 Study system and fieldwork

Our experimental site (Caracoles Ranch) is located within Doñana National Park in southwest Spain (37° 04′ N, 6° 18′ W). The area has a Mediterranean climate with mild winters and an average annual precipitation of 550-570 mm over the past 50 years. The physiognomy of the vegetation is primarily grasslands of annual grassland species, with no perennial species present. Across the experimental site, there is a slight slope of 0.16%, which causes vernal pools to form at the lower border of the ranch during the winter and spring months (November-January and March-May) while the upper parts of the ranch do not flood, except during exceptionally wet seasons. During the course of our study, an extreme flooding event occurred during the growing season of 2018. There is also a strong soil salinity-humidity gradient that is structured along this topographic gradient.

In September 2014, we established nine plots of 8.5m x 8.5m along an area measuring 1km × 200m. The plots were divided into three groups, with three plots located at the upper part of the topographic gradient, three in the middle, and the remaining three at the lower part. The average distance between these three groups of plots was 300m and the average distance between plots within each group was 30m (with a minimum distance of 20m). Each plot was further divided into 36 smaller plots of 1m × 1m, with 0.5m aisles in between for access during measurements (for a total of 324 subplots). This layout was implemented to facilitate the parameterisation of population models with which we could estimate the species niche and fitness following Spaak and De Laender (2020) definitions. These models include two types of parameters. On one hand, the intrinsic fecundity component that describes the average intrinsic ability of species to produce seeds, which is in turn discounted by the seed amount that is lost due to germination and due to seed mortality in the soil (Godoy and Levine, 2014). On the other hand, the effect of intra- and interspecific pairwise interactions that describe how this intrinsic ability of seed production is reduced by an increase of conspecific and heterospecific densities. In order to parameterise these models, the main focus of the field observations involved measuring, for each focal individual, the production of per germinant viable seeds as a function of the number and identity of neighbours within a radius of 7.5 cm, including individuals of the same species. This radius is a standard distance used in previous studies to measure competitive interactions among annual plant species (Levine and HilleRisLambers, 2009; Mayfield and Stouffer, 2017) and has been validated to capture the outcome of competition interactions at larger scales ($1m^2$) under locally homogeneous environmental conditions (Godoy and Levine, 2014). From November 2014 to September 2019, we sampled 18 species present in the study area each year (Table S4.1). There were two types of species, those considered core to be a species found every year (8 species) and non-core to be a species that was missing at our plots but not at our experimental site for at least one year (10 species) (Table S4.1). We sampled one individual per subplot for widespread species and several individuals per subplot when species were rare (max. 324 individuals/species). This sampling design ensured that all species were balanced in terms of the number of observations and that we captured the full range of observed spatial interactions among species across the study area. Furthermore, we obtained independent estimates of seed survival in the soil and seed germination rates in 2016 (see Lanuza et al. (2018) for details on obtaining these rates).

4.2.2 Estimating species interactions

We obtained pairwise per-capita interaction coefficients, both intra- and interspecific, from the empirical data in each year of sampling. In particular, we modelled the observed viable seed production per individual (yield of species i or Y_i) as a function of the identity and abundance of neighbouring individuals, following a previous study in the same study area (García-Callejas et al., 2021). The per-capita effect between each pair of species was estimated by assuming an underlying Ricker model of population dynamics (Ricker, 1954; Mayfield and Stouffer, 2017) of the form:

$$Y_i = \lambda_i e^{-\sum_{j=1}^{n}(\alpha_{ij} N_j)} \tag{4.1}$$

where λ_i is the number of seeds produced by species i in the absence of interactions, α_{ij} is the per-capita effect of species j over species i (which can be positive or negative, thus allowing both competitive and facilitative effects), and N_j is the number of individuals of species j within 7.5 cm of the focal individual. We implemented this model in a Bayesian framework, via a multilevel model with a negative binomial distribution and allowing the intercept and slope of the relationships to vary across years but not across space by including the year as a random effect. Thus, the α_{ij} values in eqn. 4.1 vary across years but are homogeneous across the whole spatial scale for the whole study area. We used the means from the obtained posterior distributions of α_{ij} values as the coefficients of our interaction matrices. For fitting the model, we used non-informative priors with MCMC settings of 5,000 iterations (of which 2,500 were warm-up) and 6 chains. We implemented the model using the "brms" R package (Bürkner, 2017).

4.2.3 Calculation of niche and fitness

With the empirical estimations of species vital rates and interaction coefficients previously described, we followed recent definitions by Spaak and De Laender (2020) to quantify the niche and fitness at the species level following previous intuitions (Adler et al., 2007). These estimations are defined within the framework of modern coexistence theory (MCT) and are also framed within invasion analyses (Grainger et al., 2019), but they differ from the traditional approach, among other things, because they are no longer calculated as a difference between pairs of species (Chesson, 2000). The advantage here is that by defining the niche at the species level, we can explore whether such estimates vary more between species than between years and whether they are predicted by species traits (indicating that it is a species property) or vary more across years than across species (indicating that it is not a species property).

The definitions of Spaak and De Laender (2020) assume that each species has a stable monoculture equilibrium and that the invasion growth rate correctly predicts coexistence. That is, for coexistence to occur, all species need to have positive invasion growth rates, defined as the growth rate of a species when it is reduced to density near to zero and the other species are at their monoculture equilibrium. The calculation of niche differences for a multispecies community is made as follows:

$$\text{Niche}_i = \frac{\overbrace{f_i(0, \mathbf{N}^{-i,*})}^{\text{Invasion growth rate}} \quad - \quad \overbrace{f_i\left(\sum_{j \neq i} c_{ij} N_j^{-i,*}, \mathbf{0}\right)}^{\text{No-niche growth rate}}}{\underbrace{f_i(0, \mathbf{0})}_{\text{Intrinsic growth rate}} \quad - \quad f_i\left(\sum_{j \neq i} c_{ij} N_j^{-i,*}, \mathbf{0}\right)}, \tag{4.2}$$

where f_i is any function that describes the per capita growth rate of species i; the term $\mathbf{N}^{-i,*}$ is the vector of equilibrium densities in the community when species i is absent; c_{ij} denotes a conversion factor that can be calculated empirically to account for the difference in resource consumption of individuals from the different species (e.g., the difference in resource use between a tree and a herb), translating the density of a species j into the density of species i; and $\mathbf{0}$ denotes the absence of all species other than i.

The framework introduces a term called no-niche growth rate of species i, which corresponds to the growth rate of species i if there was no niche differentiation, i.e., the growth rate of i at the converted monoculture density of its competitor species j (Spaak and De Laender, 2020). The niche is calculated as a comparison between the growth rate of species i when only interspecific interactions are present (invasion growth rate) and the growth rate when only intraspecific interactions are present (no-niche growth rate), divided by the comparison of the growth rate when the density of species i is close to zero (intrinsic growth rate) and the density of i is at the converted equilibrium density of species j. In other words, the niche measures the strength of the frequency dependence divided by the strength of the density dependence of species i (Adler et al., 2007).

With this in mind, the fitness of species i can be calculated for the scenario in which all species occupy the same niche (Adler et al., 2007; Barabás et al., 2018), thus measuring how well species i grows in the absence of frequency dependence, i.e., when there are only intraspecific interactions (no-niche) against its intrinsic growth rate:

$$\text{Fitness}_i = \frac{f_i\left(\sum_{j \neq i} c_{ij} N_j^{-i,*}, \mathbf{0}\right)}{f_i(0, \mathbf{0})}. \tag{4.3}$$

Both (eqn. 4.2 and 4.3) show that species abundances at equilibrium ($N*$) are necessary to define the species niche and fitness following Spaak and De Laender (2020) definitions. Therefore we computed these equilibrium abundances by parameterising a population model with estimates of λ_i and α_{ij} obtained from eqn. 4.1. The population model, which was a discrete-time logistic model, followed the same functional form (Ricker) as eqn. 4.1.

4.2.4 Functional traits and climatic variables

For each species, we measured at the beginning of the experiment (2015) during the peak of biomass of each individual species (seasonal development ranged from early February to late September), a series of plant functional traits related to particular organs such as leaves (leaf area, and specific leaf area) and roots (root diameter, specific root length, root tissue mass density, and specific root area), as well as whole-plant traits (i.e., height, canopy shape, leaf area index, C:N ratio, and C13 and N15 isotopes) (Table 4.1). These traits were selected for their recognised or assumed utility as response traits to abiotic conditions and their potential implication in plant competitive dynamics (Pérez-Ramos et al., 2019). Plant sampling and trait measurements followed standard protocols recommended by Pérez-Harguindeguy et al. (2016). Briefly, 30-50 individuals per species across their range of distribution were randomly selected to measure height and canopy shape, and leaf-level traits. Leaf size was quantified using an image analysis program (Image Pro-plus 4.5; Media Cybernetic Inc., Rockville, MD, USA). The four morphological root traits were measured in 3 to 7 individuals per species and treatment. In the field, we separated root systems from individuals of different species carefully. In the laboratory, we washed root samples to remove soil and then a representative subsample of fresh fine roots (less than 2mm in diameter) was scanned at 1200 dpi. The digital images were used to determine the length, area, volume, and mean diameter of roots using specific image-analysis software (WinRHIZO version 2003b, Regent Instruments Inc., Quebec, Canada). The root material harvested was immediately weighed, oven-dried at 60°C for 48 hours and then re-weighed. Four chemical traits (leaf N and C content, as well as the bulk isotopic composition of these elements) were determined on three composed samples per species and treatment obtained by pooling the leaves previously used for above-ground traits, using a CHN elemental analyser coupled to an isotope mass spectrometer at the laboratory facilities of Doñana Biological Station (EBD-CSIC).

We also obtained precipitation information, both annual and seasonal (winter, spring, summer, and fall) in mm (l/m^2) for each year from the nearest weather station (Aznalcazar, 10km far apart) that belongs to the Autonomous Government of Andalusia.

4.2.5 Statistical analyses

To test whether observed temporal variation in species' niche and fitness was greater between years or between species, we performed pairwise t-tests (a t-test between each year) using BH as the adjustment method to minimise true positives

(Benjamini and Hochberg, 1995). Once we had understood the source of niche and fitness variation, we tried to link this variation to properties related to species identities (functional traits) and to environmental heterogeneity (annual and seasonal precipitation). For linking niche and fitness with species functional traits, we fitted a permutational multivariate analysis of variance (PERMANOVA) with 999 permutations and euclidean method (Anderson, 2014); the results are shown in Table 4.1. Finally, to link niche and fitness with the precipitation, we applied linear (e.g., niche $\sim$ rain) and quadratic (e.g., niche rain^2 + rain) generalised linear models (GLM) for each variable (total annual rain as well as total rain during winter, spring, summer, and fall) and then selected the best model based on Akaike information criterion (AIC) (Bozdogan, 1987). All computational analyses were performed in R version 4.2.1 (R Core Team, 2022).

4.3 Results

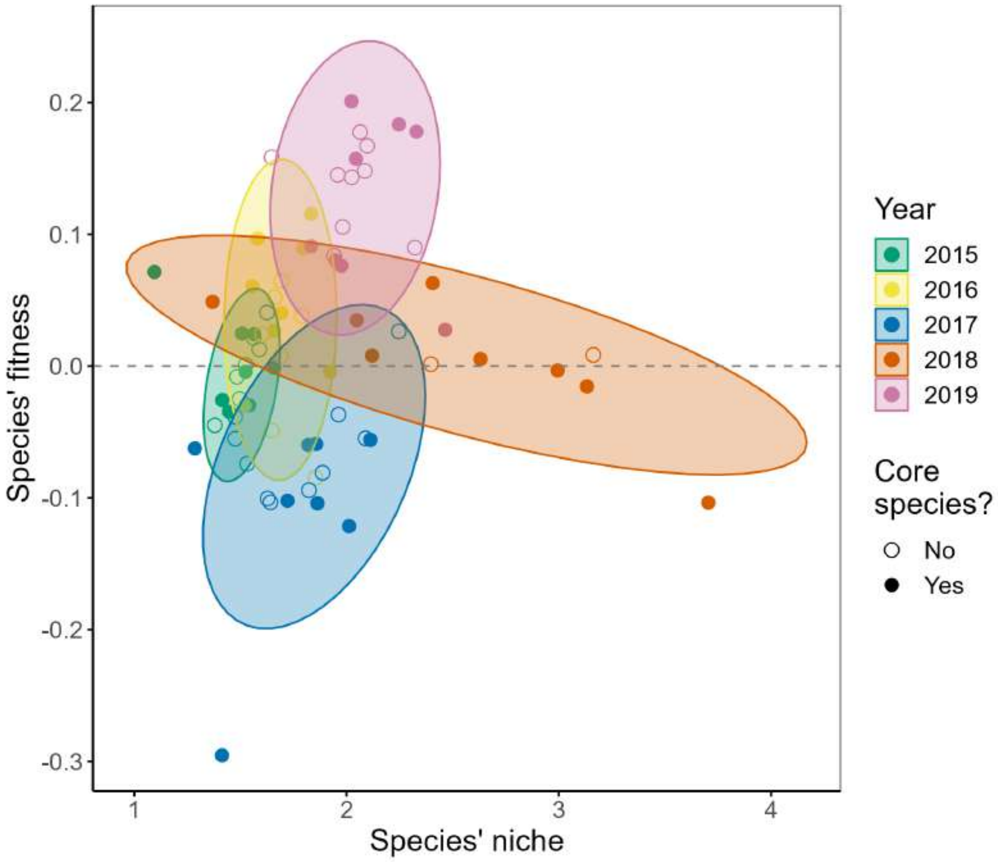

Figure 4.1: Relationship between species' niche and fitness for the 18 studied plant species across five years of field observations (2015-2019). Ellipses drawn assuming a multivariate t-distribution with 95% confidence. A species is considered core when it is present in all sampled years (see Table S4.1). Differences between years following a pairwise t-test can be found in Table S4.2 for fitness differences and in Table S4.3 for niche differences.

We estimated the niche and fitness for our 18 plant species (6 of which are core to the community, i.e., are found in all five sampled years; see Table S1) thanks to coupling a strong empirical effort with recent theoretical advances that define these characteristics at the species level. Contrary to the definition of the niche in any of its potential meanings, we observed that the niche is not a species property. That is, the position of the niche of a given species greatly varies across years (Figs. 4.1 and 4.2), and the relative differences between pairs of species were not temporally maintained (Fig. 4.1). Even more interesting is that we observed a directional change in the species' niche as well as their fitness from one year to another. This directional change indicates the critical role of the abiotic environment in defining the species niche, and in allowing species to explore different combinations of niche and fitness across years due to associated temporal variation in precipitation.

We also observed a great variation in species niche and fitness with almost all years rendering different results compared to the other four. For instance, only

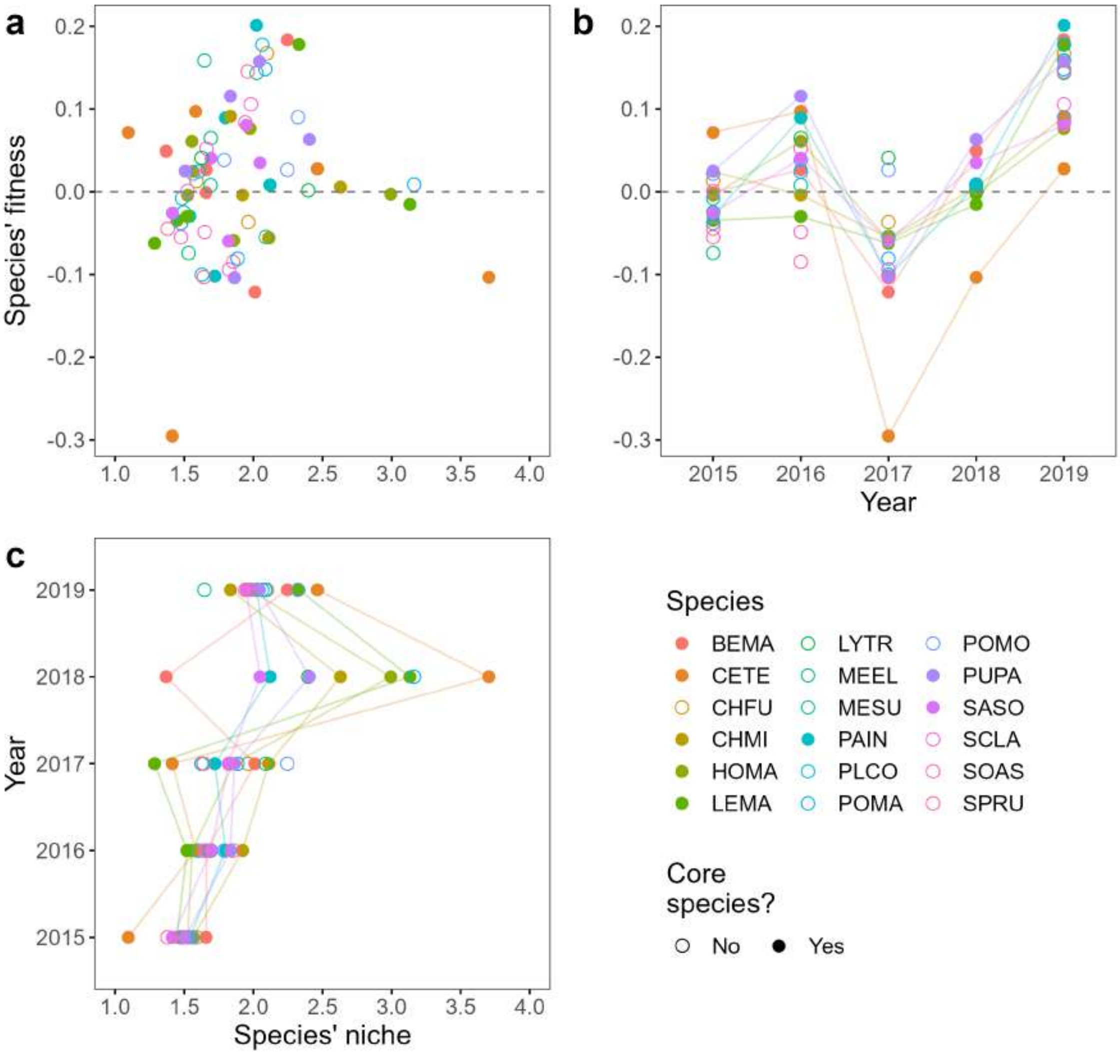

FIGURE 4.2: Annual changes in species' fitness (**a**) and niche (**c**), and (**b**) relationship between them. A species is considered core when it is present in all sampled years (see Table S4.1). Lines across years show changes in niche and fitness ranking for core species. Differences between years following a pairwise t-test can be found in Table S4.2 for fitness differences and in Table S4.3 for niche differences.

in 2018 did species show similar fitness values to 2015 and 2016 (Fig. 4.2b and Table S4.2). Moreover, we did not find two consecutive years in which the average fitness differentiation remained unchanged. In that sense, it is worth highlighting the notable differences between 2017 and 2019, with average fitness differences of -0.08 ± 0.07 and 0.13 ± 0.05, respectively. For the case of the species' niche, we found very similar patterns of variation (Fig. 4.2c and Table S4.3). Except for 2017, which showed similar niche values as 2016, we observed strong significant differences between the niches over the years. Particularly in 2018, the year in which our field site went through a big flood, the variation in species niche was the largest (2.60 ± 0.68) compared to, for instance, 2015 (1.49 ± 0.12).

As a consequence of the high variability observed for our system, we could neither assign with confidence a hierarchy of competitive dominance (Fig. 4.2b) nor

Table 4.1: Permutational multivariate analysis of variance (PERMANOVA) results for niche and fitness differences as a function of multiple species' functional traits. 99 permutations were employed. No significant differences were found, meaning that no trait was capable of explaining the variance in niche and fitness differences.

Trait	Df	Sum of squares	R2	F	Pr(>F)
Species' niche					
Height (cm)	1	0.10	0.01	0.44	0.5370
Canopy shape	1	0.06	0.00	0.25	0.6420
Leaf area (cm^2)	1	0.05	0.00	0.23	0.6240
Specific leaf area (cm^2/g)	1	0.00	0.00	0.00	0.9760
Leaf area index	1	0.27	0.02	1.14	0.2750
Root diameter (cm)	1	0.19	0.01	0.80	0.3720
Specific root length (cm/g)	1	0.19	0.01	0.80	0.3480
Root tissue mass density (g/cm^2)	1	0.00	0.00	0.01	0.9190
Specific root area (cm^2)	1	0.03	0.00	0.13	0.7150
C:N ratio	1	0.23	0.02	0.95	0.3500
C13 isotope	1	0.02	0.00	0.08	0.7990
N15 isotope	1	0.00	0.00	0.02	0.8910
Residual	57	13.60	0.92		
Total	69	14.76	1.00		
Species' fitness					
Height (cm)	1	0.00	0.00	0.11	0.7430
Canopy shape	1	0.00	0.01	0.55	0.4990
Leaf area (cm^2)	1	0.00	0.00	0.05	0.8270
Specific leaf area (cm^2/g)	1	0.00	0.00	0.07	0.7890
Leaf area index	1	0.00	0.00	0.24	0.6230
Root diameter (cm)	1	0.00	0.00	0.00	0.9970
Specific root length (cm/g)	1	0.00	0.01	0.54	0.4590
Root tissue mass density (g/cm^2)	1	0.01	0.01	0.72	0.4140
Specific root area (cm^2)	1	0.00	0.00	0.27	0.5930
C:N ratio	1	0.01	0.01	0.67	0.4200
C13 isotope	1	0.00	0.00	0.00	0.9690
N15 isotope	1	0.00	0.00	0.06	0.7970
Residual	57	0.50	0.95		
Total	69	0.53	1.00		

a niche position (Fig. 4.2c) across species. Overall, these results suggest that both central ecological concepts determining the outcome of competitive interactions were not a species' property. Indeed, some species varied more than others in their niche and fitness. This is the case of *Centaurium tenuiflorum* (CETE), which stands out for its variation. In 2015, *C. tenuiflorum* was the most competitive species according to its fitness values. However, it slightly lost dominance in 2016 and then shifted to become the less competitive species from 2017 onwards. Conversely, those years that *C. tenuiflorum* showed higher values of fitness correspond also to a low niche differentiation for the rest of the community, and those years in which this species showed lower fitness correspond to the case in which the species was most niche differentiated from the rest of the community (2018 and 2019). Additionally, we observe that core species present all years in the system were also those with higher fitness values, but core species did not present particular values of niche. For the case of rare species such as *Lythrum tribracteatum* (LYTR), only present in two of the five years (see Table S4.1), certain environmental conditions allowed this species to be the one with the highest fitness during 2017, but their special environmental requirements were not associated with particular positions of the niche.

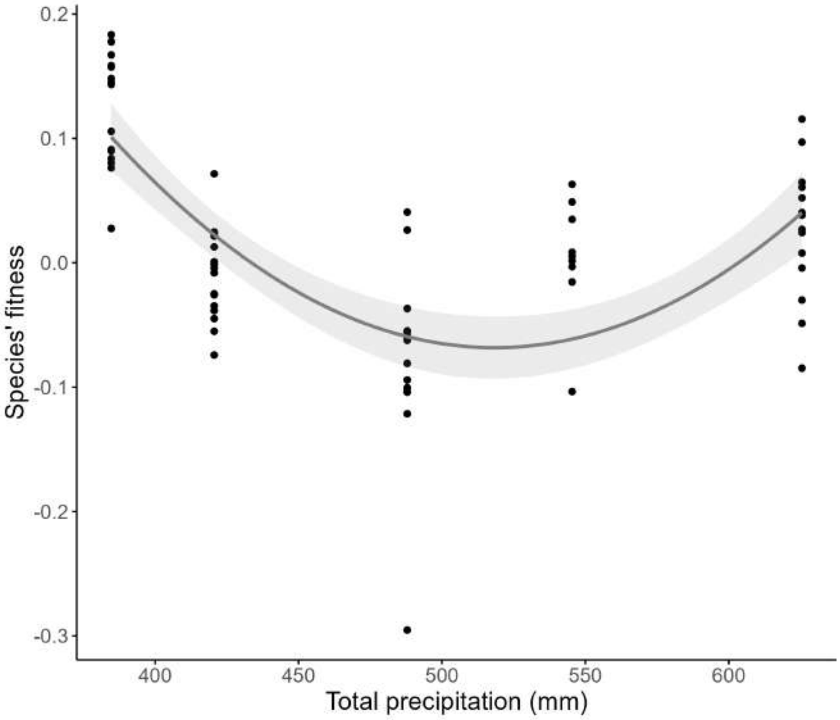

FIGURE 4.3: Quadratic relationship between species' fitness total annual precipitation. GLM statistical results are available in Table S4.4.

Following previous lines of evidence, functional traits were not explaining variation in species' niches in our community. In particular, PERMANOVA results did not significantly point to any of the twelve functional traits measured. The same happened for explaining variation in species' fitness. However, we did find that environmental variability played a role in explaining the variation found across years in species' niches and fitness. In particular, we found a quadratic (concave-up) relationship ($R^2 = 0.512$) between the total annual precipitation and the variation in species' fitness across years (Fig. 4.3 and Table S4.4). Lower species fitness was found at mid-range precipitation, but similarly, high fitness was found both at low and high precipitation conditions. However, the composition of the species with the highest fitness changed across these contrasted climatic conditions. During the dryer years, species with higher fitness correspond to *C. tenuiflorum* and *Pulicaria paludosa* (PUPA), and during the wetter years, this shifts to *Parapholis incurva* (PAIN), *Beta macrocarpa* (BEMA), and *Leontodon marocannus* (LEMA). Additionally, the precipitation in each season of the year also showed a quadratic concave-up relationship with fitness differences (Fig. S4.1), although the variation explained by each seasonal model is lower than the model including annual precipitation ($0.292 < R^2 < 0.374$; see Table S4.4).

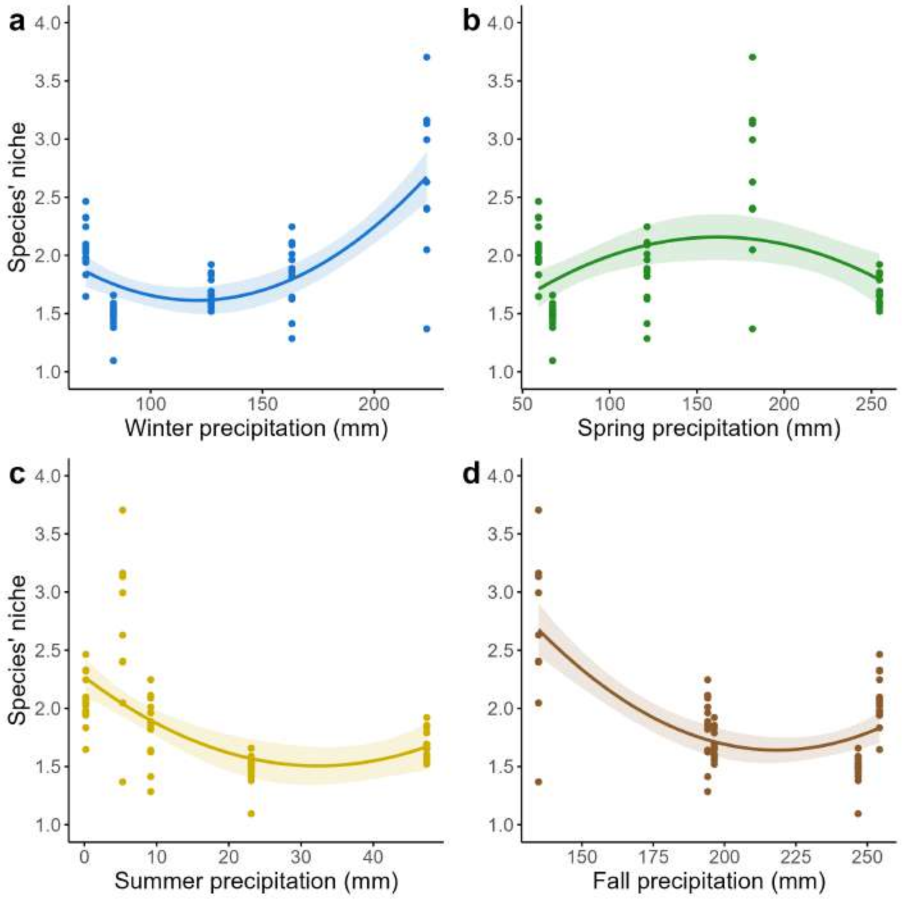

Figure 4.4: Quadratic relationship between species'niche and precipitation from the four seasons: (**a**) winter, (**b**) spring, (**c**) summer, and (**d**) fall. GLM statistical results are available in Table S4.5.

For species' niche, although the total annual precipitation fails to explain the variance found across years (Table S4.5), we did find significant quadratic relationships between the precipitation each season and the variation in species' niches (Fig. 4.4). Nevertheless, these relationships did not have the same functional form across seasons. For winter, summer and fall, either dry or wet conditions promoted niche variation across species. In particular, precipitation during winter explained 45.8% of the variance in species' niches, with either dry or very wet winters in the area promoting high values of niche. However, for spring precipitation we observed the inverse functional form (concave-down) indicating that the highest level of niche variation across species can be found at intermediate precipitation levels rather than on extremes (Fig. 4.4). Indeed, all climatic variables that showed non-quadratic relationships with variation in species niche and fitness also presented linear relationships but we selected quadratic models based on their lower AIC (Tables S4.4 and S4.5).

4.4 Discussion

Theoretical advances are of fundamental importance to make ecology a robust science, but only by combining such theoretical advances with empirical data can we learn whether they are valuable to describe natural phenomena. Among all conceptual developments in ecology, the niche has become one of the central pillars (Chase and Leibold, 2003) that serve to describe the incredible levels of biodiversity that we observe in nature (Kornfield and Smith, 2000), as well as how species can persist without excluding each other (Chesson, 2000; Levine and HilleRisLambers, 2009). However, the underlying idea that has been assumed during the last century is that the niche, in all its potential definitions (Grinnellian, Eltonian, or Hutchinsonian definitions), is a species property and therefore must be defined at the species level. Testing this assumption was not possible until recent theoretical developments, which have provided a series of mathematical tools that rigorously connect the species' niche with their population dynamics and therefore with the ability of species to persist in natural communities (Chesson, 2000).

In our study, we couple these tools with detailed field observations for five years in highly-diverse annual grasslands, and we provide solid evidence that the niche is not an ecological property that can be described at the species level. Rather, environmental variability defines the range of variation of niches a set of species can experience. In other words, a species in our system does not have a specific range of values that can be differentiated from the rest of the species, which means that species do not differ in their niches. Rather when niches vary due to changes in environmental conditions, they do vary for all species in a directional way rather than observing changes that are species-specific, and as a consequence of yearly changes in environmental conditions, species concomitantly experience yearly different niches.

Complementary to this result, there are three other lines of evidence that support our affirmation. First, we measured for each species an extensive amount of traits at the leaf, root and whole plant level that represent leading dimensions of the ability of species to compete for shared resources such as light, nutrients, water and space (Westoby, 1998; DeMalach et al., 2017; Pérez-Ramos et al., 2019), and to tolerate natural enemies such as herbivorous insects and leaf pathogens (Deraison et al., 2015*b*; Welsh et al., 2016). None of the twelve traits measured here significantly explained variation in species' niches across species. Second, we found a strong relationship between all seasonal precipitation (winter, spring, summer and autumn) with variation in species' niches across the five years. The link with precipitation, with higher niche values at higher precipitation, is not surprising as these environments are strongly water limited, and suggest that simple environmental characteristics determined by large-scale processes can influence local dynamics and the niche we quantify. Third, as variable as we show the niche of the species was, we also found similar results in terms of variation for the species' fitness. The notion that a species' fitness can be variable across years with contrasted climatic conditions is something we can expect from the previous extensive literature showing that climatic conditions alone and their interaction with soil properties strongly influence the ability of annual plant species to, for example, develop and produce seeds (Angert et al., 2009; Hallett et al., 2019; Wainwright et al., 2019). Thus, observing such a similar variation in the niche as in the fitness reinforces the idea that the niche cannot be considered a species property alone, as this does not occur in the case of the species' fitness. This study shows that both are rather mutable features that are strongly affected by environmental characteristics.

If we agreed that the variation of species' niche and fitness is something dynamic and widespread in nature, then, we believe that they could be considered two central ecological constructs that we should revisit and start a profound discussion about. The first argument arises around the concept of the niche. How should we define what is a species niche? And, how do we make this definition, if any, broad enough to be valid for any taxa? For instance, annual grasslands are known for being highly dynamic systems in which their physiognomy and their biotic interactions are reset every year (García-Callejas et al., 2021). As opposed to this system, if we had studied forests, shrublands, coral reefs or any other ecosystem in which long-lived organisms buffer their population dynamics against environmental conditions, results could have been different, with likely less variability. These results would imply that the definition of the niche is not the same for all species and it depends, at least, on a three-way interaction between environmental conditions, the evolutionary history of the species, and its demography. The second argument is that species' niche, fitness, and their differences are key concepts determining the outcome of ecological interactions between competing species, as well as the phylogenetic and functional dispersion patterns we observe in ecological communities (Adler et al., 2007; Mayfield and Levine, 2010). If these are not restricted to the species level and, instead, can be considered more complex features highly modulated by environmental conditions, then we can argue that the ecological mechanisms that we use to understand and predict the maintenance of biodiversity and the composition of ecological communities should be rethought.

The lack of role for species identities with respect to their niche and fitness values and the huge role of precipitation in modulating these ecological concepts suggest that our system follows quasi-neutral dynamics (Scheffer et al., 2018). In this emerging neutrality, the species interactions that describe density-dependent processes seem to play a minor role, and the persistence of species is more likely driven by fluctuation-dependent mechanisms (Ellner et al., 2019). For instance, it is very likely that interannual variation in precipitation promotes the storage effect in our system as we previously found two of the three following requirements of the storage effect. First, we observed that different species have higher fitness with different environmental conditions, and second, we documented a positive covariance between competition and the environment, that is wetter years promote higher abundances but also stronger competition (Johnson et al., 2023). We believe further work should attempt to evaluate how the definition of the niche and its identity at the species level matches a gradient of cases in which fluctuation-dependent and fluctuation-independent mechanisms are combined.

We were surprised to find that any of the traits correlated with the values of species niche and fitness since they are thought to play an important role in competition for light, nutrients, space, and the tolerance to shared natural enemies.

Previous findings have shown opposed results working also with annual plant species in Mediterranean grasslands of California (Kraft et al., 2015*a*) and Spain (Pérez-Ramos et al., 2019). Our system, compared to previous studies, encompasses a wide range of taxa from disparate families, and such broad phylogenetic differences might not be sensitive enough to capture differences in fitness among species. Additionally, we only measured functional traits one year, and differences in these traits were related to differences in niche and fitness across the five years. Our species do not show a high degree of phenotypic plasticity, but we agree that a better test would be to measure these traits each year. Another limitation of this study is that we followed our grasslands for only five years. Is this amount of time enough to capture the multidimensionality of the species' niche or are more years necessary? This question has never been addressed before and therefore it is difficult to give an informed answer. Our findings might be indeed biased because we have studied a very dynamical system, which opens the question to what extent we need long-term data to characterise a species niche, if possible at all.

In conclusion, we can not trace back in our study which are the ultimate sources at the local scale that drive niche variation among the species in our system, yet it is clear that a climatic factor (precipitation) showing broad scale variation modulates the species' niche variability found in our study. The effect on interannual climatic variability on modulating the variation of the species' niche strongly suggests that such niche is not a species property as ecologists have thought during the last century. If the niche is something volatile, rather than a reasonable fixed property of species, we might rethink its definition because it holds a strong weight in explaining fundamental properties of nature such as biodiversity and long-term persistence. Taken together, our results raise substantial questions about the nature of the niche as a species property, and open a discussion towards thinking which better definition, if any, would allow us to improve our understanding of the ecological dynamics of species and the maintenance of biodiversity.

Associated publication

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Open science

Data and code available through www.github.com/Granjel/Caracoles.

Author contributions

O.G. conceptualised the research. O.G., R.R.G., and L.B. performed fieldwork. D.G.-C. parameterised the population model. J.W.S. quantified niche and fitness. R.R.G. analysed and designed the visualisation of results. R.R.G. and O.G. wrote the original manuscript with input from all authors.

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Supplementary material

Supplementary figures and tables related to this chapter are available in Appendix 4.

Discussion

The rapid pace of anthropogenic global change is dramatically altering the natural world. Shifts in environmental conditions are leading to modifications of biotic interactions such as competition, mutualism, or predation (Tylianakis et al., 2008). In turn, the balance of such interactions is responsible for determining whether species are able to persist in a community or, on the contrary, they go extinct. Therefore, understanding how environmental changes and ecological interactions are shaping the mechanisms that allow species to coexist is nowadays more pressing than ever.

Throughout the chapters of this thesis, I have raised fundamental questions in community ecology and approached them from an integrative perspective that combines the most recent theoretical advances in the field of ecology with extensive empirical data. The approach used here is unmatched, as this thesis comprises the first effort towards understanding how different sources of abiotic and biotic heterogeneity affect the mechanisms of coexistence in multispecies, rather than pairwise, ecological communities. In the following lines, I discuss the main findings and developments of this thesis along with its main limitations, which open paths for future work. Finally, I present a list of the main conclusions reached as a result of this work.

In this thesis, we explore the effects for species coexistence of two primary drivers of global change: nitrogen enrichment and changes in land use intensity (Tylianakis et al., 2008). On the one hand, nitrogen (N) enrichment is currently challenging our understanding of a thoroughly studied topic in ecology such as resource competition. Much of the evidence regarding N enrichment in natural ecosystems indicates that predicted increases in the availability of this nutrient are related to shifts in species composition and biodiversity losses (Stevens et al., 2018; Midolo et al., 2019). This is suggested to happen through multiple mechanisms, such as an increase in fast-growing species leading to asymmetries in the competition for light (DeMalach et al., 2017) or a reduction in niche dimensionality that increases interspecific interactions (Harpole et al., 2016). However, our results indicate that an increment of N may not always lead to biodiversity loss (Chapter 1). We showed that the experimental addition of N to perennial plant communities did not predict losses of biodiversity. Instead, it promoted the niche differences that increase the chance of persistence and decreased the fitness differences that arise from competitive abilities and potentially lead to competitive exclusion. In light of all previous research, we claim that, although this does not indicate the existence of beneficial effects of N enrichment for natural ecosystems, higher N levels have the potential to increase niche differentiation and decrease fitness differentiation in certain years, which in turn may buffer its negative impacts on biodiversity.

On the other hand, land use is a complex concept comprehending different variables such as fertilisation, mowing, or grazing. Increasing land use intensity (LUI) has apparent negative consequences for biodiversity, including declines in plant diversity and compositional changes (Rockström et al., 2009; Newbold et al., 2015; Allan et al., 2014; Crawley et al., 2005; Pakeman, 2011; Dormann et al., 2007); however, we have little knowledge of the mechanisms behind this pattern. Based on the existing literature on the topic (e.g. Flynn et al., 2009; Laliberté and Tylianakis, 2012; Mayfield et al., 2010), we have identified three possible ways by which increases in land use intensity could hinder species persistence: by increasing competitive inequalities and thus fitness differences, by reducing the axes of niche differentiation, or by limiting the capacity of indirect interactions (discussed below) for promoting biodiversity. What we found when we analysed 150 grasslands under a gradient of LUI (Blüthgen et al., 2012) across eight years (chapter 2) is that, although fitness differences were high and restricted coexistence, they did not change in response to the gradient of LUI. Niche differences, however, show an unprecedented nonlinear relationship with LUI that implies higher chances of coexistence in lower and intermediate values of LUI than in higher values. Overall, we found relatively high niche differences across the LUI gradient. We are not able to elucidate the specific components of our LUI index that triggered such high niche differentiation, but associations with organisms that do not suffer from land-use intensification such as mycorrhizae (Hart et al., 2003), the effects of pathogens, or trade-offs with different nutrients (Harpole et al., 2016) could be plausible explanations worth exploring. Moreover, we observed high and widespread interspecific facilitation that, as pointed out in recent studies by Adler et al. (2018) and Losapio et al. (2021), could be one of the main factors behind biodiversity maintenance across environments, even in anthropised ecological systems.

Within this thesis, we also explored the role of natural enemies such as foliar fungal pathogens and herbivores for species coexistence. Fungal pathogens were studied within the same experiment as N enrichment (chapter 1) because fungal pathogens can benefit from either higher N concentration in leaves (Dordas, 2008) or a shift towards fast-growing plant species (Cappelli et al., 2020) caused by N enrichment. Pathogens, as natural enemies, are thought to promote biodiversity via stabilising and equalising mechanisms, i.e., both by suppressing increasingly common species and reducing their competitive ability (Petermann et al., 2008; Mordecai, 2011; Bagchi et al., 2014; Bever et al., 2015). However, the effects of pathogens may be complex and hard to predict because changes in pathogen abundance and composition could benefit a particular combination of specialist and generalist species, potentially resulting in neutral (Spear and Mordecai, 2018; Uricchio et al., 2019) or even detrimental effects for biodiversity maintenance. Our results indicated that the application of a fungicide treatment to suppress foliar fungal pathogens caused similar effects to those of N enrichment. In other words, plant communities with natural, unmodified pathogens showed lower niche and higher fitness differences than those plant communities with suppressed pathogens. Although we lack a detailed understanding of the pathogen community to be able to decompose the specifics by which the fungicide treatment promoted the determinants of plant coexistence, one possible explanation that we mention is that the natural pathogen community may have been composed of fungal species with overall neutral effects for coexistence (e.g., not engaging in density dependence mechanisms). Thus, applying the fungicide may have selected generalist species prone to attack the most common species in the community (see Cappelli et al. (2020)).

As well as pathogens, insect herbivores are expected to promote coexistence through negative density dependence (Ishii and Crawley, 2011; Heard and Sax, 2013; Forrister et al., 2019; Descombes et al., 2020). However, their effects are also susceptible to change depending on the composition of the herbivore community and their feeding preferences (Kempel et al., 2015). To understand this, we analysed an empirical network of perennial plants and herbivore grasshoppers (Chapter 3). For this network, we estimated the direct interactions between plants and the effects of grasshoppers on plants, but also we estimated the higher-order interactions (HOIs; modifications of a direct interaction by a third species) caused by plants and by grasshoppers; such HOIs are thought to stabilise competitive dynamics and explain unexplained variability in ecological communities (Grilli et al., 2017). What we found is that most of the variation in the plant's niche and fitness differences was explained solely by the direct interactions between plants and that neither the direct effect of herbivores nor the HOIs played a major role in species coexistence in our system. This result may be influenced by unknown determinants intrinsic to our system, but there is little doubt that this result contrasts with most previous theoretical and empirical findings and deserves further exploration.

A key decision for this study was to apply state-of-the-art statistical techniques via hierarchical group lasso regularisation (Lim and Hastie, 2015), for two reasons. One, because it helped us select the important interactions explaining the dynamics of plant species within our complex plant-herbivores network, instead of trying to quantify all interactions independently of their importance. Second, this approach also allowed us to relax our assumptions regarding the sing of effects of herbivores over plant species. As we argued, the sign of interactions had traditionally been assigned aprioristically, with herbivores being typically negative and pollinators being typically positive for plant growth (Chesson and Kuang, 2008; Bastolla et al., 2009; Rohr et al., 2014). However, we observed both positive and negative effects across ecological interactions in our network, including the effects of herbivores on plants. Thus, for interactions such as those between insect herbivores and plants, where there may be hidden mechanisms that we cannot decipher, we advocate for letting ecological models determine the sign of the effect of interactions on species dynamics.

Within the same system, we explored a key question in community ecology: how does the configuration of species interactions affect the determinants of species coexistence? To do so, we used different metrics related to the distribution of species interactions within modules, to the dominance of intraspecific over interspecific interactions, and to the prevalence of positive over negative interactions. The stabilising effect of dominant intraspecific interactions within modules was already well known within modern coexistence theory (Chesson, 2000; Barabás et al., 2016), and we corroborated it in our system. However, we found that the prevalence of stronger positive than negative interactions also promoted the axis of niche differentiation between perennial plants, which had been suggested (Losapio et al., 2021) but not explicitly shown in empirical weighted networks. As opposed to the niche differences, the axis of fitness differentiation could be explained by many more metrics, alone or interactively. For example, higher dominance and high kurtosis occurred mainly when competitive effects were prevalent (as determined by a low ratio between positive and negative interactions), representing increased fitness differences and higher chances of encountering a competitive dominant species destabilising the system. All of this suggests that niche differentiation in ecological systems may be easier to control than fitness differentiation because fewer predictors are able to capture its variability.

Applying a structural stability approach to species coexistence in Chapters 1, 2, and 3 has allowed us to explore the effects of all the topics discussed above (N enrichment, land use intensity, pathogens, herbivores, and interaction configurations) on the determinants of multispecies coexistence. One principal result of this thesis is that the number of interacting species determines the effects of different sources of abiotic and biotic heterogeneity over the niche and fitness differences that determine species coexistence. Specifically, N enrichment and pathogen suppression effects on plant coexistence (Chapter 1) differed between pairs and multispecies assemblages (three to six); changes in both niche and fitness differences were more pronounced with the gradient of LUI (Chapter 2) in three-species assemblages compared to pairs of species; herbivory and HOIs (Chapter 3) had even less relevance explaining the variation of niche and fitness differences in four-species combinations compared to three-species ones; and we found interactive effects between positive interactions and intraspecific interactions explaining niche differences in four-species combinations as opposed to three-species ones (Chapter 3). The exploration of multispecies mechanisms requires further efforts to decipher the specific causes of why different numbers of interacting species show different responses to abiotic and biotic heterogeneity. However, with only a few available studies accounting for multispecies coexistence mechanisms (Petry et al., 2018; Tabi et al., 2020; Bartomeus et al., 2021; Deng et al., 2021; García-Callejas et al., 2021; Johnson et al., 2022; Arroyo-Correa et al., 2023), the empirical results presented here are very novel and suggest that we need to explicitly incorporate the number of interacting species into coexistence studies if we want to accurately predict the effects of global change drivers and biotic interactions on biodiversity maintenance.

Additionally, another main benefit of applying a structural approach is that we are able to quantify the contribution of indirect effects, which arise in multispecies assemblages, to the determinants of species coexistence (Saavedra et al., 2017). One of the best-studied indirect effects in ecology is intransitivity, represented by the popular game of rock-paper-scissors dynamics in which rock beats scissors, scissors beat paper, and paper beats rock, stabilising an otherwise unstable community (Gallien et al., 2017; Soliveres et al., 2018). These effects are evaluated in Chapter 1 and Chapter 2 of this thesis. We found that indirect effects created new opportunities for species persistence under N enrichment and pathogen suppression (Chapter 1), but these opportunities were relevant at simpler species combinations (three and four species) and they lost importance as more species were involved (combinations of five and six species). Moreover, we did not predict changes in the importance of indirect interactions with the gradient of LUI (Chapter 2), which aligns well with previous results indicating a minor presence and role of intransitive dynamics in annual plants (Godoy et al., 2017; Matías et al., 2018) but contrasts with previous results in the study system (Soliveres et al., 2018). This may be explained by a neutralising effect of alternative indirect effects such as indirect facilitation, which occurs when a species strongly suppresses a shared competitor with a third species (Levine, 1999; Flory and Bauer, 2014). Importantly, the metrics used were useful for describing the contribution of indirect interactions to coexistence, described our empirical results effectively, and have the potential to be used in prospective studies.

In the three first chapters, we embraced the latest developments in coexistence theory, structural stability, which uses structural analogues of niche and fitness differences to understand multispecies persistence (Saavedra et al., 2017; Song et al., 2018). However, we did so without questioning the nature of such important underlying concepts. In particular, the niche has been debated for decades, and today it is thought to be a multidimensional species property accounting for all abiotic and biotic heterogeneity that makes a species thrive (Chase and Leibold, 2003). But we argue that, despite being considered a species property, the frameworks that use the niche to explain crucial ecological features such as species diversity (Chesson, 2000; Adler et al., 2007; Saavedra et al., 2017) are not able to quantify it at the species level, and instead calculate differences between pairs of species or even multispecies assemblages. Thus, we wondered whether the niche actually functions as a species property or it rather behaves as a characteristic that varies with environmental conditions. In the quest to apply the most recent theoretical developments to answer central ecological questions, we applied new definitions in modern coexistence theory that allow us to quantify species' niches and fitness at the species level (Spaak and De Laender, 2020). In our study (Chapter 4), we saw that the plant niche has a strong interannual variability that relates neither to species identity nor to species functional traits. Instead, this temporal variability can be explained by an environmental variable such as seasonal precipitation, reflecting how dry or wet each season is. Although the results of this study may be limited to short-living organisms such as annual plants, which renew their population each year and thus may be more susceptible to changes in niche and fitness, our results are robust in terms of niche variability and its lack of relation to species-level characteristics.

Despite the solid combination of theory and empirical data, the results reached in this thesis come with some limitations that are worth discussing. First, in this thesis, we have not intensively analysed the effect of spatiotemporal heterogeneity. In some studies, we have multiple years of data or different locations; for example, in Chapter 1 we have two years of experimental data and in Chapter 2 we have eight years and 150 grasslands. However, such variability is not accounted for and is rather used to improve the estimation of intrinsic growth rates and interaction coefficients between species. Multiple studies show that spatiotemporal heterogeneity is key in determining species persistence in the long term through various mechanisms (e.g., Chesson, 1985; Chesson et al., 2014; Shoemaker and Melbourne, 2016; García-Callejas et al., 2021), so explicitly accounting for such heterogeneity would be key in following empirical efforts to understand the mechanisms of multispecies coexistence. Second, although this thesis contributes to understanding how multiple key ecological factors such as natural enemies or resources drive coexistence, it does not include some fine-scale details that could be determinant to acquiring a more mechanistic understanding of these processes. For example, in Chapter 1 we suppressed fungal pathogens using a fungicide but we did not further analyse the changes in the pathogenic community; the resulting composition of such community, as we have previously indicated, could be critical to linking density-dependent mechanisms that stabilise coexistence to individual types of generalist or specialist pathogens and their associations with plants species (Mordecai, 2011, 2015; Cappelli et al., 2020). Another example is Chapter 2, where we analysed the effects of land use intensity as a single index that summarised the different factors involved (Blüthgen et al., 2012); as we have indicated, our research could not investigate the individual effects of the components of the land use index (mowing, grazing, and fertilisation) because of their high correlation (DeMalach et al., 2017), but we would benefit from deciphering the joint contribution of these different components in our system, but also of their interactions with relevant biotic drivers such as mycorrhizae (Gossner et al., 2016; Hart et al., 2003) or plant pathogens Harpole et al.. The results of this thesis would also have benefited from including more information about the different processes occurring at different development stages of a plant's life cycle. In our studies with perennial (Chapters 1, 2, and 3) and annual (Chapter 4) plants, we have focused on the interactions between adult individuals, ad we have not included competition, predation, or other relevant factors influencing the growth and survival of seeds and seedlings, although these could hold the potential to impact plant dynamics as much as phenomena occurring at later stages (e.g., Hanley and Sykes, 2009; Van Couwenberghe et al., 2013; Petry et al., 2018).

Future research opportunities are varied and extensive. The methodology used in this thesis provides the tools needed to advance our knowledge of how biodiversity is maintained. An intuitive path to follow would be to investigate ecological systems including multiple types of trophic layers, such as predator, floral visitors, or mycorrhizal associations. Moreover, the key to acquiring a global knowledge of species persistence in the system would be to quantify species interactions and growth rates not only for one trophic layer but for all, as described by (Godoy et al., 2018). The experiments and observations needed to do this are often challenging or logistically unfeasible, but some approaches have already started with simplified systems kept in experimental enclosures, such as (Bartomeus et al., 2021). Another exciting future path would be not only to explore how biotic interactions stabilise ecological communities in variable environments but also to try and identify those key biotic interactions and coexistence mechanisms that optimise the function of the system (Godoy et al., 2020), which would be critical to acquiring a global understanding of the relationship between biodiversity and ecosystem functioning. The knowledge gained from and tools applied in these perspectives can be applied in interesting ways, such as helping ecologists enhance conservation and restoration efforts (Aoyama et al., 2022). The ultimate goal of this thesis is to contribute to the scientific understanding of the mechanisms maintaining biodiversity in complex and diverse communities, serving as a valuable resource for future research efforts in the field.

Conclusions

- The use of a structural stability approach allows us to accurately predict the direct effects of global change drivers and biotic interactions on biodiversity maintenance and also to quantify the contribution of indirect effects emerging in multispecies assemblages. Future studies attempting to use empirical data to understand multispecies coexistence and ecosystem stability would benefit greatly from its application. —*Chapters 1, 2, and 3.*
- Nitrogen enrichment is considered a global change driver related to species losses from terrestrial ecosystems worldwide, yet in our study it has the ability to increase the niche differences that promote plant coexistence and also decrease the fitness differences that lead to competitive exclusion, potentially buffering its negative impacts on biodiversity. —*Chapter 1.*
- Suppressing the community of foliar fungal pathogens through the application of a fungicide is expected to reduce plant coexistence through the elimination of natural enemies. However, we observed similar effects as nitrogen enrichment in reducing niche and increasing fitness differences among multispecies assemblages. This unexpected result could only be obtained by the combination of theory with detailed empirical experiments, suggesting either that the natural pathogen community has neutral effects on coexistence or that the fungicide application may have selected generalist species that attack the most common plant species. —*Chapter 1.*
- The effects of nitrogen enrichment and foliar fungal pathogens over the structural coexistence determinants depended on the number of interacting plant species, only emerging in multispecies assemblages and not in species pairs. Also, the higher the number of interacting species, the fewer species combinations are predicted to coexist. Although nitrogen enrichment and pathogen suppression do not affect coexistence predictions, they trigger changes in community composition. All of this highlights the importance of understanding coexistence beyond pairwise interactions. —*Chapter 1.*
- Land use intensity, a complex global change driver encompassing different intensive farming practices, does not change fitness differences between plants but it does change niche differences in a nonlinear way, implying higher chances of coexistence in lower and intermediate values of land use intensity. —*Chapter 2.*
- Interspecific facilitative interactions are strong and widespread across the gradient of land use intensity observed. This facilitation seems to play a key role in maintaining biodiversity. —*Chapter 2.*
- Indirect effects emerging in multispecies systems promote coexistence but their effect is only noticeable when a few species interact; this indicates that, in more diverse communities, direct effects are the main force shaping

coexistence mechanisms rather than chains of indirect effects. Contrary to expectation, these indirect effects remain indifferent to heterogeneity sources such as nitrogen enrichment, changes in land use intensity, and modifications in the community of natural enemies such as fungal pathogens. —*Chapters 1 and 2.*

- For a complex and highly-diverse plant-herbivore network, it is possible to obtain information on direct effects between plants, between plants and grasshoppers and more complex higher-order interactions. However, direct interactions between plants play a predominant role compared to the other type of interactions in promoting the determinants of interaction outcomes, niche and fitness differences. These results contrast with previous theoretical and empirical findings and call for further exploration of the relationship between herbivores' feeding preferences and plant coexistence. —*Chapter 3.*
- Statistical techniques such as group lasso regularisation are critical to reducing the assessment of the potential set of interactions species can establish to these that explain plant population dynamics. They are also not restricted to defining a specific sign on interactions, as has traditionally been done with herbivory thought to be negative. By documenting that herbivory can have negative and positive effects on plant growth, we advocate that, instead of defining interaction signs aprioristically, this approach can help us improve our understanding of species persistence in complex, multitrophic systems. —*Chapter 3.*
- The configuration of modules of species interactions within complex food webs is key for understanding species persistence. The prevalence of dominant intraspecific interactions and positive rather than negative interactions supports niche differentiation between perennial plants, whereas fitness differentiation is influenced by the interactive effect of multiple network metrics, making niche differentiation easier to predict and control. —*Chapter 3.*
- Although existing frameworks for explaining species diversity consider the niche as a species-level property, they could only quantify it as differences between pairs of species or multispecies assemblages. A new theoretical advancement to quantify the niche at the species level allows us to show that plant niches in annual systems have a strong temporal variation, with changes that are greater between years than between species. —*Chapter 4.*
- The fact that this interannual variation in species niches can be explained by a climatic variable such as precipitation instead of being linked to species properties such as functional characteristics challenges the predetermined understanding of the niche as a species property, suggesting that it may be dependent on the environment. —*Chapter 4.*

Conclusiones

- El uso de un enfoque de estabilidad estructural nos permite predecir con precisión los efectos directos de los motors de cambio global y las interacciones bióticas en el mantenimiento de la biodiversidad, así como cuantificar la contribución de los efectos indirectos que surgen en comunidades multiespecíficas. Futuros estudios que intenten utilizar datos empíricos para comprender la coexistencia multiespecífica y la estabilidad del ecosistema se verían beneficiados de su aplicación. —*Capítulos 1, 2, and 3.*

- El enriquecimiento de nitrógeno está considerado un motor de cambio global relacionado con la pérdida de especies en ecosistemas terrestres de todo el mundo. Sin embargo, en nuestro estudio tiene la habilidad de incrementar las diferencias de nicho que promueven la coexistencia de plantas y también de reducir las diferencias de capacidad competitiva que llevan a exclusión, tamponando potencialmente sus impactos negativos sobre la biodiversidad. —*Capítulo 1.*

- Se espera que suprimir la comunidad de hongos patógenos de hoja a través de la aplicación de un fungicida reduzca la coexistencia de plantas mediante la eliminación de enemigos naturales. Sin embargo, hemos observado efectos similares al enriquecimiento de nitrógeno, en tanto a reducir las diferencias de nicho y de capacidad competitiva entre combinaciones multiespecífos. Este inesperado resultado solo pudo obtenerse de la combinación de teoría con detallados experimentos empíricos, lo que sugiere o que las comunidades de patógenos naturales tienen efectos neutros sobre la coexistencia o que la aplicación del fungicida puede haber seleccionado especies generalistas que atacan a las especies más comunes. —*Capítulo 1.*

- Los efectos del enriquecimiento de nitrógeno y los hongos patógenos foliares sobre los determinantes de la coexistencia estructural dependen del número de especies de plantas que interactúan, solo surgiendo en combinaciones multiespecíficas y no en parejas de especies. Además, a medida que aumenta el número de especies que interactúan, se predice que hay menos combinaciones de especies que coexisten. Aunque el enriquecimiento de nitrógeno y la supresión de patógenos no afectan a las predicciones de coexistencia, provocan cambios en la composición de la comunidad. Todo esto destaca la importancia de comprender la coexistencia más allá de las interacciones entre pares de especies. —*Capítulo 1.*

- La intensidad de uso del suelo, un complejo motor de cambio global que comprende diferentes prácticas agroganaderas intensivas, no cambia las diferencias de capacidad competitiva entre plantas pero sí cambia las diferencias de nicho de una manera no lineal, lo que implica mayores posibilidades de coexistencia en valores más bajos e intermedios de intensidad del uso del suelo. —*Capítulo 2.*

- Las interacciones interespecíficas de facilitación son fuertes y ampliamente extendidas a lo largo del gradiente de intensidad de uso del suelo observado. Esta facilitación parece desempeñar un papel clave en el mantenimiento de la biodiversidad. —*Chapter 2.*

- Los efectos indirectos que surgen en sistemas multiespecíficos promueven la coexistencia, pero su efecto solo es patente cuando pocas especies interactúan; esto indica que, en comunidades más diversas, los efectos directos son la fuerza principal que moldea los mecanismos de coexistencia y no las cadenas de efectos indirectos. Al contrario que lo esperado, estos efectos indirectos permanecen indiferentes a las fuentes de heterogeneidad como el enriquecimiento de nitrógeno, los cambios en la intensidad de uso del suelo y las modificaciones en la comunidad de enemigos naturales como los hongos patógenos. —*Capítulos 1 and 2.*

- Para una red compleja y altamente diversa de plantas y herbívoros, es posible obtener información sobre efectos directos entre plantas, entre plantas y saltamontes, así como interacciones complejas de alto orden. Sin embargo, las interacciones directas entre plantas juegan un papel predominante en comparación con otros tipos de interacciones a la hora de promover los determinantes de lla persistencia de especies, diferencias de nicho y de capacidad competitiva. Estos resultados contrastan con hallazgos teóricos y empíricos previos y llaman a una mayor exploración de la relación entre las preferencias de alimentación de los herbívoros y la coexistencia de las plantas. —*Capítulo 3.*

- Técnicas estadísticas como la regularización de grupo lasso son críticas para reducir la evaluación del conjunto potencial de interacciones que las especies pueden establecer a aquellas que realmente explican la dinámica poblacional de las plantas. Asimismo, no están restringidas a definir un signo específico en las interacciones, como se ha hecho tradicionalmente con la herbivoría, considerada negativa. Al documentar que la herbivoría puede tener efectos negativos y positivos en el crecimiento de las plantas, abogamos por que, en lugar de definir los signos de interacción a priori, este enfoque pueda ayudarnos a mejorar nuestra comprensión de la persistencia de las especies en sistemas complejos multitróficos. —*Capítulo 3.*

- La configuración de los módulos de interacción entre especies dentro de redes tróficas complejas es clave para entender la persistencia de las especies. La prevalencia de interacciones intraspecíficas dominantes e interacciones positivas en lugar de negativas promueve la diferenciación de nicho entre plantas perennes, mientras que la diferenciación de capacidad competitiva se ve influenciada por el efecto interactivo de múltiples métricas de red, lo que hace que sea más fácil predecir y controlar la diferenciación de nicho. —*Capítulo 3.*

- Aunque los marcos existentes para explicar la diversidad de especies consideran al nicho como una propiedad a nivel de especie, solo son capaces de cuantificarlo como diferencias entre pares de especies o combinaciones de múltiples especies. Un avance teórico reciente hacia la cuantificación del nicho a nivel de especie nos permite mostrar que los nichos de las plantas en sistemas anuales tienen una fuerte variación temporal, con cambios que son mayores entre años que entre especies. —*Capítulo 4.*

- El hecho de que esta variación interanual en los nichos se pueda explicar por una variable climática como la precipitación, en lugar de estar vinculada a propiedades de las especies como las características funcionales, cuestiona nuestro entendimiento predeterminado sobre el nicho como una propiedad de la especie, sugiriendo que puede actuar como una propiedad dependiente del ambiente. —*Capítulo 4.*

Appendix 1 — Chapter 1

Supplementary Figures

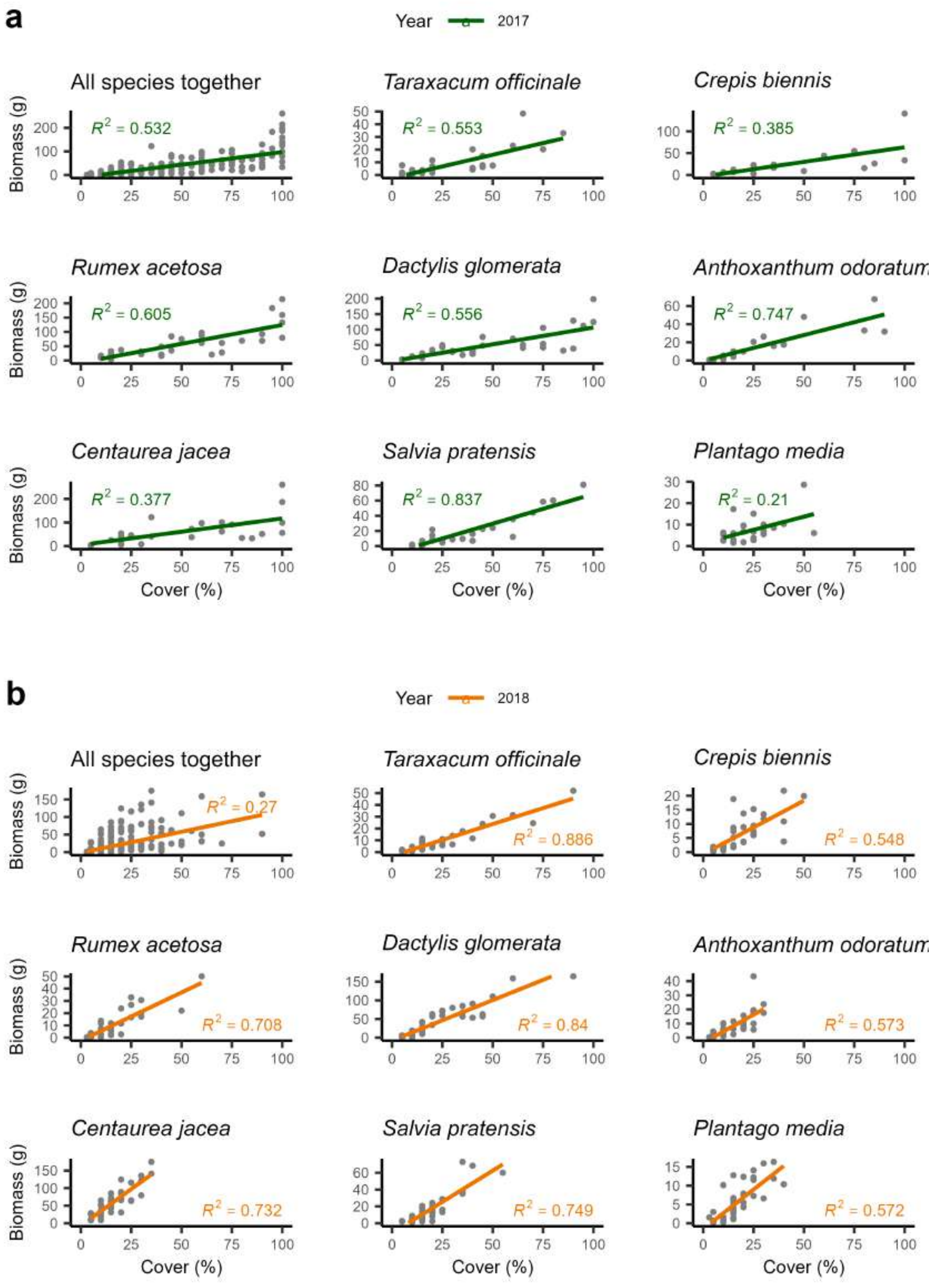


Figure S1.1: Relationship between biomass (g) and cover (%) for all 8 species together and individually, in (**a**) 2017 and (**b**) 2018. The R^2 coefficient is shown for each linear model.

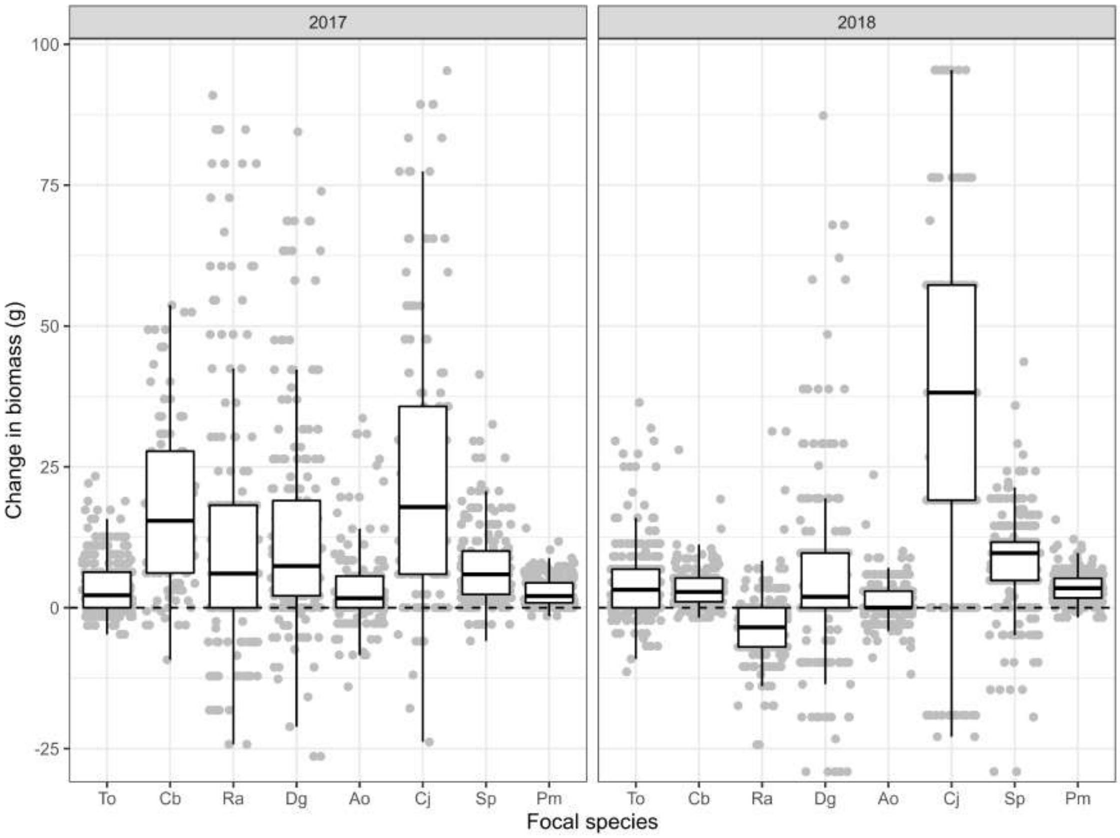


Figure S1.2: Change in biomass (g) in 2017 and 2018 for the 8 studied plant species: (To) *Taraxacum officinale*, (Cb) *Crepis biennis*, (Ra) *Rumex acetosa*, (Dg) *Dactylis glomerata*, (Ao) *Anthoxanthum odoratum*, (Cj) *Centaurea jacea*, (Sp) *Salvia pratensis*, and (Pm) *Plantago media*. The dashed horizontal line denotes the limit between positive and negative change in biomass. Note that *Rumex acetosa* in 2018 is the only species with a negative change in biomass.

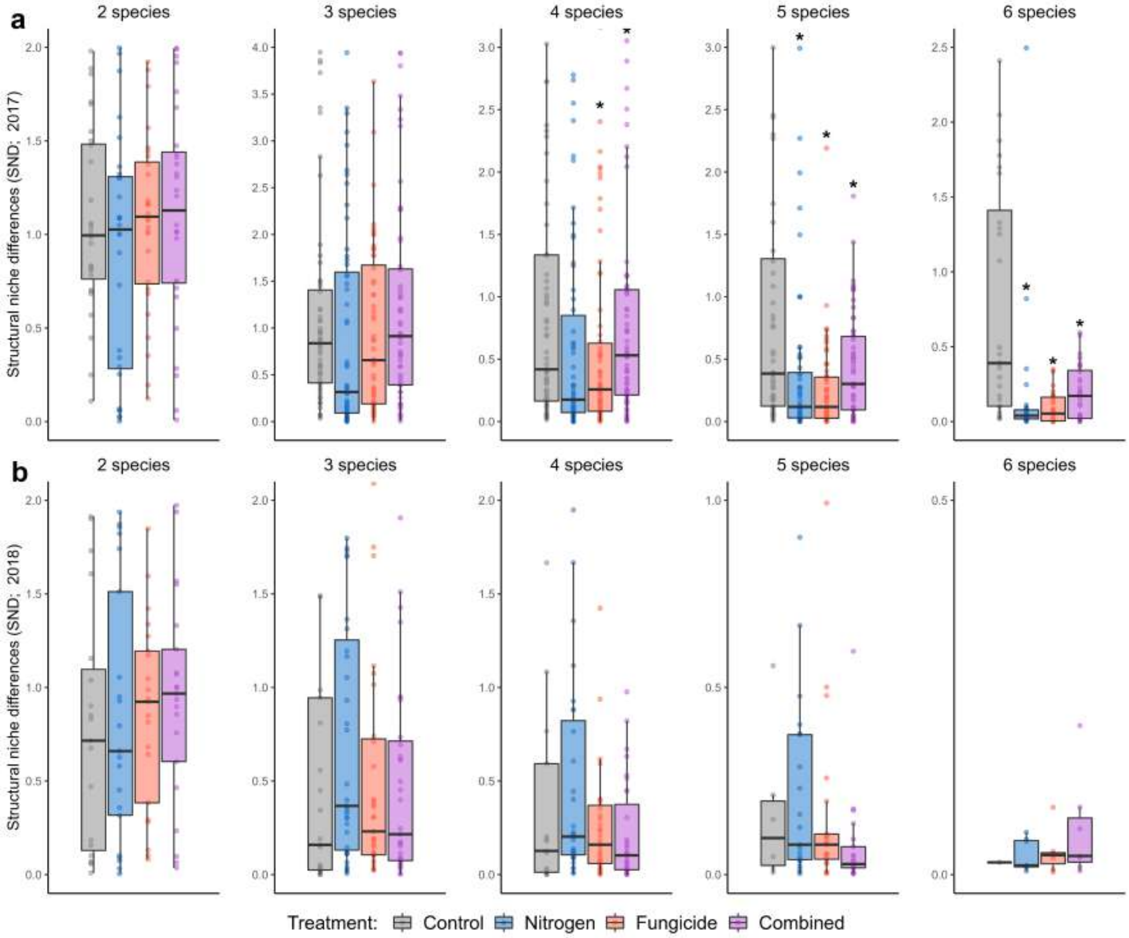


Figure S1.3: Structural niche differences (SND) for all combinations of two-six species, in the four experimental treatments (control, nitrogen, fungicide, and combined), for the years (**a**) 2017 and (**b**) 2018. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.9—S1.10). Note that the axis scales differ between panels because results can be compared across treatments within each species combination but not between species combinations (see main text).

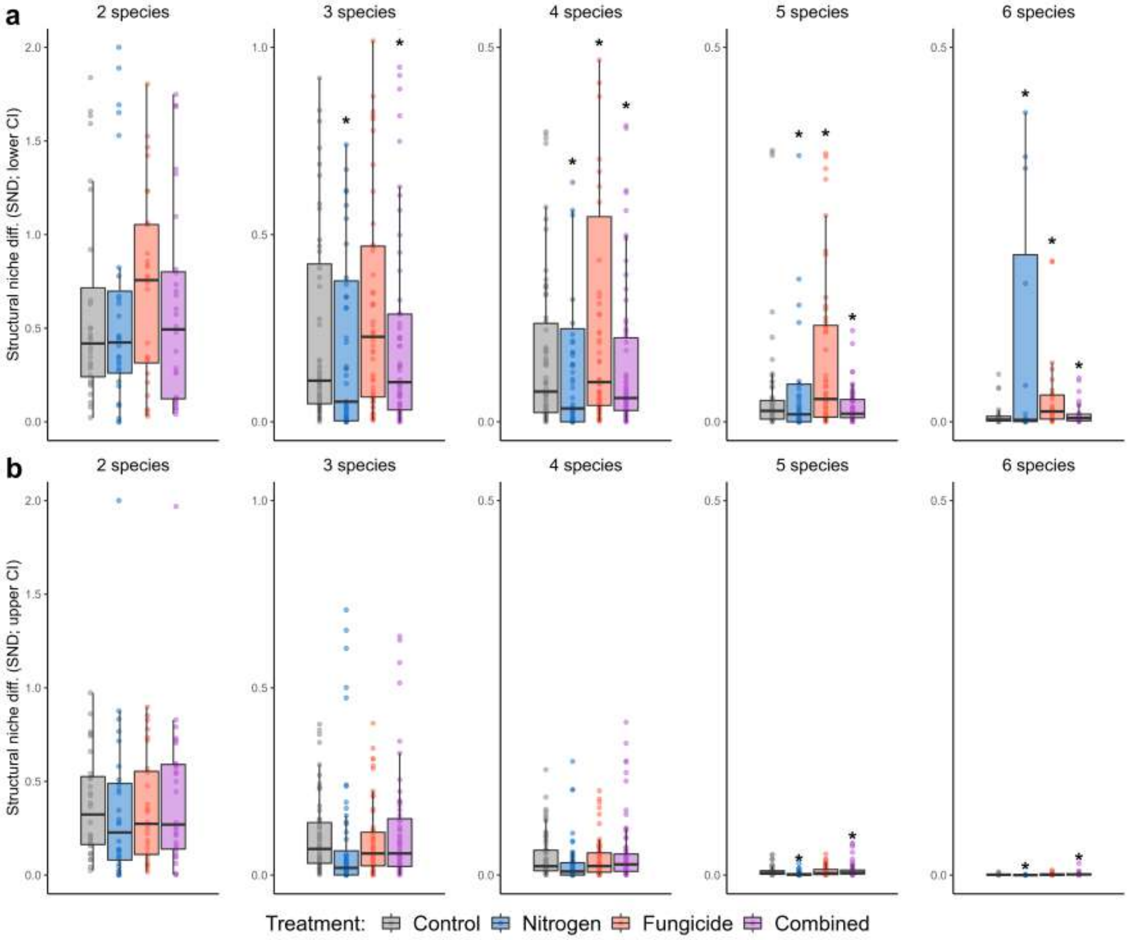


Figure S1.4: Structural niche differences (SND) for all combinations of two to six species, in the four experimental treatments (control, nitrogen, fungicide, and combined), calculated using the coefficients estimated for the (**a**) lower and (**b**) upper limits of the 95% confidence interval. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.13 and Table S1.15). Note that the axis scales differ between panels because results can be compared across treatments within each species combination but not between species combinations (see main text).

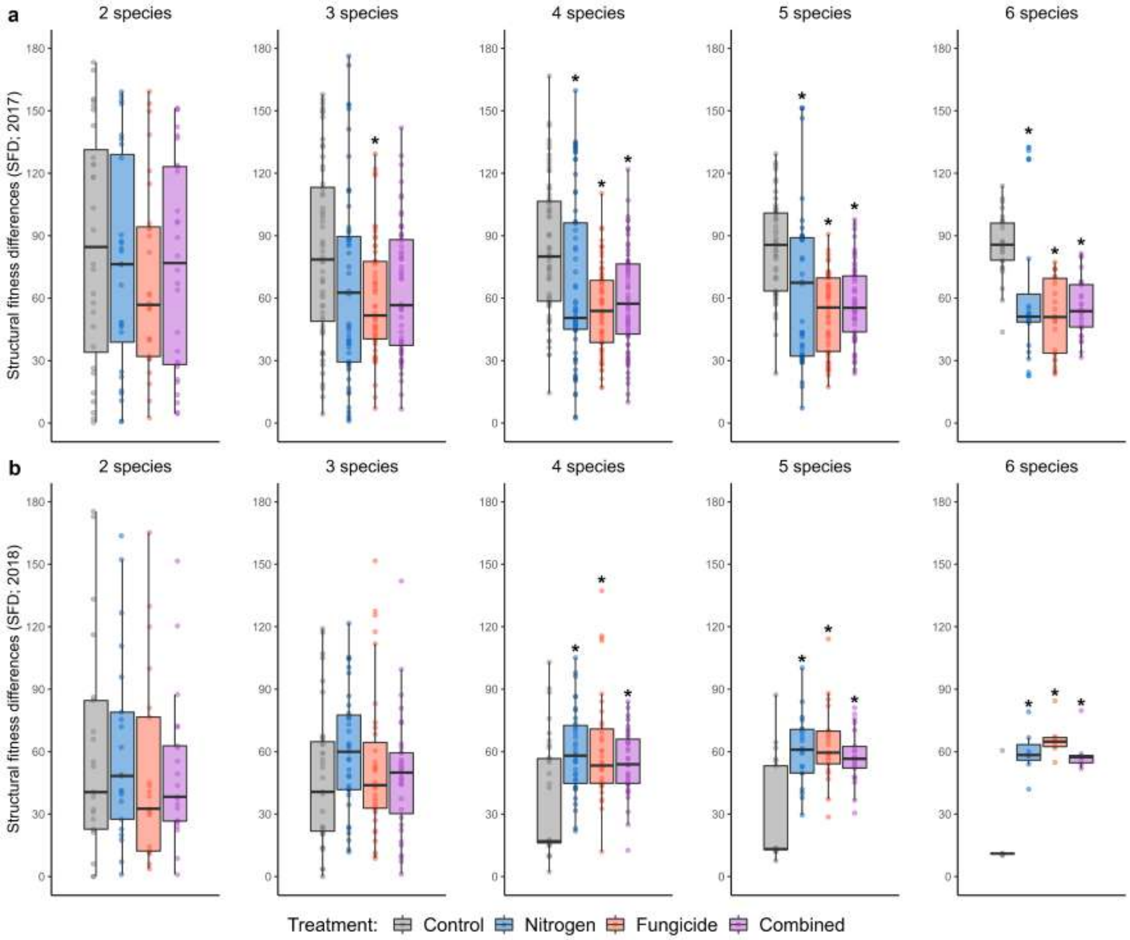


Figure S1.5: Structural fitness differences (SFD) for all combinations of two-six species, in the four experimental treatments (control, nitrogen, fungicide, and combined), for the years (**a**) 2017 and (**b**) 2018. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.11—S1.12). Note that the axis scales differ between panels because results can be compared across treatments within each species combination but not between species combinations (see main text).

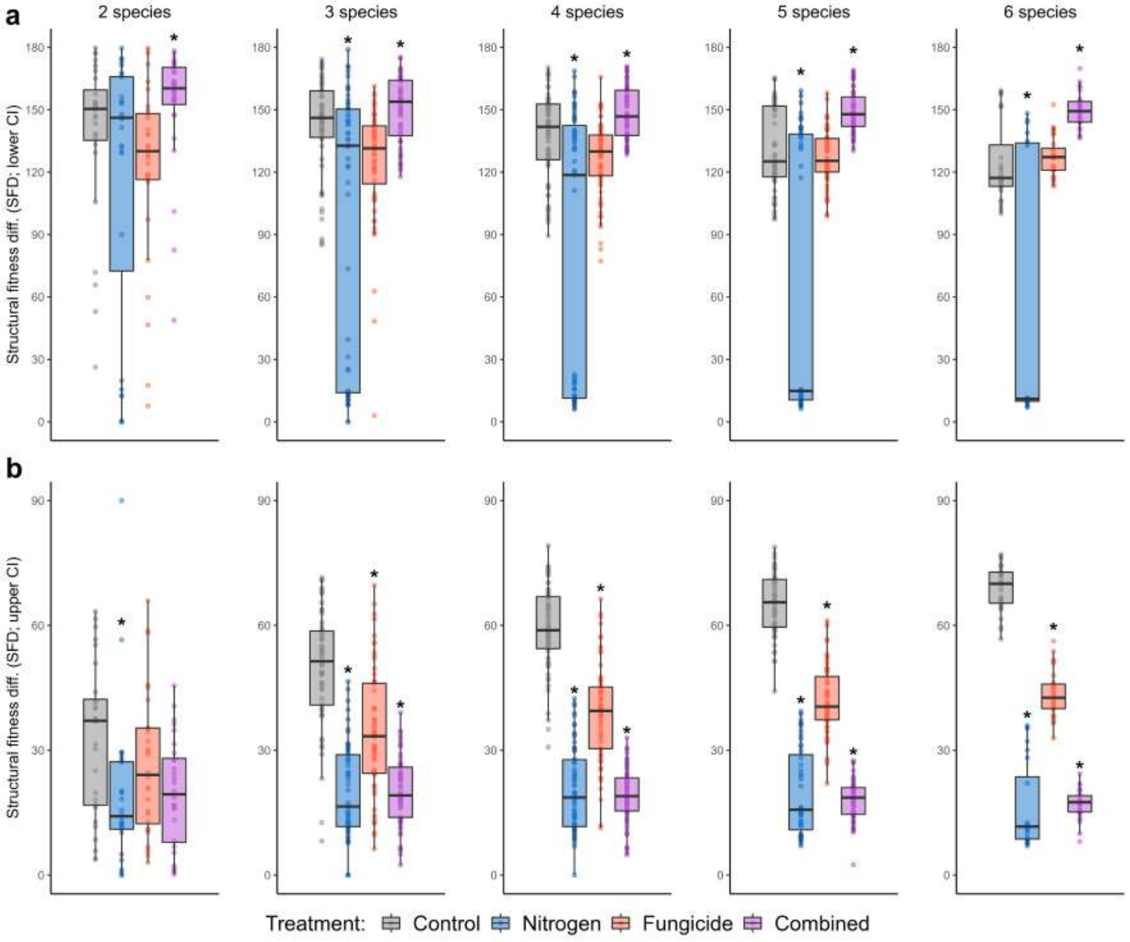


Figure S1.6: Structural fitness differences (SFD) for all combinations of two to six species, in the four experimental treatments (control, nitrogen, fungicide, and combined), calculated using the coefficients estimated for the (**a**) lower and (**b**) upper limits of the 95% confidence interval. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.14 and Table S1.16). Note that the axis scales differ between panels because results can be compared across treatments within each species combination but not between species combinations (see main text).

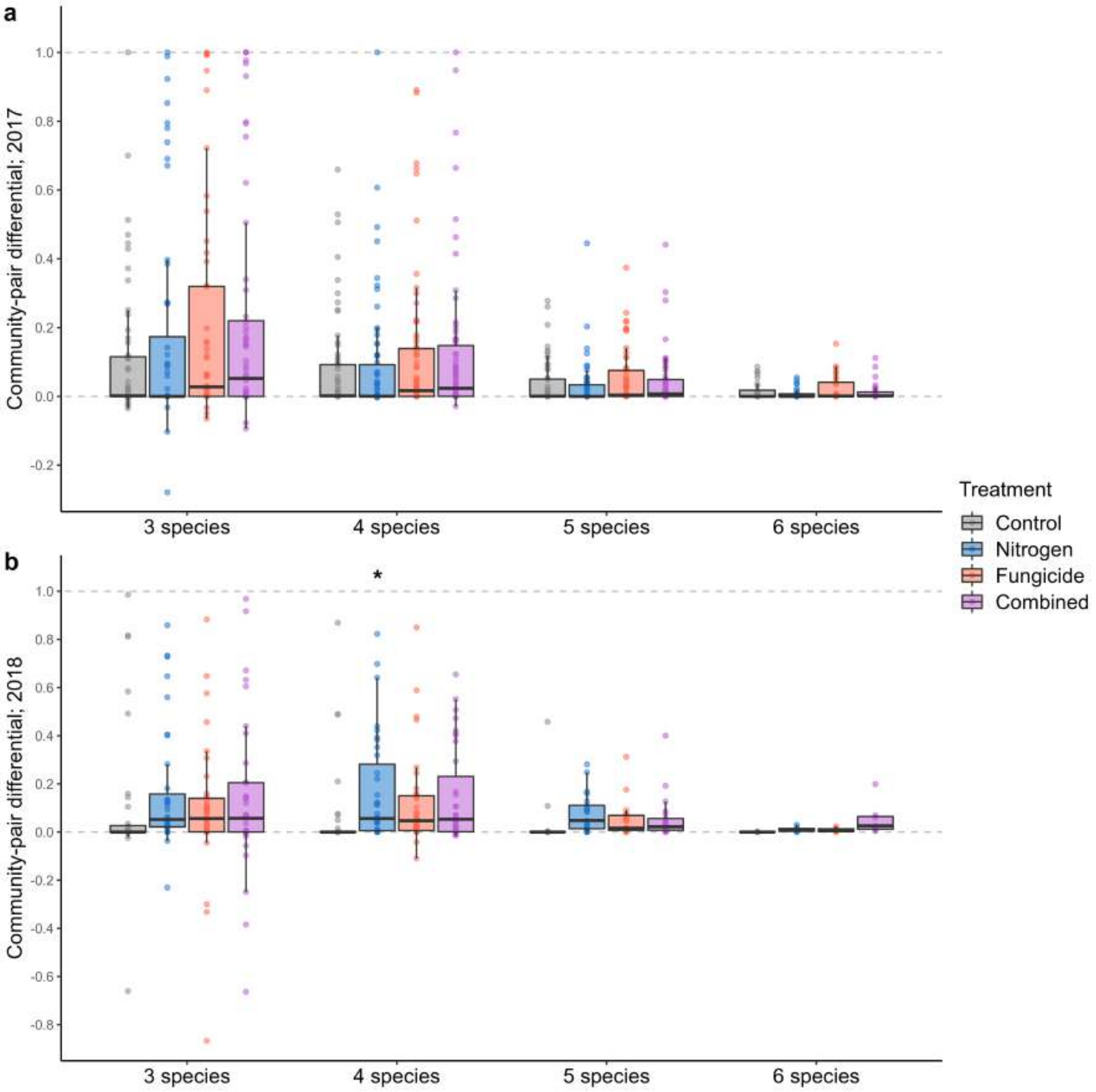


Figure S1.7: Community-pair differential for combinations from three to six species simultaneously, in the four experimental treatments (control, nitrogen, fungicide, and combined), for the years (**a**) 2017 and (**b**) 2018. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.18—S1.19.

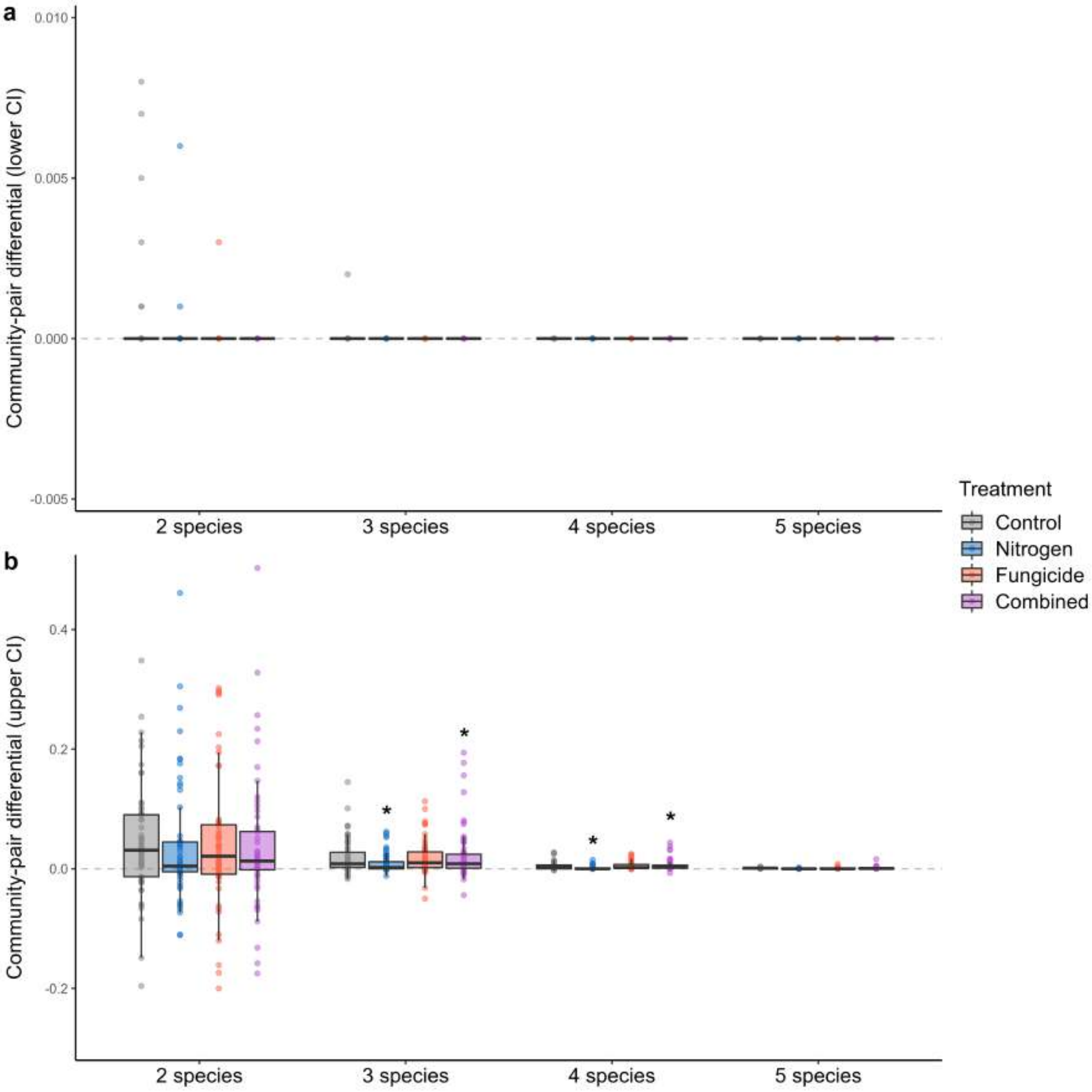


Figure S1.8: Community-pair differential for combinations from three to six species simultaneously, in the four experimental treatments (control, nitrogen, fungicide, and combined), calculated using the coefficients estimated for the (**a**) lower and (**b**) upper limits of the 95% confidence interval. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.20—S1.21.

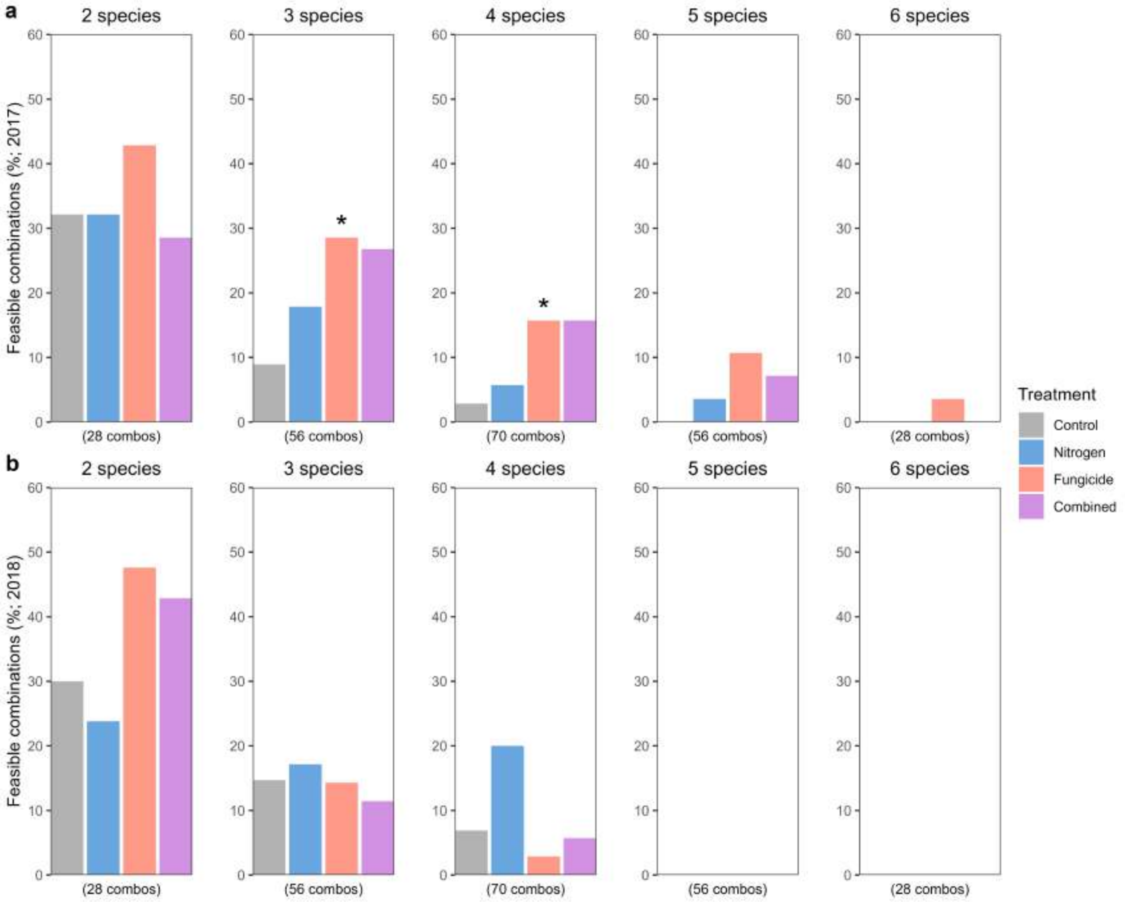


Figure S1.9: Feasible combinations (% of the total) of two to six species, in the four experimental treatments (control, nitrogen, fungicide, and combined), for the years (**a**) 2017 and (**b**) 2018. A feasible combination is one in which all species are predicted to coexist. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.23—S1.24). The total number of possible combinations (combos) of two-six species is shown at the bottom.

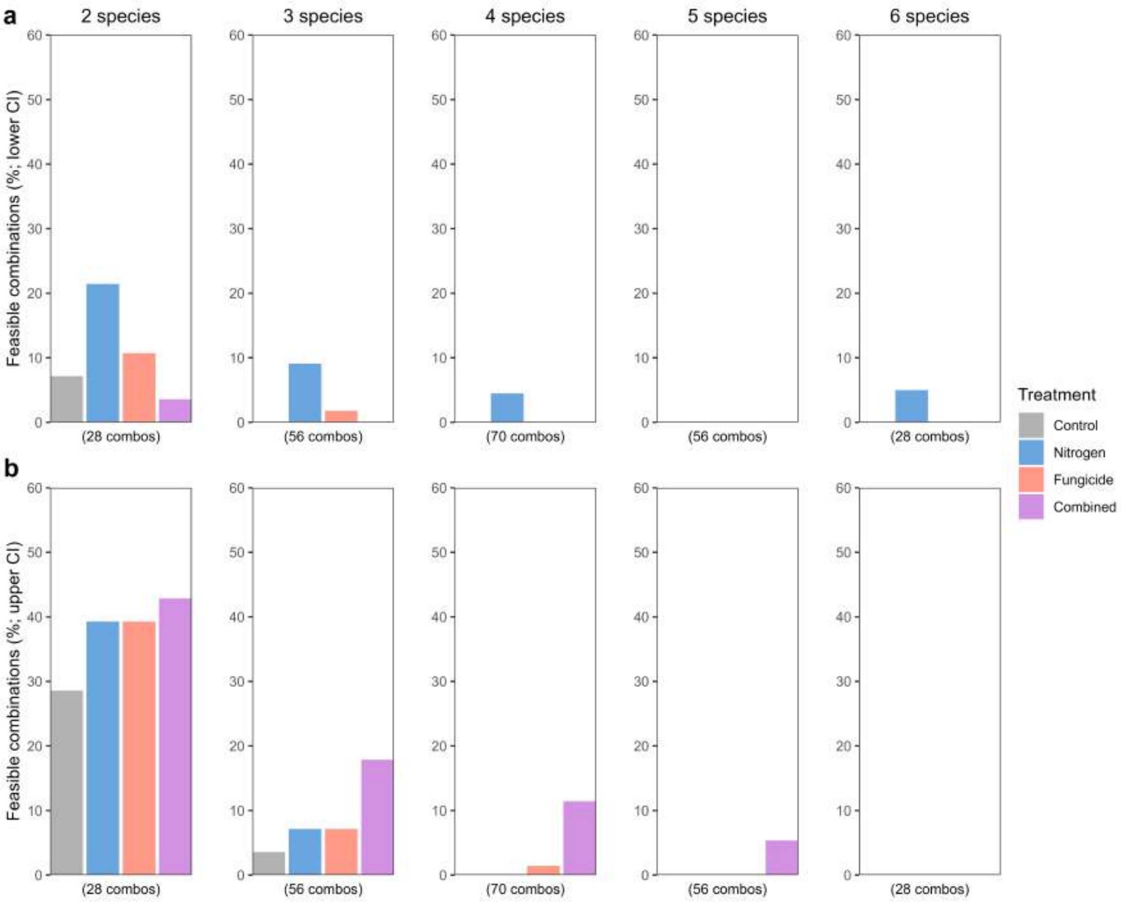


Figure S1.10: Feasible combinations (% of the total) of two to six species, in the four experimental treatments (control, nitrogen, fungicide, and combined), calculated using the coefficients estimated for the (**a**) lower and (**b**) upper limits of the 95% confidence interval. A feasible combination is one in which all species are predicted to coexist. Asterisks above boxes indicate significant differences ($p < 0.05$) between the corresponding treatment and the control (Table S1.25—S1.26). The total number of possible combinations (combos) of two-six species is shown at the bottom.

Supplementary Tables

Table S1.1: Set of 20 common Central Europe perennial grassland species that were used to assemble the experimental plant communities at the PaNDiv experiment (Bern, Switzerland). The species used for the current piece of research are highlighted in bold.

Species	Family
Poa trivialis	Poaceae
Lolium perenne	Poaceae
Holcus lanatus	Poaceae
Helictotrichon pubescens	Poaceae
Festuca rubra	Poaceae
Dactylis glomerata	**Poaceae**
Bromus erectus	Poaceae
Anthoxanthum odoratum	**Poaceae**
Centaurea jacea	**Asteraceae**
Crepis biennis	**Asteraceae**
Taraxacum officinale	**Asteraceae**
Achillea millefolium	Asteraceae
Anthriscus sylvestris	Apiaceae
Daucus carota	Apiaceae
Heracleum sphondylium	Apiaceae
Salvia pratensis	**Lamiaceae**
Prunella grandiflora	Lamiaceae
Plantago media	**Plantaginaceae**
Galium album	Rubiaceae
Rumex acetosa	**Plygonaceae**

Table S1.2: Selected variants of the population model expressed in eqn. 1.1 (see Chapter 1), based on their AIC values. Remember that model variant 1 does not account for the effect of plant initial biomass on other parameters, model variant 2 includes a common effect of initial biomass on other parameters, and model variant 3 account for the species-specific effects of initial plant biomass.

	model1	model2	model3	selected
Control treatment				
Anthoxanthum odoratum	122.08	112.28	123.64	model2
Centaurea jacea	133.31	123.49	118.42	model3
Crepis biennis	208.95	206.80	214.91	model2
Dactylis glomerata	289.62	277.63	287.66	model2
Plantago media	142.67	136.41	147.66	model2
Rumex acetosa	144.62	145.54	157.38	model1
Salvia pratensis	201.07	169.94	170.49	model2
Taraxacum officinale	195.14	181.29	183.00	model2
Nitrogen treatment				
Anthoxanthum odoratum	248.88	253.61	258.35	model1
Centaurea jacea	191.83	159.32	167.85	model2
Crepis biennis	143.93	149.00	151.21	model1
Dactylis glomerata	280.22	274.62	285.78	model2
Plantago media	100.64	93.77	104.96	model2
Rumex acetosa	83.95	86.93	98.77	model1
Salvia pratensis	235.82	218.60	228.88	model2
Taraxacum officinale	210.07	211.28	221.04	model1
Fungicide treatment				
Anthoxanthum odoratum	250.24	245.47	254.51	model2
Centaurea jacea	161.17	140.75	152.51	model2
Crepis biennis	171.91	172.74	176.88	model1
Dactylis glomerata	242.39	228.00	238.75	model2
Plantago media	112.83	102.74	110.51	model2
Rumex acetosa	119.69	121.95	129.00	model1
Salvia pratensis	310.84	288.15	303.04	model2
Taraxacum officinale	206.47	199.61	204.97	model2
Combined treatment				
Anthoxanthum odoratum	236.81	231.45	248.95	model2
Centaurea jacea	320.15	283.75	315.12	model2
Crepis biennis	176.74	160.86	169.02	model2
Dactylis glomerata	295.00	279.88	292.53	model2
Plantago media	207.95	203.91	211.86	model2
Rumex acetosa	98.26	101.11	111.35	model1
Salvia pratensis	188.75	173.87	185.65	model2
Taraxacum officinale	299.63	298.45	309.14	model2

Table S1.3: Estimated pairwise α interaction matrix and instrinsic growth rate coefficients (λ) in the control treatment ± 95% standard error. Rows show focal species and columns display neighbouring plants. Species codes are as follows: **Ao**, *Anthoxanthum odoratum*; **Cj**, *Centaurea jacea*; **Cb**, *Crepis biennis*; **Dg**, *Dactylis glomerata*; **Pm**, *Plantago media*; **Ra**, *Rumex acetosa*; **Sp**, *Salvia pratensis*; **To**, *Taraxacum officinale*.

	Ao	**Cj**	**Cb**	**Dg**	**Pm**	**Ra**	**Sp**	**To**	λ
Ao	-2.2e-07 ± 2.8e-05	1.8e-05 ± 2.2e-05	7.6e-06 ± 1.1e-03	1.7e-06 ± 3.4e-05	4.6e-06 ± 1.2e-04	3.0e-05 ± 4.9e-05	8.7e-08 ± 4.9e-05	1.6e-02 ± 1.4e-05	0.377 ± 0.403
Cj	2.3e-06 ± 2.3e-05	1.9e-06 ± 2.3e-05	-7.7e-08 ± 9.7e-04	9.0e-07 ± 4.2e-05	-1.5e-06 ± 3.5e-05	2.2e-05 ± 2.9e-05	-1.3e-06 ± 8.6e-06	1 ± 8.3e-06	3.221 ± 0.284
Cb	3.7e-06 ± 2.5e-05	1.6e-06 ± 1.6e-05	-5.8e-07 ± 1.4e-03	2.3e-06 ± 1.2e-05	1.8e-06 ± 6.4e-06	-4.5e-07 ± 1.2e-05	-9.4e-08 ± 2.9e-06	4.0e-03 ± 1.4e-05	2.365 ± 2.4e+01
Dg	7.6e-06 ± 5.7e-06	3.3e-06 ± 4.3e-05	-2.6e-06 ± 1.2e-03	-2.5e-06 ± 1.4e-05	1.2e-05 ± 3.7e-05	1.5e-06 ± 9.9e-06	9.4e-06 ± 2.2e-05	4.2e-02 ± 3.2e-05	0.775 ± 0.529
Pm	7.0e-06 ± 2.9e-05	3.7e-07 ± 1.3e-05	1.5e-06 ± 1.1e-03	1.4e-05 ± 9.2e-06	3.7e-06 ± 8.9e-06	1.9e-06 ± 3.0e-05	4.8e-06 ± 7.5e-06	4.9e-02 ± 7.9e-06	5.523 ± 2.246
Ra	1.7e-05 ± 1.4e-04	1.2e-05 ± 7.2e-06	5.9e-08 ± 4.2e-06	-7.8e-06 ± 1.1e-04	3.6e-06 ± 9.1e-06	-1.5e-06 ± 4.7e-06	-6.4e-07 ± 7.6e-07	1.2e-06 ± 1.2e-05	0.817 ± 0.949
Sp	-1.2e-06 ± 8.3e-06	-2.5e-06 ± 3.9e-06	1.6e-06 ± 1.1e-03	-1.0e-06 ± 1.7e-05	1.1e-06 ± 8.1e-06	-2.2e-06 ± 1.4e-05	2.0e-06 ± 2.0e-05	-4.8e-03 ± 1.4e-05	4.159 ± 0.762
To	1.1e-07 ± 9.8e-06	-9.4e-07 ± 4.4e-05	-1.6e-06 ± 1.0e-03	-7.5e-07 ± 1.6e-05	1.8e-05 ± 5.0e-05	1.1e-06 ± 9.5e-06	2.7e-06 ± 8.4e-06	1.1e-02 ± 4.5e-05	1.6e+04 ± 0.522

Table S1.4: Estimated pairwise α interaction matrix and instrinsic growth rate coefficients (λ) in the nitrogen treatment ± 95% standard error. Rows show focal species and columns display neighbouring plants. Species codes are as follows: **Ao**, *Anthoxanthum odoratum*; **Cj**, *Centaurea jacea*; **Cb**, *Crepis biennis*; **Dg**, *Dactylis glomerata*; **Pm**, *Plantago media*; **Ra**, *Rumex acetosa*; **Sp**, *Salvia pratensis*; **To**, *Taraxacum officinale*.

	Ao	**Cj**	**Cb**	**Dg**	**Pm**	**Ra**	**Sp**	**To**	λ
Ao	-1.5e-06	6.4e-06	2.7e-06	3.6e-06	-5.7e-07	-3.3e-06	8.1e-07	6.4e-06	0.33
	± 2.3e-06	± 2.9e-06	± 1.9e-06	± 1.4e-04	± 2.9e-06	± 3.2e-06	± 3.3e-06	± 1.5e-06	± 0.197
Cj	8.8e-06	-2.0e-06	9.2e-02	-8.2e-07	2.8e-06	-1.6e-06	2.1e-06	1.5e-06	5.704
	± 2.9e-06	± 3.5e-05	± 5.2e-06	± 1.5e-05	± 5.1e-06	± 8.6e-05	± 3.5e-04	± 1.3e-05	± 0.588
Cb	0.13	4.2e-02	0.111	1	4.2e-02	-9.2e-02	2.3e-02	0.101	3.3e+05
	± 3.3e-06	± 3.2e-06	± 1.4e-06	± 2.0e-04	± 2.8e-06	± 2.4e-06	± 1.7e-06	± 3.5e-06	± 1.162
Dg	3.9e-06	8.9e-08	5.1e-02	-1.0e-06	-5.7e-07	5.1e-06	-1.9e-08	-2.3e-07	0.455
	± 1.1e-05	± 3.4e-05	± 9.5e-06	± 4.6e-05	± 2.9e-06	± 4.3e-05	± 5.4e-04	± 1.0e-05	± 3.158
Pm	8.5e-07	4.8e-07	6.7e-02	1.2e-06	2.4e-07	2.4e-06	-9.9e-07	1.7e-07	1.495
	± 1.0e-05	± 1.2e-05	± 2.8e-06	± 4.5e-05	± 1.3e-06	± 7.3e-05	± 3.1e-04	± 6.9e-06	± 0.391
Ra	2.2e-06	-8.7e-06	-9.3e-07	4.3e-06	6.4e-06	3.3e-06	-6.0e-06	2.5e-06	0.496
	± 1.2e-05	± 5.0e-05	± 1.7e-06	± 1.5e-04	± 1.0e-05	± 4.5e-06	± 4.6e-06	± 3.7e-06	± 0.392
Sp	1.3e-06	-2.2e-06	5.5e-02	-8.9e-07	-7.8e-07	5.1e-06	8.3e-08	1.1e-05	2.606
	± 3.4e-06	± 1.0e-05	± 1.1e-05	± 4.0e-05	± 3.3e-06	± 3.1e-06	± 5.0e-04	± 2.6e-06	± 2.276
To	7.8e-06	1.4e-06	5.6e-06	-1.7e-05	9.4e-09	-4.1e-06	5.5e-06	-3.9e-08	1.608
	± 1.3e-06	± 5.8e-07	± 8.0e-07	± 3.7e-05	± 6.7e-07	± 4.8e-06	± 2.5e-07	± 1.5e-06	± 0.333

Table S1.5: Estimated pairwise α interaction matrix and instrinsic growth rate coefficients (λ) in the fungicide treatment ± 95% standard error. Rows show focal species and columns display neighbouring plants. Species codes are as follows: **Ao**, *Anthoxanthum odoratum*; **Cj**, *Centaurea jacea*; **Cb**, *Crepis biennis*; **Dg**, *Dactylis glomerata*; **Pm**, *Plantago media*; **Ra**, *Rumex acetosa*; **Sp**, *Salvia pratensis*; **To**, *Taraxacum officinale*.

	Ao	**Cj**	**Cb**	**Dg**	**Pm**	**Ra**	**Sp**	**To**	λ
Ao	-1.2e-02 ± 7.4e-02	-1.7e-02 ± 0.144	0.182 ± 8.4e-02	-2.6e-02 ± 6.6e-02	1.5e-02 ± 0.299	-5.0e-02 ± 0.546	6.8e-02 ± 0.831	-6.4e-02 ± 0.533	± 0.414 ± 0.413
Cj	3.2e-02 ± 2.5e-02	-1.8e-03 ± 0.269	-4.9e-04 ± 0.106	-7.8e-03 ± 1.0e-02	-4.4e-03 ± 4.3e-02	-4.7e-02 ± 1.271	2.9e-03 ± 0.233	2.5e-02 ± 8.5e-02	± 1.667 ± 6.0e-02
Cb	0.194 ± 1.2e-02	-6.5e-04 ± 4.9e-03	1.1e-02 ± 3.3e-03	-2.1e-01 ± 0.342	2.0e-02 ± 3.6e-03	5.8e-02 ± 8.4e-03	0.625 ± 4.8e-03	4.2e-02 ± 3.1e-03	± 4.128 ± 0.675
Dg	0.288 ± 6.8e-02	-1.1e-02 ± 1.605	2.2e-02 ± 0.178	-4.9e-03 ± 0.176	2.7e-04 ± 0.394	3.8e-02 ± 1.335	2.0e-02 ± 5.1e-02	-8.2e-02 ± 0.146	± 0.815 ± 5.2e-02
Pm	5.7e-02 ± 0.387	0.15 ± 0.605	2.3e-02 ± 5.4e-02	0.276 ± 0.212	7.3e-03 ± 3.4e-02	9.3e-02 ± 1.311	-1.3e-02 ± 1.152	-6.1e-04 ± 9.6e-02	± 1.883 ± 0.318
Ra	-9.5e-03 ± 5.2e-02	-2.0e-02 ± 5.0e-02	-5.4e-03 ± 4.4e-02	-1.1e-01 ± 1.372	0.147 ± 4.8e-02	-6.7e-03 ± 4.1e-02	-2.8e-02 ± 0.236	2.7e-02 ± 9.3e-02	± 0.664 ± 0.456
Sp	3.0e-02 ± 0.101	-3.5e-03 ± 0.349	1 ± 0.201	-2.8e-03 ± 7.2e-02	1.8e-03 ± 4.3e-02	-8.8e-02 ± 1.877	-2.8e-03 ± 2.017	6.0e-02 ± 4.5e-02	± 1.833 ± 1.569
To	-1.7e-02 ± 0.196	3.5e-03 ± 0.255	3.8e-02 ± 5.4e-02	4.1e-02 ± 0.263	-4.8e-03 ± 3.6e-02	0.141 ± 1.518	-9.9e-03 ± 0.34	0.131 ± 5.4e-02	± 4.556 ± 0.982

Table S1.6: Estimated pairwise α interaction matrix and instrinsic growth rate coefficients (λ) in the combined treatment ± 95% standard error. Rows show focal species and columns display neighbouring plants. Species codes are as follows: **Ao**, *Anthoxanthum odoratum*; **Cj**, *Centaurea jacea*; **Cb**, *Crepis biennis*; **Dg**, *Dactylis glomerata*; **Pm**, *Plantago media*; **Ra**, *Rumex acetosa*; **Sp**, *Salvia pratensis*; **To**, *Taraxacum officinale*.

	Ao	**Cj**	**Cb**	**Dg**	**Pm**	**Ra**	**Sp**	**To**	λ
Ao	-4.6e-04 ± 5.8e-03	-5.5e-04 ± 9.5e-04	-1.5e-04 ± 1.8e-03	4.6e-04 ± 3.1e-02	1.3e-03 ± 3.8e-03	0.376 ± 0.573	-8.4e-04 ± 6.8e-03	-9.7e-04 ± 3.8e-03	0.281 ± 0.387
Cj	8.9e-04 ± 1.6e-02	-4.2e-04 ± 6.6e-03	6.4e-04 ± 1.2e-02	1.6e-02 ± 5.0e-03	1.4e-03 ± 4.3e-03	0.327 ± 0.268	2.1e-04 ± 9.5e-04	4.7e-04 ± 2.1e-03	1.655 ± 0.439
Cb	4.7e-04 ± 6.3e-04	2.1e-04 ± 4.0e-03	-8.9e-05 ± 3.7e-03	3.7e-04 ± 2.4e-02	-3.7e-05 ± 2.4e-03	1 ± 0.53	-4.7e-05 ± 6.9e-04	1.2e-03 ± 2.9e-03	0.979 ± 0.838
Dg	3.2e-04 ± 2.4e-03	5.5e-03 ± 2.5e-03	-4.5e-04 ± 8.7e-03	-5.0e-04 ± 9.8e-02	-5.7e-04 ± 8.8e-03	0.414 ± 0.545	1.4e-04 ± 1.0e-03	8.3e-04 ± 1.8e-02	1.064 ± 1.293
Pm	4.0e-04 ± 7.8e-03	-8.0e-04 ± 2.9e-03	2.4e-04 ± 2.5e-02	4.5e-04 ± 2.8e-02	-6.3e-06 ± 3.8e-03	0.399 ± 0.474	-3.4e-04 ± 4.7e-03	1.4e-02 ± 3.6e-03	1.886 ± 0.576
Ra	5.7e-04 ± 6.7e-03	9.5e-05 ± 2.4e-03	4.6e-03 ± 2.6e-03	-3.1e-04 ± 0.207	-2.1e-04 ± 1.4e-02	-1.1e-03 ± 3.5e-03	-5.0e-04 ± 4.2e-03	-6.7e-04 ± 1.8e-03	0.265 ± 1.115
Sp	2.1e-03 ± 2.1e-03	-7.9e-05 ± 1.5e-02	1.8e-03 ± 1.5e-02	7.6e-05 ± 2.8e-03	-2.6e-04 ± 3.3e-03	0.664 ± 0.604	-2.5e-04 ± 3.1e-03	-8.5e-04 ± 3.1e-03	1.51 ± 0.169
To	-6.4e-04 ± 5.7e-03	-5.9e-04 ± 2.0e-03	-4.6e-04 ± 2.7e-03	2.2e-04 ± 6.8e-03	2.5e-03 ± 3.4e-03	-9.5e-01 ± 0.47	9.6e-03 ± 1.9e-04	2.2e-04 ± 2.6e-02	0.663 ± 0.687

Table S1.7: GLM model output for structural niche differences (SND).

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	1.071	0.115	9.339	0.000	*
Nitrogen	-0.216	0.147	-1.471	0.144	
Fungicide	-0.212	0.147	-1.445	0.151	
Nitrogen × Fungicide	0.577	0.217	2.661	0.009	*
3 species					
Intercept	0.601	0.089	6.740	0.000	*
Nitrogen	0.566	0.217	2.612	0.010	*
Fungicide	0.233	0.153	1.530	0.128	
Nitrogen × Fungicide	0.547	0.192	2.845	0.005	*
4 species					
Intercept	0.290	0.051	5.643	0.000	*
Nitrogen	1.356	0.372	3.651	0.000	*
Fungicide	0.441	0.139	3.167	0.002	*
Nitrogen × Fungicide	-1.051	0.434	-2.419	0.016	*
5 species					
Intercept	0.149	0.058	2.556	0.011	*
Nitrogen	1.087	0.726	1.497	0.136	
Fungicide	0.373	0.212	1.758	0.080	
Nitrogen × Fungicide	-0.822	0.814	-1.009	0.314	
6 species					
Intercept	0.017	0.005	3.530	0.001	*
Nitrogen	0.431	0.224	1.925	0.057	
Fungicide	0.131	0.042	3.103	0.003	*
Nitrogen × Fungicide	-0.240	0.247	-0.969	0.335	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.8: GLM model output for structural fitness differences (SFD).

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	55.883	8.199	6.816	0.000	*
Nitrogen	4.780	12.101	0.395	0.694	
Fungicide	12.413	12.947	0.959	0.340	
Nitrogen × Fungicide	14.654	20.310	0.721	0.472	
3 species					
Intercept	59.742	4.950	12.069	0.000	*
Nitrogen	-11.945	6.385	-1.871	0.063	
Fungicide	-2.585	6.851	-0.377	0.706	
Nitrogen × Fungicide	22.673	9.739	2.328	0.021	*
4 species					
Intercept	65.359	3.809	17.160	0.000	*
Nitrogen	-21.011	4.796	-4.381	0.000	*
Fungicide	-15.561	4.788	-3.250	0.001	*
Nitrogen × Fungicide	27.391	6.492	4.219	0.000	*
5 species					
Intercept	69.367	3.649	19.011	0.000	*
Nitrogen	-30.378	4.601	-6.603	0.000	*
Fungicide	-24.560	4.344	-5.654	0.000	*
Nitrogen × Fungicide	30.587	5.686	5.379	0.000	*
6 species					
Intercept	73.206	4.396	16.653	0.000	*
Nitrogen	-38.133	5.634	-6.768	0.000	*
Fungicide	-32.121	5.041	-6.372	0.000	*
Nitrogen × Fungicide	32.891	6.516	5.047	0.000	*

Formula: SFD ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.9: GLM model output for structural niche differences (SND) in the year 2017 alone.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	1.207	0.125	9.645	0.000	*
Nitrogen	-0.486	0.146	-3.333	0.001	*
Fungicide	-0.057	0.173	-0.331	0.741	
Nitrogen × Fungicide	0.379	0.217	1.744	0.084	
3 species					
Intercept	1.144	0.170	6.743	0.000	*
Nitrogen	-0.361	0.206	-1.758	0.080	
Fungicide	0.051	0.245	0.208	0.836	
Nitrogen × Fungicide	0.011	0.299	0.038	0.970	
4 species					
Intercept	0.887	0.186	4.780	0.000	*
Nitrogen	-0.277	0.225	-1.228	0.221	
Fungicide	0.147	0.285	0.516	0.606	
Nitrogen × Fungicide	0.043	0.355	0.121	0.904	
5 species					
Intercept	0.838	0.224	3.740	0.000	*
Nitrogen	-0.446	0.247	-1.804	0.073	
Fungicide	-0.105	0.298	-0.353	0.725	
Nitrogen × Fungicide	0.059	0.329	0.180	0.857	
6 species					
Intercept	0.474	0.228	2.084	0.040	*
Nitrogen	-0.247	0.252	-0.977	0.331	
Fungicide	-0.071	0.299	-0.239	0.811	
Nitrogen × Fungicide	-0.063	0.321	-0.197	0.844	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.10: GLM model output for structural niche differences (SND) in the year 2018 alone.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	0.709	0.124	5.718	0.000	*
Nitrogen	0.348	0.223	1.562	0.122	
Fungicide	-0.102	0.163	-0.622	0.536	
Nitrogen × Fungicide	0.064	0.304	0.211	0.833	
3 species					
Intercept	0.530	0.118	4.505	0.000	*
Nitrogen	0.269	0.213	1.264	0.208	
Fungicide	0.066	0.177	0.371	0.711	
Nitrogen × Fungicide	0.129	0.334	0.386	0.700	
4 species					
Intercept	0.245	0.079	3.089	0.002	*
Nitrogen	0.641	0.297	2.156	0.033	*
Fungicide 0.020	0.117	0.171	0.864		
Nitrogen × Fungicide	0.107	0.451	0.238	0.812	
5 species					
Intercept	0.046	0.025	1.869	0.065	
Nitrogen	0.609	0.352	1.733	0.087	
Fungicide	0.095	0.079	1.192	0.237	
Nitrogen × Fungicide	0.432	0.728	0.594	0.554	
6 species					
Intercept	0.001	0.000	2.155	0.041	*
Nitrogen	0.572	0.266	2.151	0.042	*
Fungicide	0.028	0.013	2.085	0.048	*
Nitrogen × Fungicide	0.589	0.614	0.961	0.346	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.11: GLM model output for structural fitness differences (SFD) in the year 2017 alone.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	95.734	11.324	8.454	0.000	*
Nitrogen	-20.359	14.412	-1.413	0.161	
Fungicide	-28.981	13.805	-2.099	0.038	*
Nitrogen × Fungicide	46.853	19.792	2.367	0.020	*
3 species					
Intercept	82.312	5.668	14.522	0.000	*
Nitrogen	-0.887	7.973	-0.111	0.911	
Fungicide	-18.993	7.151	-2.656	0.008	*
Nitrogen × Fungicide	26.919	10.974	2.453	0.015	*
4 species					
Intercept	71.767	3.708	19.355	0.000	*
Nitrogen	10.566	5.643	1.872	0.062	
Fungicide	-13.109	4.789	-2.737	0.007	*
Nitrogen × Fungicide	16.074	7.775	2.067	0.040	*
5 species					
Intercept	59.579	2.949	20.204	0.000	*
Nitrogen	21.624	4.985	4.338	0.000	*
Fungicide	-4.315	4.022	-1.073	0.285	
Nitrogen × Fungicide	5.374	6.994	0.768	0.443	
6 species					
Intercept	48.233	2.778	17.363	0.000	*
Nitrogen	32.651	5.424	6.020	0.000	*
Fungicide	4.313	4.108	1.050	0.296	
Nitrogen × Fungicide	-3.562	7.790	-0.457	0.648	

Formula: SFD ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.12: GLM model output for structural fitness differences (SFD) in the year 2018 alone.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	60.192	9.240	6.515	0.000	*
Nitrogen	33.611	17.109	1.965	0.053	
Fungicide	2.184	13.306	0.164	0.870	
Nitrogen × Fungicide	7.271	25.211	0.288	0.774	
3 species					
Intercept	46.224	4.024	11.488	0.000	*
Nitrogen	52.153	9.462	5.512	0.000	*
Fungicide	17.823	6.876	2.592	0.011	*
Nitrogen × Fungicide	-36.885	12.972	-2.843	0.005	*
4 species					
Intercept	41.373	2.815	14.698	0.000	*
Nitrogen	52.548	6.983	7.525	0.000	*
Fungicide	18.274	4.939	3.700	0.000	*
Nitrogen × Fungicide	-41.413	9.403	-4.404	0.000	*
5 species					
Intercept	38.603	2.702	14.289	0.000	*
Nitrogen	51.119	6.836	7.478	0.000	*
Fungicide	21.147	4.978	4.248	0.000	*
Nitrogen × Fungicide	-45.168	9.239	-4.889	0.000	*
6 species					
Intercept	36.687	3.352	10.945	0.000	*
Nitrogen	50.102	8.608	5.820	0.000	*
Fungicide	26.850	6.703	4.006	0.001	*
Nitrogen × Fungicide	-51.339	11.841	-4.336	0.000	*

Formula: SFD ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.13: GLM model output for structural niche differences (SND) in the lower 95% confidence interval for species interaction coefficients.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	0.609	0.100	6.070	0.000	*
Nitrogen	0.003	0.142	0.023	0.982	
Fungicide	0.098	0.154	0.640	0.523	
Nitrogen × Fungicide	-0.111	0.209	-0.531	0.597	
3 species					
Intercept	0.269	0.053	5.082	0.000	*
Nitrogen	0.289	0.130	2.227	0.027	*
Fungicide	0.152	0.098	1.549	0.123	
Nitrogen × Fungicide	-0.479	0.161	-2.977	0.003	*
4 species					
Intercept	0.107	0.023	4.619	0.000	*
Nitrogen	0.604	0.188	3.222	0.001	*
Fungicide	0.191	0.069	2.784	0.006	*
Nitrogen × Fungicide	-0.819	0.199	-4.111	0.000	*
5 species					
Intercept	0.040	0.010	4.213	0.000	*
Nitrogen	0.491	0.160	3.072	0.002	*
Fungicide	0.059	0.025	2.314	0.022	*
Nitrogen × Fungicide	-0.569	0.162	-3.521	0.001	*
6 species					
Intercept	0.009	0.003	3.210	0.002	*
Nitrogen	0.258	0.110	2.343	0.021	*
Fungicide	0.024	0.011	2.256	0.026	*
Nitrogen × Fungicide	-0.280	0.110	-2.532	0.013	*

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.14: GLM model output for structural fitness differences (SND) in the lower 95% confidence interval for species interaction coefficients.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	138.170	9.868	14.002	0.000	*
Nitrogen	-22.257	12.881	-1.728	0.087	
Fungicide	-16.693	13.140	-1.270	0.207	
Nitrogen × Fungicide	53.069	18.960	2.799	0.006	*
3 species					
Intercept	141.603	6.976	20.300	0.000	*
Nitrogen	-45.190	8.463	-5.340	0.000	*
Fungicide	-16.898	9.295	-1.818	0.070	
Nitrogen × Fungicide	70.222	12.798	5.487	0.000	*
4 species					
Intercept	136.817	6.843	19.993	0.000	*
Nitrogen	-56.553	7.979	-7.088	0.000	*
Fungicide	-10.269	9.322	-1.102	0.272	
Nitrogen × Fungicide	78.755	12.613	6.244	0.000	*
5 species					
Intercept	131.034	8.127	16.123	0.000	*
Nitrogen	-63.383	9.306	-6.811	0.000	*
Fungicide	-3.734	11.331	-0.330	0.742	
Nitrogen × Fungicide	85.087	15.308	5.558	0.000	*
6 species					
Intercept	124.534	12.034	10.348	0.000	*
Nitrogen	-69.297	13.591	-5.099	0.000	*
Fungicide	2.762	17.208	0.161	0.873	
Nitrogen × Fungicide	91.687	23.350	3.927	0.000	*

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.15: GLM model output for structural niche differences (SND) in the upper 95% confidence interval for species interaction coefficients.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	0.373	0.066	5.619	0.000	*
Nitrogen	-0.020	0.091	-0.224	0.823	
Fungicide	-0.022	0.091	-0.245	0.807	
Nitrogen × Fungicide	0.085	0.133	0.637	0.526	
3 species					
Intercept	0.107	0.018	5.808	0.000	*
Nitrogen	-0.003	0.026	-0.115	0.908	
Fungicide	-0.015	0.024	-0.630	0.529	
Nitrogen × Fungicide	0.032	0.037	0.858	0.392	
4 species					
Intercept	0.025	0.004	6.118	0.000	*
Nitrogen	-0.006	0.005	-1.098	0.273	
Fungicide	-0.004	0.005	-0.682	0.496	
Nitrogen × Fungicide	0.015	0.008	1.818	0.070	
5 species					
Intercept	0.005	0.001	5.691	0.000	*
Nitrogen	-0.003	0.001	-2.511	0.013	*
Fungicide	0.000	0.001	-0.083	0.934	
Nitrogen × Fungicide	0.004	0.002	2.255	0.025	*
6 species					
Intercept	0.001	0.000	3.731	0.000	*
Nitrogen	-0.001	0.000	-2.508	0.014	*
Fungicide	0.000	0.000	0.826	0.411	
Nitrogen × Fungicide	0.001	0.001	2.000	0.048	*

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.16: GLM model output for structural fitness differences (SFD) in the upper 95% confidence interval for species interaction coefficients.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	32.444	4.476	7.248	0.000	*
Nitrogen	-13.401	5.191	-2.582	0.011	*
Fungicide	-5.829	5.790	-1.007	0.316	
Nitrogen × Fungicide	6.427	6.912	0.930	0.354	
3 species					
Intercept	49.714	2.925	16.994	0.000	*
Nitrogen	-29.663	3.154	-9.404	0.000	*
Fungicide	-15.329	3.557	-4.310	0.000	*
Nitrogen × Fungicide	15.474	3.931	3.936	0.000	*
4 species					
Intercept	59.295	2.431	24.387	0.000	*
Nitrogen	-38.867	2.578	-15.078	0.000	*
Fungicide	-20.423	2.907	-7.024	0.000	*
Nitrogen × Fungicide	19.079	3.130	6.095	0.000	*
5 species					
Intercept	64.981	2.644	24.573	0.000	*
Nitrogen	-45.311	2.785	-16.270	0.000	*
Fungicide	-23.251	3.143	-7.398	0.000	*
Nitrogen × Fungicide	21.600	3.343	6.460	0.000	*
6 species					
Intercept	68.792	4.278	16.080	0.000	*
Nitrogen	-52.617	4.414	-11.921	0.000	*
Fungicide	-25.315	5.061	-5.002	0.000	*
Nitrogen × Fungicide	26.114	5.283	4.943	0.000	*

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.17: GLM model output for community-pair differential.

	Estimate	SE	*t* value	*p*	
3 species					
Intercept	1.124	0.042	27.078	0.000	*
Nitrogen	-0.026	0.058	-0.456	0.649	
Fungicide	-0.029	0.058	-0.508	0.612	
Nitrogen × Fungicide	0.036	0.082	0.435	0.664	
4 species					
Intercept	1.080	0.023	46.779	0.000	*
Nitrogen	0.038	0.033	1.138	0.256	
Fungicide	0.007	0.033	0.213	0.832	
Nitrogen × Fungicide	-0.012	0.047	-0.249	0.804	
5 species					
Intercept	1.036	0.015	68.570	0.000	*
Nitrogen	0.008	0.021	0.370	0.712	
Fungicide	0.030	0.022	1.374	0.171	
Nitrogen × Fungicide	-0.026	0.031	-0.852	0.395	
6 species					
Intercept	1.005	0.009	111.826	0.000	*
Nitrogen	0.006	0.013	0.460	0.647	
Fungicide	0.022	0.013	1.696	0.093	
Nitrogen × Fungicide	0.006	0.018	0.343	0.733	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.18: GLM model output for community-pair differential in the year 2017 alone.

	Estimate	SE	*t* value	*p*	
3 species					
Intercept	1.120	0.037	29.975	0.000	*
Nitrogen	0.014	0.053	0.254	0.800	
Fungicide	0.033	0.054	0.624	0.533	
Nitrogen × Fungicide	-0.056	0.075	-0.739	0.460	
4 species					
Intercept	1.057	0.016	67.226	0.000	*
Nitrogen	-0.012	0.022	-0.521	0.603	
Fungicide	0.049	0.023	2.144	0.033	*
Nitrogen × Fungicide	-0.071	0.031	-2.253	0.025	*
5 species					
Intercept	1.041	0.010	105.160	0.000	*
Nitrogen	-0.029	0.014	-2.076	0.039	*
Fungicide	0.003	0.014	0.248	0.804	
Nitrogen × Fungicide	-0.011	0.020	-0.589	0.556	
6 species					
Intercept	1.022	0.007	141.520	0.000	*
Nitrogen	-0.021	0.010	-2.048	0.043	*
Fungicide	0.000	0.010	0.021	0.983	
Nitrogen × Fungicide	-0.001	0.014	-0.090	0.929	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.19: GLM model output for community-pair differential in the year 2018 alone.

	Estimate	SE	*t* value	*p*	
3 species					
Intercept	1.058	0.038	28.148	0.000	*
Nitrogen	0.026	0.054	0.475	0.636	
Fungicide	0.083	0.055	1.510	0.133	
Nitrogen × Fungicide	-0.057	0.078	-0.728	0.468	
4 species					
Intercept	1.014	0.019	54.233	0.000	*
Nitrogen	0.021	0.027	0.803	0.423	
Fungicide	0.019	0.027	0.702	0.484	
Nitrogen × Fungicide	0.017	0.038	0.455	0.650	
5 species					
Intercept	1.002	0.014	71.046	0.000	*
Nitrogen	0.001	0.020	0.053	0.958	
Fungicide	0.047	0.020	2.305	0.024	*
Nitrogen × Fungicide	-0.029	0.029	-1.018	0.312	
6 species					
Intercept	1.000	0.008	126.193	0.000	*
Nitrogen	0.000	0.011	0.000	1.000	
Fungicide	0.009	0.011	0.787	0.439	
Nitrogen × Fungicide	0.011	0.016	0.680	0.503	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.20: GLM model output for community-pair differential in the lower 95% confidence interval for species interaction coefficients.

	Estimate	SE	*t* value	*p*	
3 species					
Intercept	1.000	0.009	1.088400e+02	0.000	*
Nitrogen	0.015	0.013	1.124000e+00	0.262	
Fungicide	0.011	0.013	8.540000e-01	0.394	
Nitrogen × Fungicide	-0.026	0.019	-1.423000e+00	0.156	
4 species					
Intercept	1.000	0.000	7.000200e+04	0.000	*
Nitrogen	0.000	0.000	-1.414000e+00	0.158	
Fungicide	0.000	0.000	-1.414000e+00	0.158	
Nitrogen × Fungicide	0.000	0.000	1.000000e+00	0.318	
5 species					
Intercept	1.000	0.000	2.075349e+15	0.000	*
Nitrogen	0.000	0.000	1.350000e+00	0.178	
Fungicide	0.000	0.000	1.350000e+00	0.178	
Nitrogen × Fungicide	0.000	0.000	-9.540000e-01	0.341	
6 species					
Intercept	1.000	0.000	2.305807e+15	0.000	*
Nitrogen	0.000	0.000	-1.368000e+00	0.174	
Fungicide	0.000	0.000	-1.368000e+00	0.174	
Nitrogen × Fungicide	0.000	0.000	9.680000e-01	0.335	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.21: GLM model output for community-pair differential in the upper 95% confidence interval for species interaction coefficients.

	Estimate	SE	*t* value	*p*	
3 species					
Intercept	1.044	0.015	71.027	0.000	*
Nitrogen	-0.004	0.021	-0.213	0.832	
Fungicide	-0.008	0.021	-0.402	0.688	
Nitrogen × Fungicide	0.005	0.029	0.186	0.853	
4 species					
Intercept	1.019	0.004	288.561	0.000	*
Nitrogen	-0.010	0.005	-2.052	0.041	*
Fungicide	-0.002	0.005	-0.386	0.699	
Nitrogen × Fungicide	0.015	0.007	2.122	0.035	*
5 species					
Intercept	1.005	0.001	1045.214	0.000	*
Nitrogen	-0.004	0.001	-2.946	0.004	*
Fungicide	0.000	0.001	-0.171	0.865	
Nitrogen × Fungicide	0.005	0.002	2.731	0.007	*
6 species					
Intercept	1.001	0.000	2717.305	0.000	*
Nitrogen	-0.001	0.001	-1.509	0.134	
Fungicide	0.000	0.001	0.411	0.682	
Nitrogen × Fungicide	0.001	0.001	1.745	0.084	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.22: GLM model output for feasibility.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	0.288	0.382	0.753	0.451	
Nitrogen	-0.145	0.538	-0.269	0.788	
Fungicide	-1.035	0.556	-1.860	0.063	
Nitrogen × Fungicide	0.145	0.785	0.184	0.854	
3 species					
Intercept	-1.526	0.349	-4.374	0.000	*
Nitrogen	-0.083	0.505	-0.165	0.869	
Fungicide	0.000	0.493	0.000	1.000	
Nitrogen × Fungicide	-0.713	0.772	-0.923	0.356	
4 species					
Intercept	-3.526	0.717	-4.915	0.000	*
Nitrogen	2.022	0.798	2.534	0.011	*
Fungicide	1.479	0.810	1.826	0.068	
Nitrogen × Fungicide	-2.172	0.968	-2.244	0.025	*
5 species					
Intercept	-4.007	1.009	-3.972	0.000	*
Nitrogen	0.640	1.433	0.447	0.655	
Fungicide	2.061	1.087	1.897	0.058	
Nitrogen × Fungicide	1.136	1.171	0.970	0.332	
6 species					
Intercept	-19.566	2032.317	-0.010	0.992	
Nitrogen	17.369	2032.318	0.009	0.993	
Fungicide	17.001	2032.318	0.008	0.993	
Nitrogen × Fungicide	-16.596	2032.318	-0.008	0.993	

Formula: SND ∼ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.23: GLM model output for community-pair feasibility in the year 2017 alone.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	-1.792	0.540	-3.318	0.001	*
Nitrogen	0.693	0.694	0.998	0.318	
Fungicide	1.504	0.661	2.274	0.023	*
Nitrogen × Fungicide	-1.504	0.905	-1.663	0.096	
3 species					
Intercept	-1.946	0.404	-4.816	0.000	*
Nitrogen	0.293	0.544	0.539	0.590	
Fungicide	0.420	0.534	0.786	0.432	
Nitrogen × Fungicide	-0.420	0.741	-0.566	0.571	
4 species					
Intercept	-2.565	0.464	-5.527	0.000	*
Nitrogen	-0.541	0.751	-0.721	0.471	
Fungicide	0.651	0.586	1.112	0.266	
Nitrogen × Fungicide	-17.111	1285.351	-0.013	0.989	
5 species					
Intercept	-3.296	0.720	-4.577	0.000	*
Nitrogen	-18.270	3906.349	-0.005	0.996	
Fungicide	0.424	0.933	0.455	0.649	
Nitrogen × Fungicide	-0.424	5524.412	0.000	1.000	
6 species					
Intercept	-3.296	1.018	-3.236	0.001	*
Nitrogen	-19.270	9108.215	-0.002	0.998	
Fungicide	-19.270	9108.215	-0.002	0.998	
Nitrogen × Fungicide	19.270	15775.891	0.001	0.999	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.24: GLM model output for community-pair feasibility in the year 2018 alone.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	-1.447	0.556	-2.604	0.009	*
Nitrogen	-0.804	0.928	-0.867	0.386	
Fungicide	0.284	0.756	0.375	0.707	
Nitrogen × Fungicide	0.176	1.230	0.143	0.886	
3 species					
Intercept	-1.576	0.448	-3.513	0.000	*
Nitrogen	-0.792	0.752	-1.052	0.293	
Fungicide	-0.472	0.695	-0.679	0.497	
Nitrogen × Fungicide	0.036	1.174	0.031	0.976	
4 species					
Intercept	-2.803	0.728	-3.850	0.000	*
Nitrogen	-0.723	1.249	-0.579	0.563	
Fungicide	-0.723	1.249	-0.579	0.563	
Nitrogen × Fungicide	1.446	1.766	0.819	0.413	
5 species					
Intercept	-26.566	77712.628	0.000	1.000	
Nitrogen	0.000	109902.252	0.000	1.000	
Fungicide	0.000	109902.252	0.000	1.000	
Nitrogen × Fungicide	0.000	155425.256	0.000	1.000	
6 species					
Intercept	-25.566	81640.373	0.000	1.000	
Nitrogen	0.000	115456.923	0.000	1.000	
Fungicide	0.000	115456.923	0.000	1.000	
Nitrogen × Fungicide	0.000	163280.746	0.000	1.000	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.25: GLM model output for community-pair feasibility in the lower 95% confidence interval for species interaction coefficients.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	-2.565	0.734	-3.495	0.000	*
Nitrogen	1.266	0.866	1.461	0.144	
Fungicide	0.445	0.955	0.466	0.641	
Nitrogen × Fungicide	-2.441	1.470	-1.661	0.097	
3 species					
Intercept	-21.566	3906.349	-0.006	0.996	
Nitrogen	19.263	3906.349	0.005	0.996	
Fungicide	17.559	3906.349	0.004	0.996	
Nitrogen × Fungicide	-36.822	5524.412	-0.007	0.995	
4 species					
Intercept	-22.566	5760.541	-0.004	0.997	
Nitrogen	19.506	5760.541	0.003	0.997	
Fungicide	0.000	8146.635	0.000	1.000	
Nitrogen × Fungicide	-19.506	9977.549	-0.002	0.998	
5 species					
Intercept	-26.566	47589.071	-0.001	1.000	
Nitrogen	0.000	70049.216	0.000	1.000	
Fungicide	0.000	67301.110	0.000	1.000	
Nitrogen × Fungicide	0.000	67301.110	0.000	1.000	
6 species					
Intercept	-22.566	9108.215	-0.002	0.998	
Nitrogen	19.622	9108.215	0.002	0.998	
Fungicide	0.000	12880.961	0.000	1.000	
Nitrogen × Fungicide	-19.622	15775.891	-0.001	0.999	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Table S1.26: GLM model output for community-pair feasibility in the upper 95% confidence interval for species interaction coefficients.

	Estimate	SE	*t* value	*p*	
2 species					
Intercept	-0.916	0.418	-2.190	0.028	*
Nitrogen	0.481	0.570	0.8441	0.399	
Fungicide	0.481	0.570	0.8441	0.399	
Nitrogen × Fungicide	-0.333	0.788	-0.423	0.672	
3 species					
Intercept	-3.296	0.720	-4.577	0.000	*
Nitrogen	0.731	0.888	0.823	0.410	
Fungicide	0.731	0.888	0.823	0.410	
Nitrogen × Fungicide	0.308	1.086	0.284	0.777	
4 species					
Intercept	-20.566	2119.185	-0.010	0.992	
Nitrogen	0.000	3030.342	0.000	1.000	
Fungicide	16.332	2119.185	0.008	0.994	
Nitrogen × Fungicide	2.186	3030.342	0.001	0.999	
5 species					
Intercept	-22.566	6440.480	-0.004	0.997	
Nitrogen	0.000	9534.281	0.000	1.000	
Fungicide	0.000	9108.215	0.000	1.000	
Nitrogen × Fungicide	19.694	6440.480	0.003	0.998	
6 species					
Intercept	-26.566	67301.110	0.000	1.000	
Nitrogen	0.000	99064.551	0.000	1.000	
Fungicide	0.000	95178.142	0.000	1.000	
Nitrogen × Fungicide	0.000	137377.815	0.000	1.000	

Formula: SND ~ Nitrogen * Fungicide; family = Gamma (link = identity).

Appendix 2 — Chapter 2

Supplementary Figures

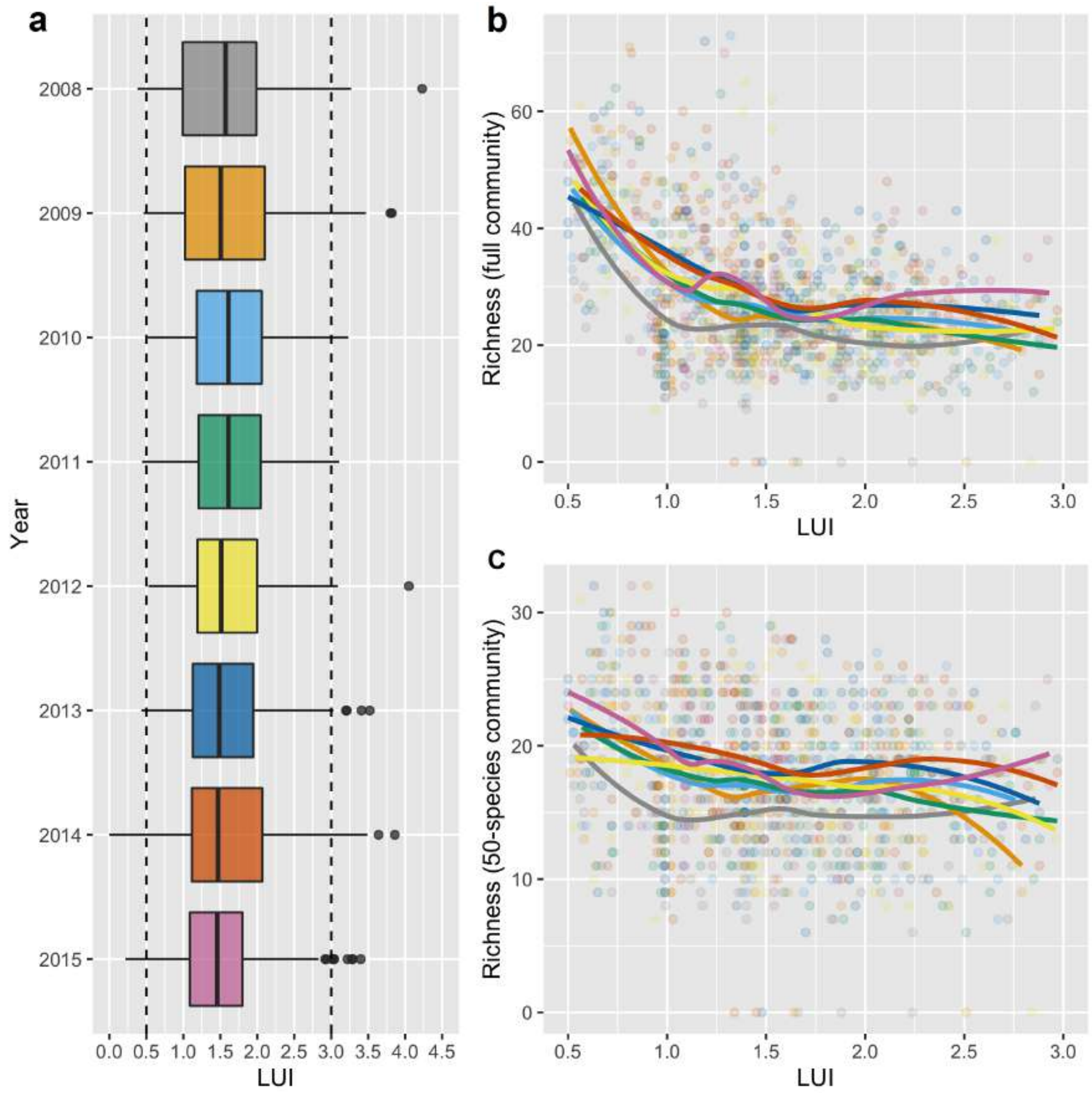


Figure S2.1: Range of LUI values for each year considered in the study from 2008 to 2015. (**a**) According to this information, we decide to take a conservative approach and restrict our analyses to LUI values between 0.5 and 3.0. (**b**) Variation in richness across the LUI gradient for all species observed in our 150 plots across all years. Each color represents a year. (**c**) Variation in species richness across the LUI gradient for the 50 most common species selected for the analyses.

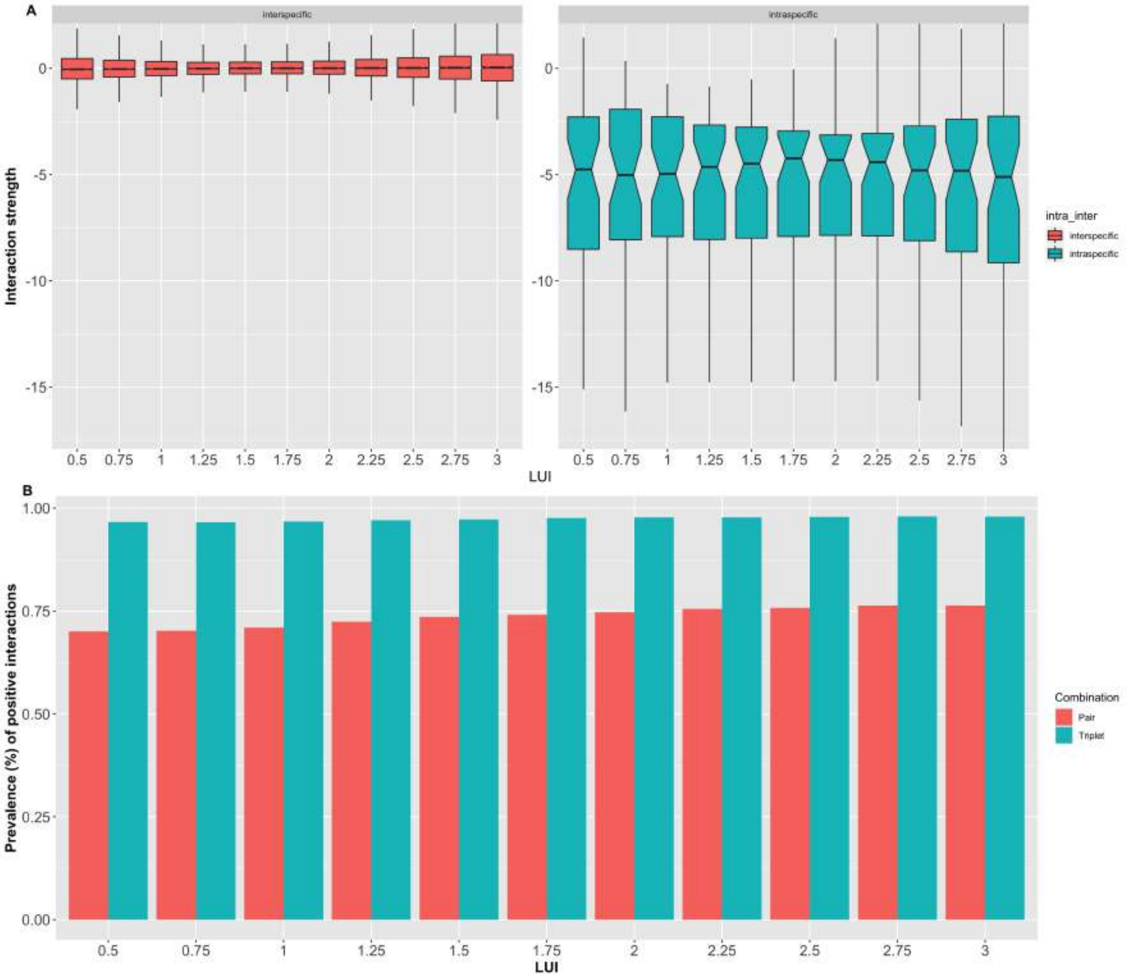


Figure S2.2: Land use intensity changes the strength of species interactions. Boxplots show median and range in values for all species at each level of LUI. LUI increases the variance in interspecific competition strength (upper left panel, **a**). LUI also increases the variance of intraspecific competition (intraspecific interactions become more negative as LUI increases) (upper right panel, **a**). Despite these changes, the proportion of combinations of two or three species with at least one positive interaction remained constant across the LUI gradient suggesting that facilitation between species is widespread in our system (bottom panel, **b**).

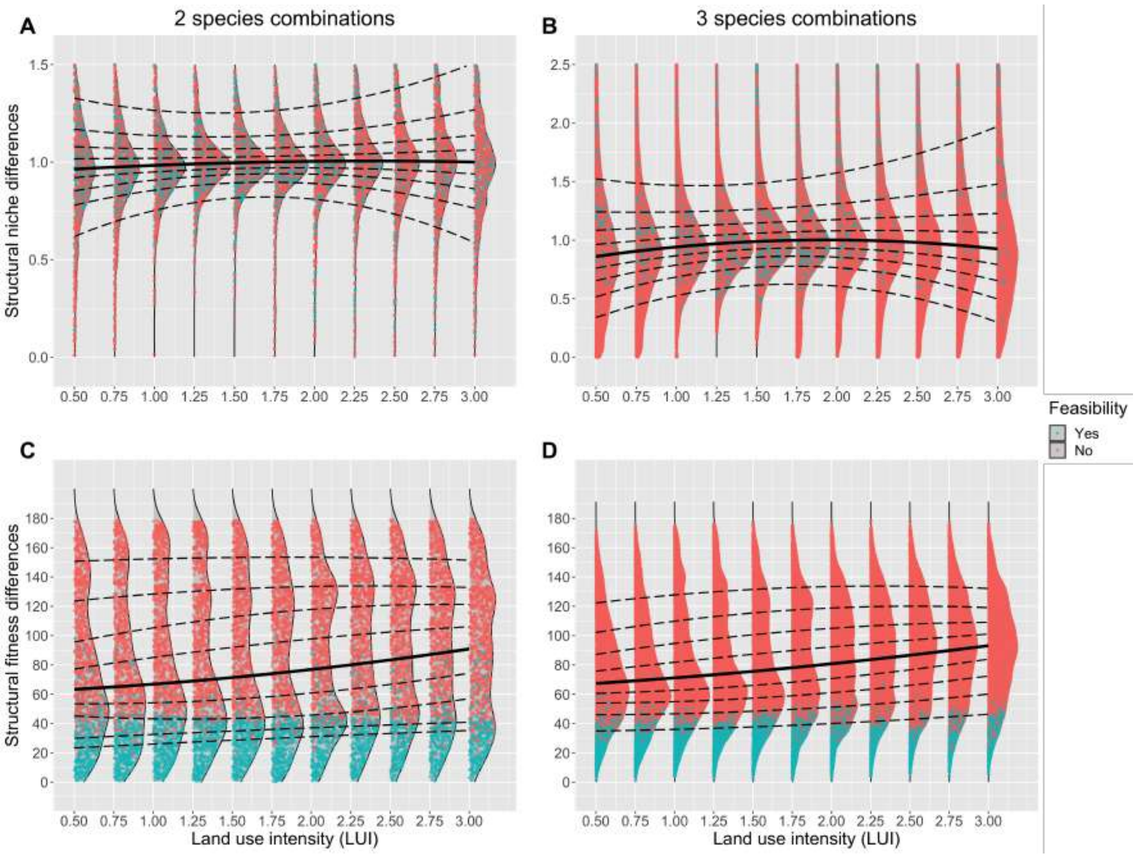


Figure S2.3: Mean and standard error values of estimates of quantile regression evaluating whether LUI changes structural niche and fitness differences for combinations of two and three species when values of interaction coefficients were randomised. All quantile regressions were significant. We evaluated 9 quantiles in total from 0.1 to 0.9. (0.5 corresponds to the median). Note that the range of values of the y-axis for both structural niche and fitness differences is similar compared to those presented in the main document (Fig. 2.1). Yet, there are two main differences: while the structural niche fitness differences remain constant across LUI, structural fitness differences increase under the randomisation, and accordingly, the proportion of species combinations predicted to persist is substantially reduced.

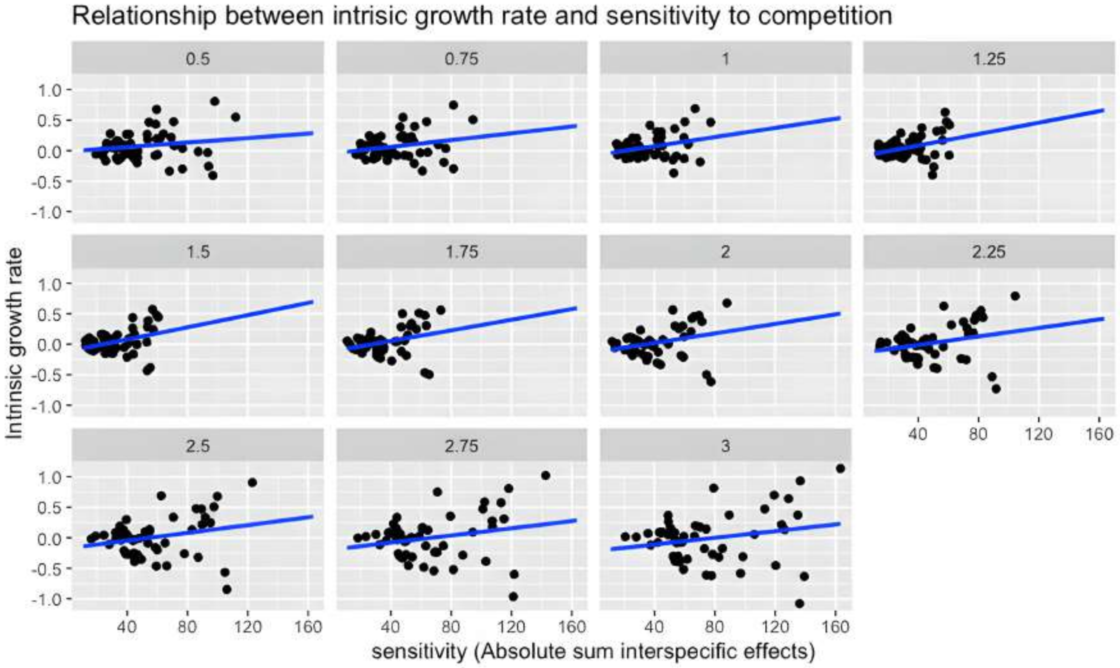


Figure S2.4: Relationship between intrinsic growth rate (y-axis) and sensitivity to interspecific interactions (x-axis). Across the LUI gradient, we observe a positive correlation between species' intrinsic growth rate and their sensitivity to interact measured as the absolute sum by rows of interspecific interaction coefficients. This means that species that grow more are also more susceptible to be affected by competition (or in some cases affected by facilitation) with other species. This trade-off between being a species that grows well but suffers more from competition could explain why we do not find variation in structural fitness differences across the LUI gradient.

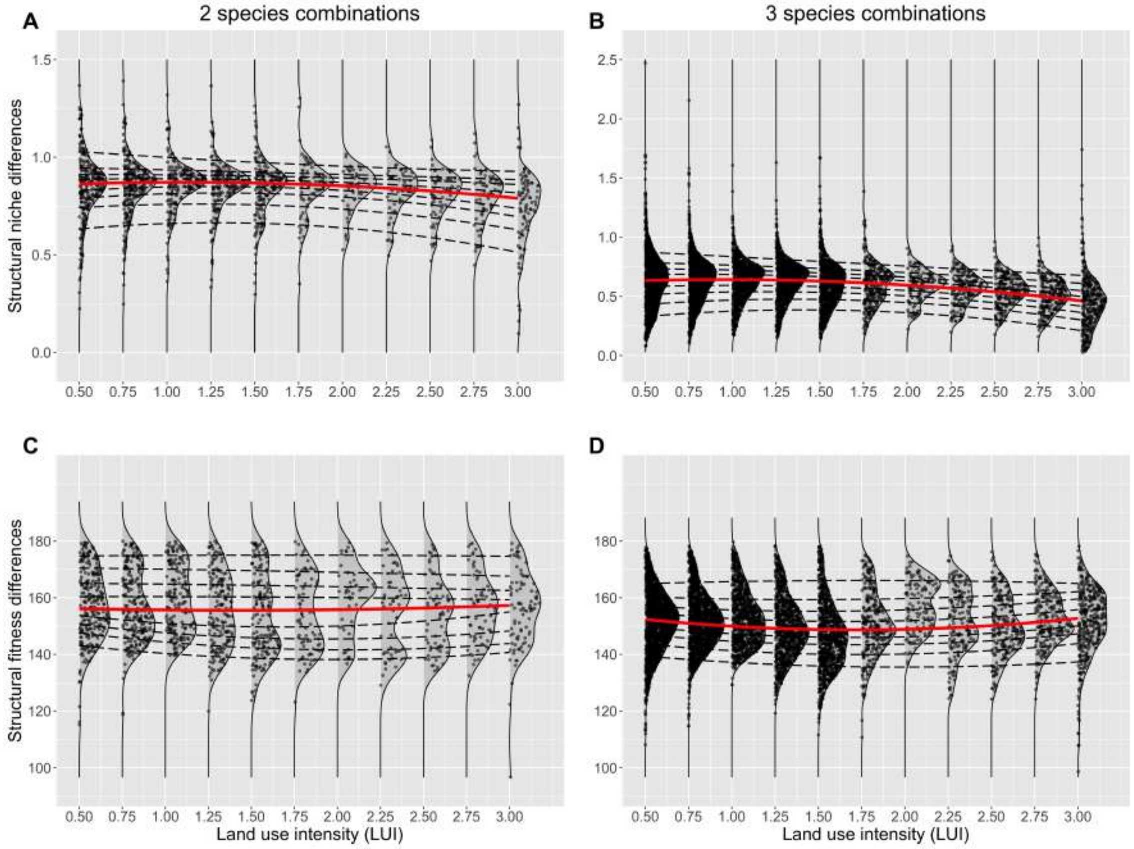


Figure S2.5: Distribution of structural niche (panels **a** and **b**) and structural fitness differences (panels **c** and **d**) taking into consideration the lower estimate of species' intrinsic growth rate and interaction coefficients with a confidence interval of 95%. The distribution of structural niche and fitness differences is shown for two (**a** and **c**) and three (**b** and **d**) species combinations, across the LUI gradient. Each point corresponds to a species combination. The lines across the graph correspond to nonlinear quantile regressions evaluating whether LUI changes structural niche and fitness differences for combinations of two and three species. We performed 9 nonlinear quantile regressions (using a polynomial form $y \sim a * LUI2 + b * LUI + c$) including the median (thicker red solid line) for each panel. Statistical significance is provided in Table S2.2.

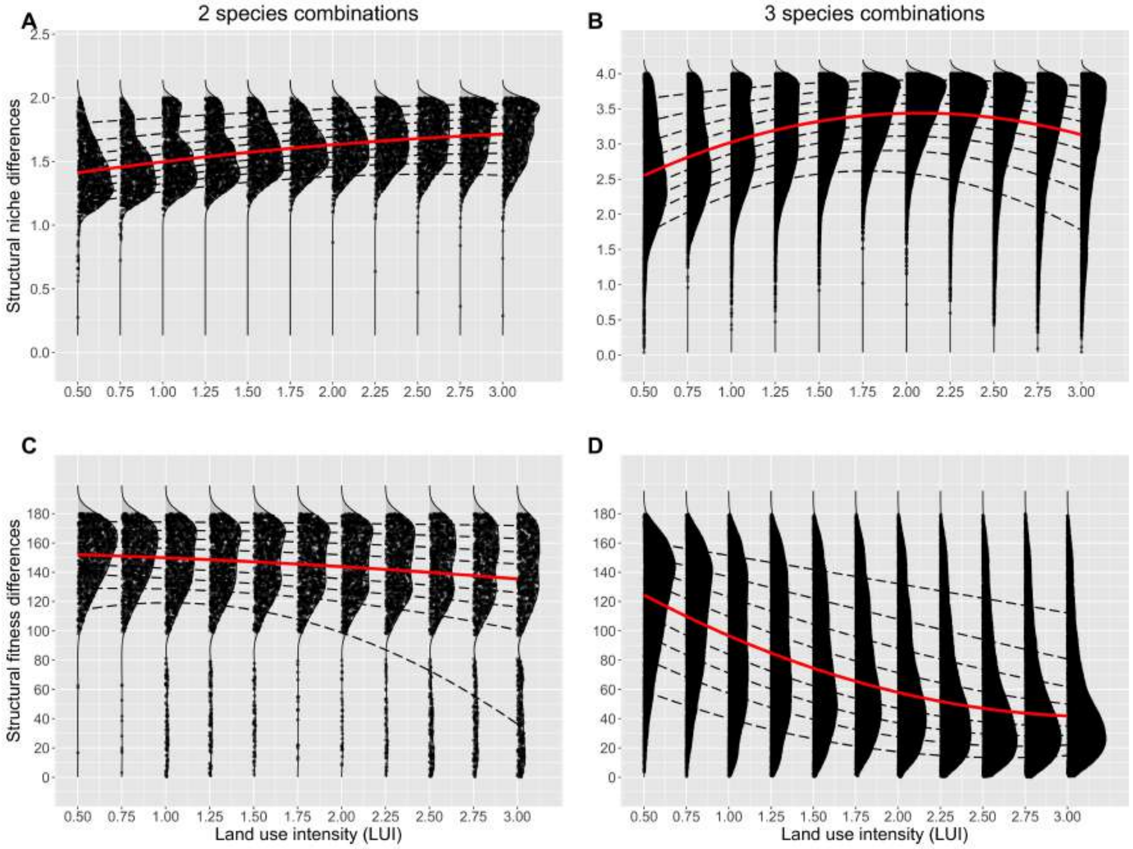


Figure S2.6: Distribution of structural niche (panels **a** and **b**) and structural fitness differences (panels **c** and **d**) taking into consideration the upper estimate of species' intrinsic growth rate and interaction coefficients with a confidence interval of 95%. The distribution of structural niche and fitness differences is shown for two (**a** and **c**) and three (**b** and **d**) species combinations, across the LUI gradient. Each point corresponds to a species combination. The lines across the graph correspond to nonlinear quantile regressions evaluating whether LUI changes structural niche and fitness differences for combinations of two and three species. We performed 9 nonlinear quantile regressions (using a polynomial form $y \sim a * LUI2 + b * LUI + c$) including the median (thicker red solid line) for each panel. Statistical significance is provided in Table S2.3.

Supplementary Tables

Table S2.1: Mean ± standard error estimates and ($p < 0.01$, denoted in bold) of the quadratic and the linear coefficients of the quantile regressions in Fig. 2.1.

	2 species				3 species			
	SND		SFD		SND		SFD	
Quantile	Quadratic	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	Linear
10	**-0.164**	**0.530**	**-1.286**	**4.912**	**-0.288**	**0.924**	**-3.213**	**11.974**
	±0.0160	**±0.053**	**±0.500**	**±1.799**	**±0.006**	**±0.022**	**±0.196**	**±0.705**
20	**-0.085**	**0.254**	**-2.149**	**8.478**	**-0.220**	**0.674**	**-3.567**	**12.818**
	±0.008	**±0.030**	**±0.553**	**±1.913**	**±0.005**	**±0.018**	**±0.184**	**±0.643**
30	**-0.054**	**0.157**	**-2.066**	**8.741**	**-0.160**	**0.475**	**-3.411**	**12.171**
	±0.0046	**±0.014**	**±0.635**	**±2.298**	**±0.004**	**±0.016**	**±0.185**	**±0.648**
40	**-0.033**	**0.102**	**-2.332**	**9.180**	**-0.121**	**0.352**	**-3.218**	**11.309**
	±0.003	**±0.012**	**±0.742**	**±2.670**	**±0.003**	**±0.011**	**±0.149**	**±0.543**
50	**-0.020**	**0.061**	**-2.284**	**8.277**	**-0.091**	**0.262**	**-2.991**	**10.478**
	±0.061	**±0.011**	**±0.602**	**±2.133**	**±0.002**	**±0.009**	**±0.141**	**±0.490**
60	**-0.010**	**0.030**	**-2.064**	**7.821**	**-0.065**	**0.187**	**-2.466**	**8.855**
	±0.003	**±0.012**	**±0.457**	**±1.518**	**±0.003**	**±0.010**	**±0.167**	**±0.568**
70	-0.003	0.005	**-1.556**	**5.743**	**-0.038**	**0.103**	**-2.051**	**7.674**
	±0.005	±0.017	**±0.481**	**±1.687**	**±0.003**	**±0.012**	**±0.178**	**±0.591**
80	**0.026**	**-0.096**	**-0.623**	**2.421**	-0.001	-0.005	**-1.280**	**5.486**
	±0.009	**±0.030**	**±0.480**	**±1.680**	±0.004	±0.015	**±0.238**	**±0.755**
90	**0.082**	**-0.272**	-0.275	1.452	**0.062**	**-0.196**	0.091	2.347
	±0.016	**±0.060**	±0.659	±2.148	**±0.010**	**±0.042**	±0.327	±0.972

Table S2.2: Mean ± standard error estimates and ($p < 0.01$, denoted in bold) of the quadratic and the linear coefficients of the quantile regressions in Fig. S2.5.

	2 species				3 species			
	SND		SFD		SND		SFD	
Quantile	Quadratic	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	Linear
10	**-0.051**	0.132	**2.319**	**-9.085**	**-0.069**	**0.194**	**1.747**	**-6.931**
	±**0.015**	±0.055	±**0.637**	±**2.024**	±**0.006**	±**0.023**	±**0.358**	±**1.122**
20	-0.040	0.095	**2.509**	**-10.285**	**-0.062**	**0.167**	**2.424**	**-8.442**
	±0.016	±0.056	±**0.713**	±**2.389**	±**0.006**	±**0.021**	±**0.318**	±**1.051**
30	**-0.042**	**0.110**	**2.509**	**-9.709**	**-0.053**	**0.127**	**2.394**	**-8.260**
	±**0.011**	±**0.036**	±**0.846**	±**2.761**	±**0.006**	±**0.022**	±**0.332**	±**1.067**
40	**-0.037**	**0.093**	**2.996**	**-9.986**	**-0.044**	**0.089**	**2.504**	**-8.647**
	±**0.007**	±**0.025**	±**1.082**	±**3.693**	±**0.005**	±**0.017**	±**0.256**	±**0.874**
50	**-0.022**	0.049	0.689	-1.957	**-0.042**	**0.080**	**2.484**	**-8.545**
	±**0.007**	±0.024	±1.047	±3.783	±**0.004**	±**0.014**	±**0.315**	±**0.976**
60	**-0.017**	0.032	-0.230	0.377	**-0.037**	**0.058**	**2.347**	**-7.799**
	±**0.006**	±0.019	±0.954	±3.234	±**0.004**	±**0.014**	±**0.280**	±**0.932**
70	-0.008	0.010	-0.240	-0.161	**-0.026**	0.017	**1.906**	**-5.689**
	±0.006	±0.020	±0.896	±3.050	±**0.005**	±0.016	±**0.304**	±**1.029**
80	-0.001	-0.018	-0.163	-0.573	**-0.014**	-0.021	**1.126**	-3.212
	±0.006	±0.022	±1.095	±3.746	±**0.004**	±0.013	±**0.355**	±1.262
90	0.009	-0.072	-0.220	0.733	-0.001	**-0.079**	-0.713	2.525
	±0.011	±0.038	±0.745	±2.421	±0.007	±**0.024**	±0.422	±1.439

Table S2.3: Mean ± standard error estimates and ($p < 0.01$, denoted in bold) of the quadratic and the linear coefficients of the quantile regressions in Fig. S2.6.

	2 species				3 species			
	SND		SFD		SND		SFD	
Quantile	Quadratic	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	Linear
10	**-0.054**	**0.280**	**-20.993**	**42.534**	**-0.560**	**1.988**	**10.063**	**-52.904**
	±0.004	**±0.014**	**±1.233**	**±4.007**	**±0.005**	**±0.018**	**±0.201**	**±0.809**
20	**-0.050**	**0.280**	**-5.347**	7.405	**-0.476**	**1.814**	**12.070**	**-65.881**
	±0.004	**±0.014**	**±1.081**	±3.271	**±0.004**	**±0.015**	**±0.181**	**±0.702**
30	**-0.040**	**0.255**	-1.617	-3.293	**-0.428**	**1.699**	**12.730**	**-72.156**
	±0.004	**±0.014**	±0.658	±2.193	**±0.004**	**±0.015**	**±0.201**	**±0.764**
40	**-0.031**	**0.233**	-1.147	-3.680	**-0.393**	**1.603**	**12.417**	**-74.059**
	±0.005	**±0.020**	±0.607	±2.035	**±0.003**	**±0.013**	**±0.203**	**±0.762**
50	**-0.025**	**0.210**	-1.151	-2.609	**-0.358**	**1.487**	**11.293**	**-72.558**
	±0.005	**±0.018**	±0.669	±2.278	**±0.003**	**±0.013**	**±0.236**	**±0.850**
60	**-0.024**	**0.207**	-0.934	-1.632	**-0.317**	**1.331**	**8.475**	**-63.710**
	±0.004	**±0.015**	±0.628	±2.004	**±0.003**	**±0.013**	**±0.214**	**±0.755**
70	**-0.016**	**0.178**	**-1.413**	1.207	**-0.264**	**1.116**	**4.388**	**-48.163**
	±0.005	**±0.020**	**±0.463**	±1.597	**±0.003**	**±0.013**	**±0.271**	**±0.902**
80	**-0.017**	**0.162**	-1.208	2.047	**-0.193**	**0.817**	**0.740**	**-30.970**
	±0.005	**±0.019**	±0.471	±1.561	**±0.003**	**±0.012**	**±0.267**	**±0.882**
90	**-0.013**	**0.110**	-0.290	-0.029	**-0.104**	**0.441**	-0.343	**-18.238**
	±0.005	**±0.020**	±0.369	±1.273	**±0.002**	**±0.008**	±0.291	**±0.898**

Appendix 3 — Chapter 3

Group lasso regularisation and `glinternet`

Given a general regression with J factors,

$$Y = \sum_{j=1}^{J} X_j \theta_j + \varepsilon, \tag{S3.1}$$

where Y is an $n \times 1$ vector, $\varepsilon \sim N_n(0, \sigma^2 I)$, X_j is an $n \times p_j$ matrix corresponding to the jth factor and θ_j is a coefficient vector of size p_j, $j = 1, \ldots, J$, abbreviated as $Y = X\theta + \varepsilon$; the goal is to decide whether to set the vector θ_j to zero vectors for each j, i.e., selecting important factors for accurate estimation in eqn. S3.1. For this, Tibshirani (1996) proposed the lasso regularisation, which consists of a penalty term added to the objective function of the model and can be described as

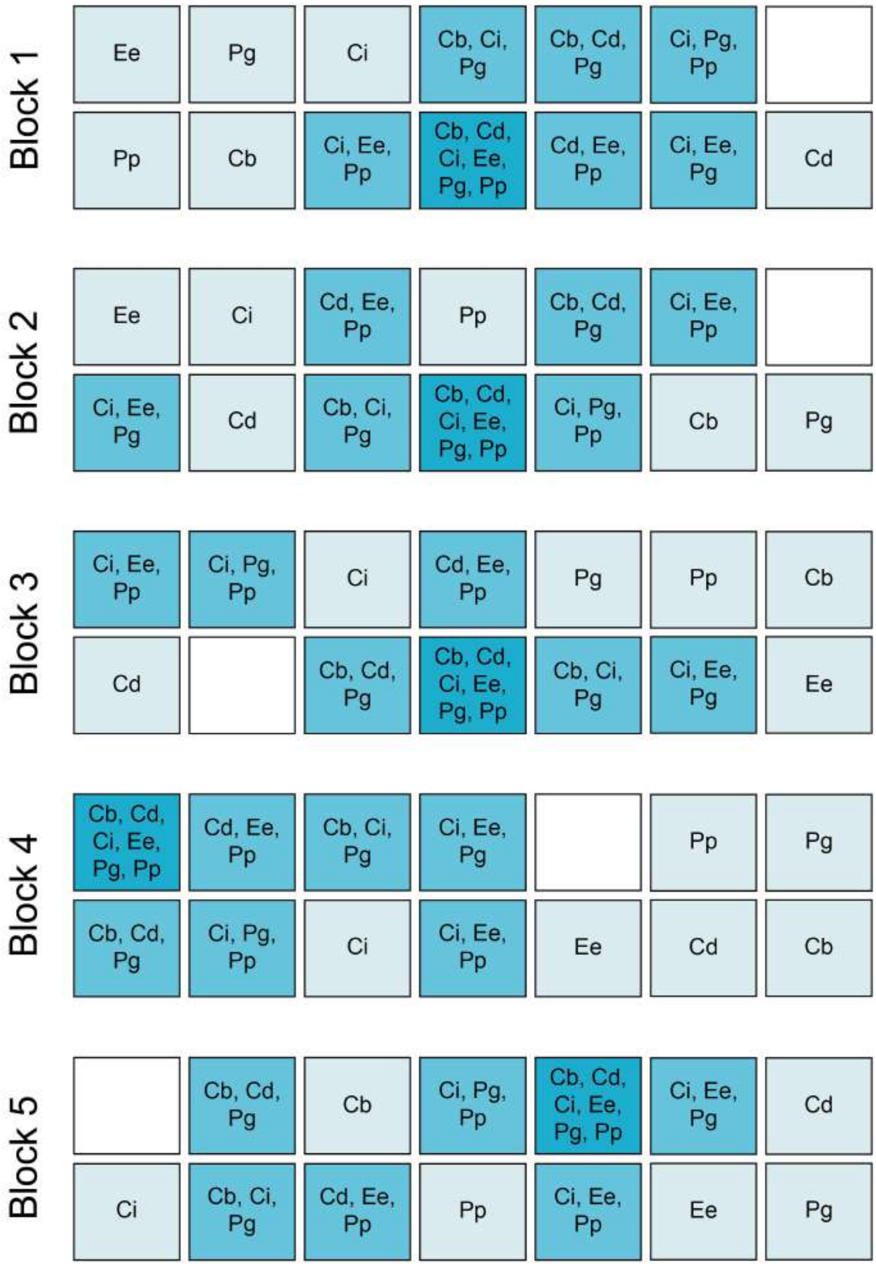
$$\widehat{\theta}_\lambda = \arg\min_\theta \left(\|Y - X\theta\|^2 + \lambda \|\theta\|_{l_1} \right), \tag{S3.2}$$

where $\lambda \geq 0$ is a tuning parameter and $\|\cdot\|_{l_1}$ is the penalty term vector l_1-norm. This penalty induced sparsity in the solution and helps an optimisation algorithm that shrinks the coefficients of less important variables to zero, effectively removing them from the model. The advantage of this method is that, as the solution paths are piecewise linear, they can be computed very efficiently. However, l_1 is designed to select individual input variables and therefore, more often than not results in selecting more factors than needed. To this regard, the group lasso overcomes this problems by extending the lasso penalty to encourage sparsity to the factor, not the individual level, allowing us to do variable selection at the group level. The group lasso estimate is defined as the solution to

$$\frac{1}{2} \left\| Y - \sum_{j=1}^{J} X_j \theta_j \right\|^2 + \lambda \sum_{j=1}^{J} \left\| \theta_j \right\| K_j, \tag{S3.3}$$

where K_j are positive definite kernel matrices $K_1, K_2, \ldots, K_J$, such as $K_j = I_{p_j}$ or $K_j = p_j I_{p_j}$. Note that the group lasso expansion (eqn. S3.3) collapses into the lasso (eqn. S3.2) when there are no groups to make, i.e., when $p_1 = \ldots = p_J = 1$. Importantly, the tuning parameter λ regulates the penalty term in such a way that when $\lambda = 0$ all parameters are selected and when $\lambda = \infty$ all parameters are set to zero, making the choice of a tuning parameter non-trivial.

Supplementary Figures



Block 1	Ee	Pg	Ci	Cb, Ci, Pg	Cb, Cd, Pg	Ci, Pg, Pp	
	Pp	Cb	Ci, Ee, Pp	Cb, Cd, Ci, Ee, Pg, Pp	Cd, Ee, Pp	Ci, Ee, Pg	Cd
Block 2	Ee	Ci	Cd, Ee, Pp	Pp	Cb, Cd, Pg	Ci, Ee, Pp	
	Ci, Ee, Pg	Cd	Cb, Ci, Pg	Cb, Cd, Ci, Ee, Pg, Pp	Ci, Pg, Pp	Cb	Pg
Block 3	Ci, Ee, Pp	Ci, Pg, Pp	Ci	Cd, Ee, Pp	Pg	Pp	Cb
	Cd		Cb, Cd, Pg	Cb, Cd, Ci, Ee, Pg, Pp	Cb, Ci, Pg	Ci, Ee, Pg	Ee
Block 4	Cb, Cd, Ci, Ee, Pg, Pp	Cd, Ee, Pp	Cb, Ci, Pg	Ci, Ee, Pg		Pp	Pg
	Cb, Cd, Pg	Ci, Pg, Pp	Ci	Ci, Ee, Pp	Ee	Cd	Cb
Block 5		Cb, Cd, Pg	Cb	Ci, Pg, Pp	Cb, Cd, Ci, Ee, Pg, Pp	Ci, Ee, Pg	Cd
	Ci	Cb, Ci, Pg	Cd, Ee, Pp	Pp	Ci, Ee, Pp	Ee	Pg

Figure S3.1: Herbivore treatments within five blocks over a two-year experiment. One square represents one cage containing 24 grasshopper individuals per square meter with a constant sex ratio of one. Each cage corresponds to a 1 m^3 enclosure cage made from transparent insect proof netting (PE 22.30, 920×920μ, DIATEX, Saint Genis Laval, France). The letters correspond to the six grasshopper species selected in the experiment: Cb: *Chorthippus biguttulus*, Cd: *Chorthippus dorsatus*, Ci: *Calliptamus italicus*, Ee: *Euchorthippus elegantulus*, Pg: *Pezotettix giornae*, Pp: *Pseudochorthippus parallelus*. Those squares withour a grasshopper code refer to the control treatment with no grasshoppers. The experiment was performed using a randomised block design with 70 cages in total (14 per block). After Deraison et al. (2015*b*).

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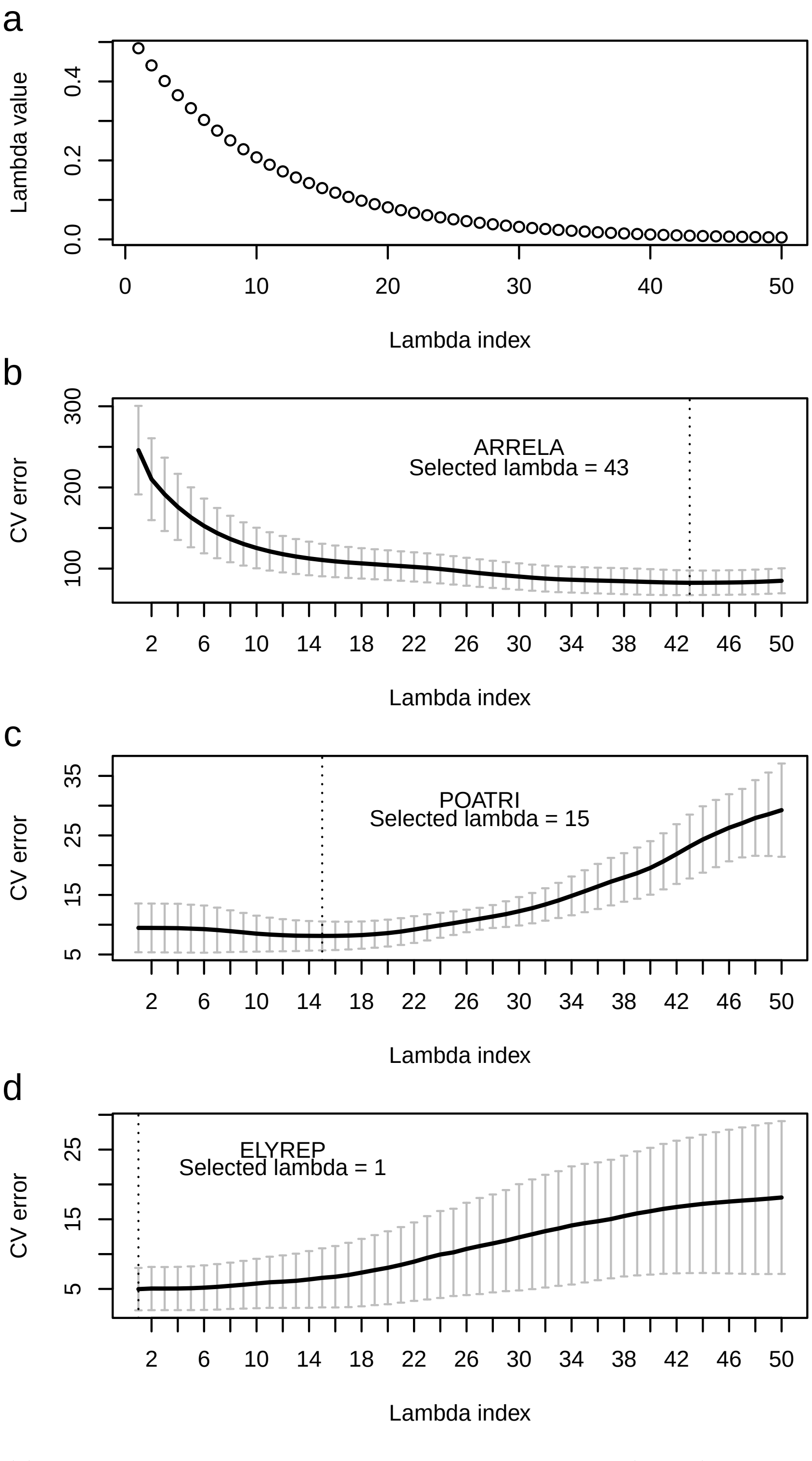

Figure S3.2: (**a**) Inverse relationship between the tuning parameter λ (y-axis) and the lambda index used by `glinternet.cv`. (**b—d**) Three examples of cross validation error against the lambda index in our `glinternet.cv` models. Vertical dotted lines indicate the selected value of lambda index. Whiskers indicate the standard error for each value of lambda index. ARRELA, *Arrhenatherum elatius*; POATRI, *Poa trivialis*; ELYREP, *Elytrigia repens*.

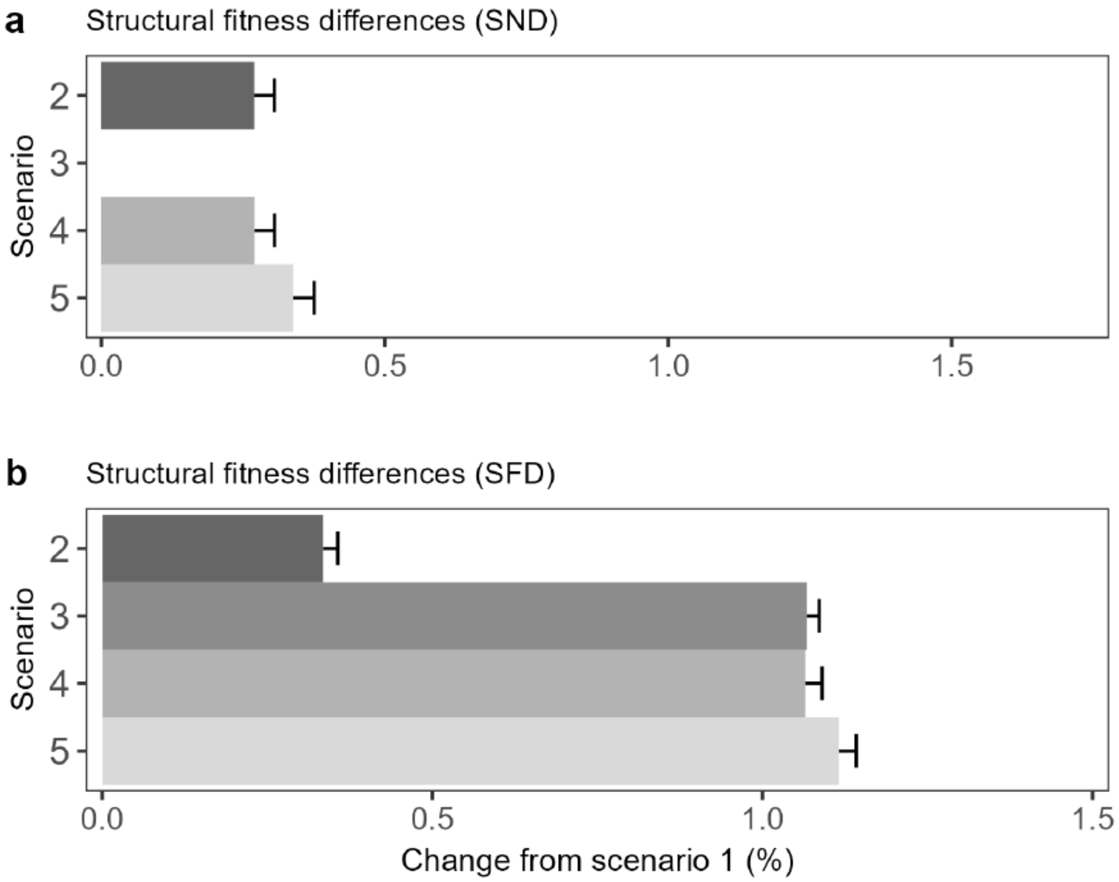

Figure S3.3: Contribution to the coexistence mechanisms ((**a**) structural niche differences and (**b**) structural fitness differences) of the effects of grasshoppers on plants (γ) and the additive higher-order effects of plants on plant-plant interactions (β) for modules of 4 species. This contribution is measured as the change (%) in the coexistence mechanisms compared to a community with only plant-plant interactions (α). Whiskers represent the standard error for each bar.

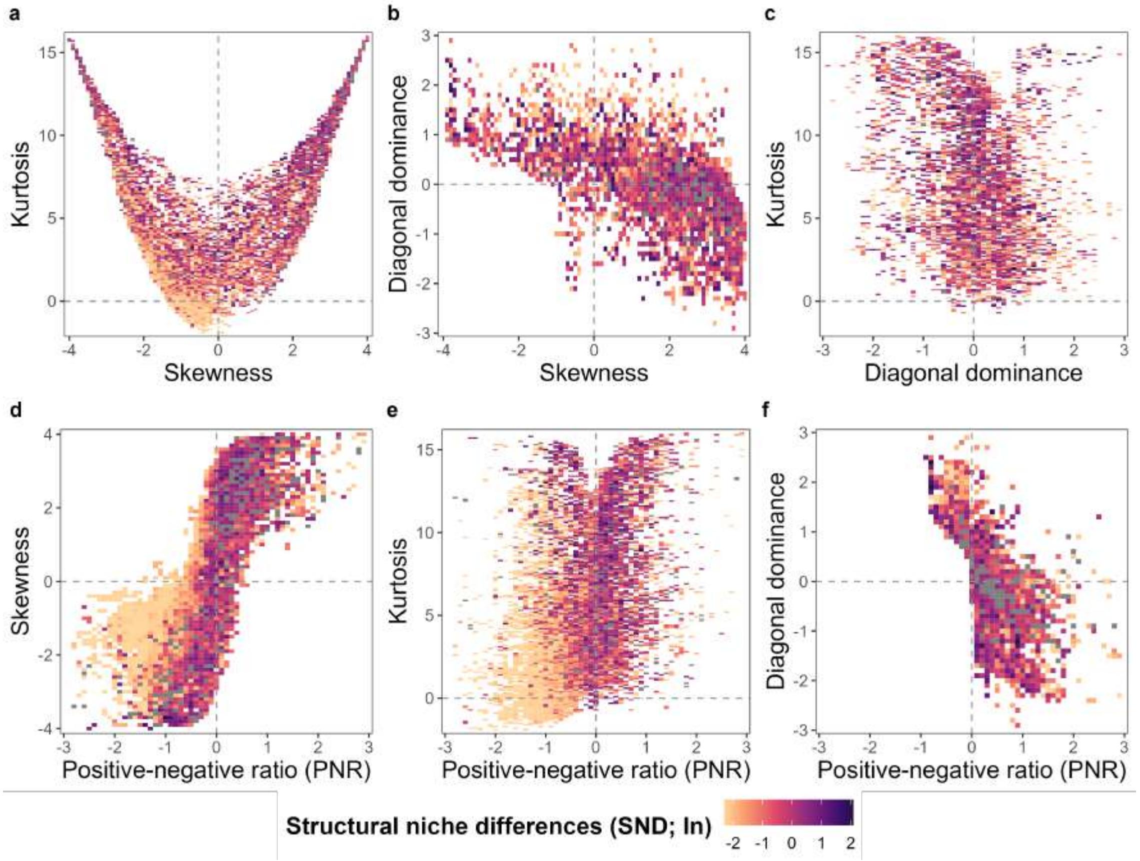

Figure S3.4: Structural niche differences (**a—f**) as a function of four metrics (skewness, kurtosis, diagonal dominance, and PNR) measuring the configuration of interaction strengths within each four-species module in the community. Skewness describes how much a distribution is shifted to the left (stronger negative interactions) or right (stronger positive interactions) of its centre. Kurtosis describes the peak and tails of a distribution: higher values indicate a higher peak and thinner tails (more weak interactions) and lower values indicate a lower peak and thicker tails (more strong interactions). Diagonal dominance is an index describing the relative difference in the strength of the diagonal of the interaction matrix (intraspecific interactions) compared to its non-diagonal elements (interspecific interactions), with logarithmic values below zero indicating higher intra- than interspecific effects and vice versa. Positive-negative ratio (PNR) is an index describing the preeminence of positive (PNR > 0) or negative (PNR < 0) interactions within each module. See methods for a description of each metric.

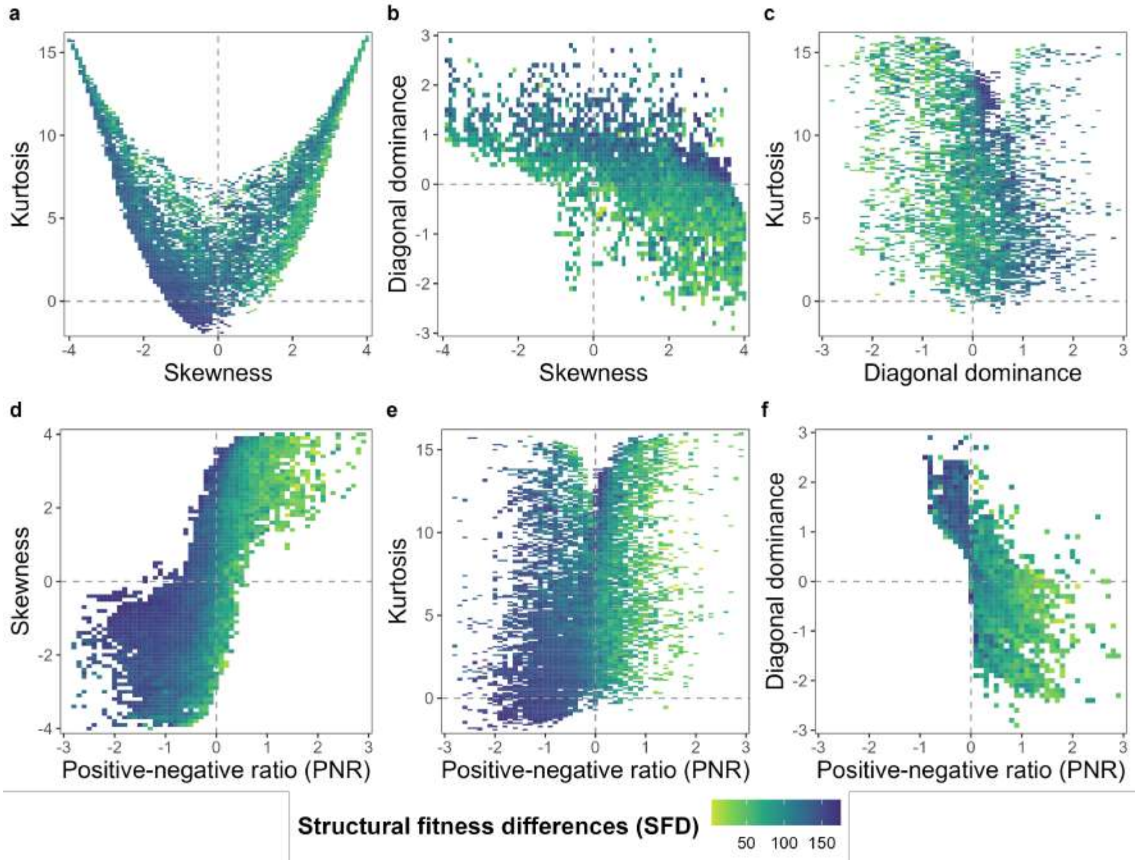

Figure S3.5: Structural fitness differences (**a—f**) as a function of four metrics (skewness, kurtosis, diagonal dominance, and PNR) measuring the configuration of interaction strengths within each four-species module in the community. Skewness describes how much a distribution is shifted to the left (stronger negative interactions) or right (stronger positive interactions) of its centre. Kurtosis describes the peak and tails of a distribution: higher values indicate a higher peak and thinner tails (more weak interactions) and lower values indicate a lower peak and thicker tails (more strong interactions). Diagonal dominance is an index describing the relative difference in the strength of the diagonal of the interaction matrix (intraspecific interactions) compared to its non-diagonal elements (interspecific interactions), with logarithmic values below zero indicating higher intra- than interspecific effects and vice versa. Positive-negative ratio (PNR) is an index describing the preeminence of positive (PNR > 0) or negative (PNR < 0) interactions within each module. See methods for a description of each metric.

Supplementary Tables

Table S3.1: List of plant species present in the experimental enclosure and analysed in this manuscript. Species code using throughout the analysis, abbreviation for visualisation (e.g., Fig. 3.2a).

Code	Abbrev.	Species	Cover (%)	Ind. (%)
ARRELA	Ae	*Arrhenatherum elatius*	19.0	6.7
BROERE	Be	*Bromus erectus*	17.8	7.4
MEDARA	Ma	*Medicago arabica*	7.1	5.1
DACGLO	Dg	*Dactylis glomerata*	6.4	6.0
DAUCAR	Dc	*Daucus carota*	5.3	5.1
PLALAN	Pl	*Plantago lanceolata*	4.6	5.2
TRIPRA	Tp	*Trifolium pratensis*	4.0	3.9
RANACR	Ra	*Ranunculus acris*	3.7	3.5
TRIFLA	Tf	*Trifolium flavescens*	3.3	2.9
GALVER	Gv	*Galium verum*	3.2	4.1
SALPRA	Sp	*Salvia pratensis*	2.3	3.0
FESRUB	Fr	*Festuca rubra*	2.2	1.8
POAANG	Pa	*Poa angustifolia*	2.2	3.7
GERDIS	Gd	*Geranium dissectum*	2.1	2.8
POATRI	Pt	*Poa trivialis*	1.4	1.5
CONARV	Ca	*Convolvulus arvense*	1.2	2.6
CENJAC	Cj	*Centaurea jacea*	1.1	1.8
LEUVUL	Lv	*Leucanthemum vulgare*	1.0	1.5
ANTODO	Ao	*Anthoxanthum odoratum*	0.9	0.9
ERYNGE	Em	*Eryngium* sp.	0.9	1.7
POAPRA	Pp	*Poa pratensis*	0.9	2.0
TAROFF	To	*Taraxacum officinale*	0.9	1.6
ACHMIL	Am	*Achillea millefolium*	0.6	1.5
FESARU	Fa	*Festuca arundinacea*	0.6	0.9
LOTCOR	Lc	*Lotus corniculatus*	0.6	1.2
ONOREP	Or	*Ononis repens*	0.6	1.2
PICECH	Pe	*Picris echoides*	0.6	0.7
PICHIE	Ph	*Picris hieracoides*	0.6	0.7
CREPIS	Cr	*Crepis* sp.	0.5	0.9
GERROT	Gr	*Geranium rotondifolium*	0.5	1.0
RUMACE	Ra	*Rumex acetosa*	0.5	1.2
SONCHU	So	*Sonchus* sp.	0.5	1.5
LOLPER	Lp	*Lolium perenne*	0.4	1.2
VERPER	Vp	*Veronica persica*	0.4	1.5
ELYREP	Er	*Elytrigia repens*	0.3	1.0
VERBOF	Vb	*Verbena officinalis*	0.3	1.0

Table S3.2: GLM results for SND and metrics (diagonal dominance, skewness, kurtosis, and positive-negative ratio or PNR) for modules of 3 species. Formula: SND + e ~ dominance * skewness * kurtosis * PNR; family = Gamma (link = log).

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.4370	0.0533	26.96	0.0000	*
dominance	-0.1901	0.0698	-2.72	0.0069	*
skewness	0.0108	0.0458	0.24	0.8142	
kurtosis	-0.0204	0.0156	-1.31	0.1914	
PNR	-0.4144	0.1465	-2.83	0.0050	*
dominance:skewness	0.0041	0.0514	0.08	0.9372	
dominance:kurtosis	0.0207	0.0195	1.06	0.2892	
skewness:kurtosis	0.0028	0.0087	0.32	0.7480	
dominance:PNR	0.1811	0.0948	1.91	0.0570	
skewness:PNR	0.1476	0.0951	1.55	0.1217	
kurtosis:PNR	0.0292	0.0255	1.14	0.2534	
dominance:skewness:kurtosis	-0.0030	0.0094	-0.32	0.7516	
dominance:skewness:PNR	-0.0548	0.0520	-1.05	0.2926	
dominance:kurtosis:PNR	-0.0129	0.0157	-0.82	0.4120	
skewness:kurtosis:PNR	-0.0105	0.0124	-0.84	0.4003	
dominance:skewness:kurtosis:PNR	0.0070	0.0057	1.23	0.2200	

Table S3.3: GLM results for SFD and metrics (diagonal dominance, skewness, kurtosis, and positive-negative ratio or PNR) for modules of 3 species. Formula: SFD ∼ dominance * skewness * kurtosis * PNR; family = Gamma (link = log).

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	4.7512	0.0792	60.01	0.0000	*
dominance	-0.0954	0.1039	-0.92	0.3591	
skewness	-0.0075	0.0679	-0.11	0.9125	
kurtosis	-0.0987	0.0230	-4.28	0.0000	*
PNR	-1.1694	0.2177	-5.37	0.0000	*
dominance:skewness	-0.0609	0.0762	-0.80	0.4249	
dominance:kurtosis	0.0790	0.0289	2.73	0.0067	*
skewness:kurtosis	0.0553	0.0128	4.34	0.0000	*
dominance:PNR	0.2895	0.1406	2.06	0.0404	*
skewness:PNR	0.2864	0.1407	2.03	0.0429	*
kurtosis:PNR	-0.1062	0.0371	-2.86	0.0045	*
dominance:skewness:kurtosis	-0.0045	0.0139	-0.32	0.7459	
dominance:skewness:PNR	-0.2410	0.0772	-3.12	0.0020	*
dominance:kurtosis:PNR	0.0641	0.0228	2.81	0.0054	*
skewness:kurtosis:PNR	0.0238	0.0181	1.31	0.1906	
dominance:skewness:kurtosis:PNR	-0.0084	0.0084	-0.99	0.3210	

Table S3.4: GLM results for SND and metrics (diagonal dominance, skewness, kurtosis, and positive-negative ratio or PNR) for modules of 4 species. Formula: SND + e ~ dominance * skewness * kurtosis * PNR; family = Gamma (link = log).

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.3525	0.0393	34.44	0.0000	*
dominance	-0.0843	0.0454	-1.86	0.0633	
skewness	0.0260	0.0227	1.15	0.2510	
kurtosis	-0.0012	0.0064	-0.19	0.8471	
PNR	-0.3336	0.1158	-2.88	0.0040	*
dominance:skewness	-0.0253	0.0250	-1.01	0.3127	
dominance:kurtosis	0.0004	0.0079	0.05	0.9584	
skewness:kurtosis	-0.0001	0.0025	-0.03	0.9788	
dominance:PNR	0.2314	0.0868	2.67	0.0077	*
skewness:PNR	0.0836	0.0560	1.49	0.1354	
kurtosis:PNR	-0.0054	0.0135	-0.40	0.6867	
dominance:skewness:kurtosis	0.0025	0.0028	0.89	0.3723	
dominance:skewness:PNR	-0.0569	0.0358	-1.59	0.1120	
dominance:kurtosis:PNR	-0.0120	0.0088	-1.36	0.1734	
skewness:kurtosis:PNR	0.0001	0.0044	0.03	0.9759	
dominance:skewness:kurtosis:PNR	0.0040	0.0025	1.60	0.1102	

Table S3.5: GLM results for SFD and metrics (diagonal dominance, skewness, kurtosis, and positive-negative ratio or PNR) for modules of 4 species. Formula: SFD ∼ dominance * skewness * kurtosis * PNR; family = Gamma (link = log).

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	4.8267	0.0312	154.76	0.0000	*
dominance	-0.1585	0.0361	-4.39	0.0000	*
skewness	0.0032	0.0179	0.18	0.8592	
kurtosis	-0.0538	0.0051	-10.64	0.0000	*
PNR	-1.3858	0.0916	-15.13	0.0000	*
dominance:skewness	0.0120	0.0198	0.60	0.5465	
dominance:kurtosis	0.0388	0.0063	6.16	0.0000	*
skewness:kurtosis	0.0196	0.0019	10.14	0.0000	*
dominance:PNR	0.2801	0.0688	4.07	0.0000	*
skewness:PNR	0.3888	0.0441	8.82	0.0000	*
kurtosis:PNR	-0.0398	0.0106	-3.76	0.0002	*
dominance:skewness:kurtosis	-0.0032	0.0022	-1.47	0.1427	
dominance:skewness:PNR	-0.1694	0.0284	-5.97	0.0000	*
dominance:kurtosis:PNR	0.0105	0.0070	1.50	0.1327	
skewness:kurtosis:PNR	-0.0075	0.0034	-2.17	0.0302	*
dominance:skewness:kurtosis:PNR	-0.0004	0.0020	-0.18	0.8577	

Appendix 4 — Chapter 4

Supplementary Figures

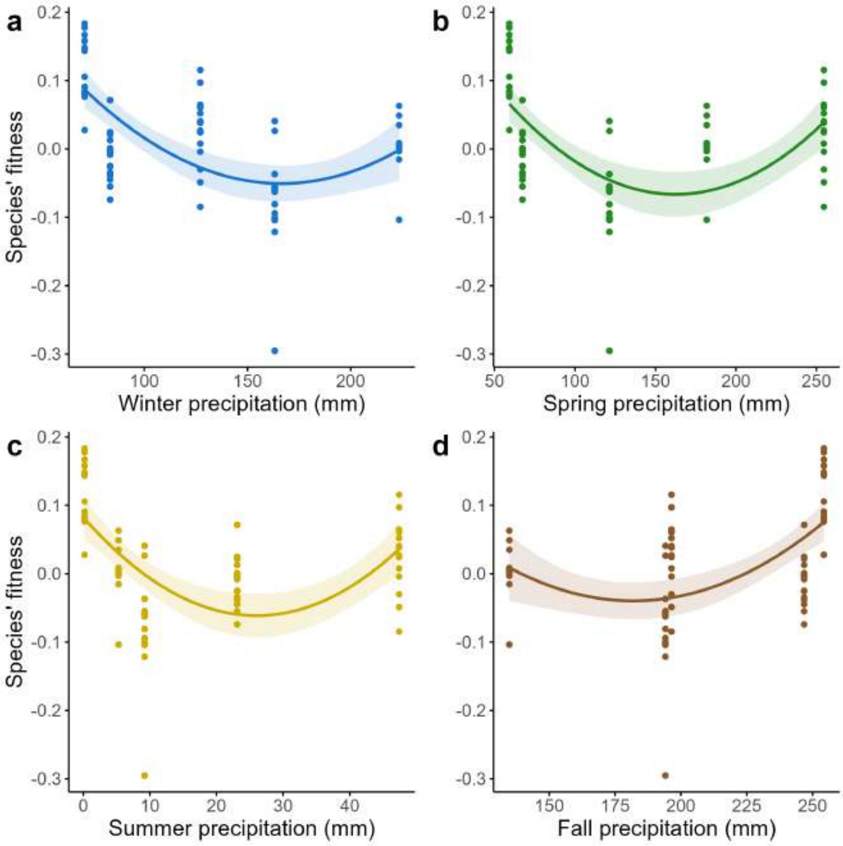

Figure S4.1: Quadratic relationship between species' fitness and total precipitation from the four seasons: (**a**) winter, (**b**) spring, (**c**) summer, and (**d**) fall. GLM statistical results are available in Table S4.4 and a visualisation of the total annual precipitation can be found in Fig. 4.3.

Supplementary Tables

Table S4.1: Plant species list from Caracoles Ranch (Doñana National Park, Spain). Codes used in the analyses, species scientific names, number of years (1 to 5) in which the species are found, and percentage of individuals per species with respect to the total across years, are shown. Codes in bold indicate core species, i.e., species found in all sampled years.

Code	Species	Years (number)	Individuals (%)
BEMA	*Beta macrocarpa*	5.00	0.41
CETE	*Centaurium tenuiflorum*	5.00	3.63
CHFU	*Chamaemelum fuscatum*	3.00	9.12
CHMI	*Chamaemelum mixtum*	5.00	2.65
HOMA	*Hordeum marinum*	5.00	20.22
LEMA	*Leontodon maroccanus*	5.00	7.48
LYTR	*Lythrum tribracteatum*	2.00	1.59
MEEL	*Melilotus elengans*	3.00	0.57
MESU	*Melilotus sulcatus*	4.00	1.21
PAIN	*Parapholis incurva*	5.00	3.73
PLCO	*Plantago coronopus*	4.00	13.09
POMA	*Polypogon maritimus*	4.00	9.87
POMO	*Polypogon monspeliensis*	4.00	1.06
PUPA	*Pulicaria paludosa*	5.00	0.37
SASO	*Salsola soda*	5.00	22.10
SCLA	*Scorzonera laciniata*	4.00	2.12
SOAS	*Sonchus asper*	3.00	0.43
SPRU	*Spergularia rubra*	4.00	0.35

Table S4.2: (**a**) Mean and standard deviation of species' fitness in all five years studied (2015-2019). (**b**) Pairwise comparisons of fitness differences between years using t-tests with pooled standard deviation (*p*-value adjustment method: "BH").

(**a**)

Year	Fitness (mean)	Fitness (sd)
2015	-0.01	0.04
2016	0.03	0.06
2017	-0.08	0.07
2018	0.00	0.04
2019	0.13	0.05

(**b**)

t-test	2015	2016	2017	2018
2016	**0.0430**			
2017	**0.0007**	**7.4e-07**		
2018	0.4633	0.2814	**0.0004**	
2019	**3.3e-10**	**2.6e-06**	**2.5e-16**	**4.1e-07**

Table S4.3: (**a**) Mean and standard deviation of species' niche in all five years studied (2015-2019). (**b**) Pairwise comparisons of fitness differences between years using t-tests with pooled standard deviation (*p*-value adjustment method: "BH").

(**a**)

Year	Niche (mean)	Niche (sd)
2015	1.49	0.12
2016	1.70	0.12
2017	1.81	0.25
2018	2.60	0.68
2019	2.06	0.20

(**b**)

t-test	2015	2016	2017	2018
2016	**0.0499**			
2017	**0.0033**	0.2973		
2018	**4.4e-13**	**1.0e-09**	**2.3e-08**	
2019	**8.4e-07**	**0.0018**	**0.0243**	**4.1e-05**

Table S4.4: Linear and quadratic generalised linear models (GLM) results for species' fitness as a function of climatic variables (total annual precipitation, and precipitation in every season: winter, spring, summer, and fall, across five years (2015-2019).

	Estimate	Std. Error	t value	Pr(>|t|)
Total precipitation, **linear** — AIC: -150.52, R^2: 0.065				
(Intercept)	0.1409	0.0561	2.51	0.0143
prec	-0.0003	0.0001	-2.25	0.0277
Total precipitation, **quadratic** — AIC: -197.33, R^2: 0.512				
(Intercept)	2.5547	0.2998	8.52	**0.0000**
prec	-0.0101	0.0012	-8.32	**0.0000**
prec^2	0.0000	0.0000	8.13	**0.0000**
Winter precipitation, **linear** — AIC: -162.75, R^2: 0.205				
(Intercept)	0.1137	0.0241	4.71	**0.0000**
winter	-0.0008	0.0002	-4.34	**0.0000**
Winter precipitation, **quadratic** — AIC: -178.65, R^2: 0.374				
(Intercept)	0.3729	0.0627	5.95	**0.0000**
winter	-0.0051	0.0010	-5.13	**0.0000**
winter^2	0.0000	0.0000	4.41	**0.0000**
Spring precipitation, **linear** — AIC: -147.30, R^2: 0.024				
(Intercept)	0.0406	0.0205	1.98	0.0520
spring	-0.0002	0.0001	-1.33	0.1874
Spring precipitation, **quadratic** — AIC: -173.92, R^2: 0.333				
(Intercept)	0.2673	0.0428	6.25	**0.0000**
spring	-0.0041	0.0007	-5.97	**0.0000**
spring^2	0.0000	0.0000	5.78	**0.0000**
Summer precipitation, **linear** — AIC: -147.03, R^2: 0.020				
(Intercept)	0.0297	0.0146	2.03	0.0462
summer	-0.0007	0.0006	-1.22	0.2247
Summer precipitation, **quadratic** — AIC: -174.49, R^2: 0.338				
(Intercept)	0.0861	0.0154	5.57	**0.0000**
summer	-0.0116	0.0019	-6.07	**0.0000**
summer^2	0.0002	0.0000	5.89	**0.0000**
Fall precipitation, **linear** — AIC: -156.85, R^2: 0.140				
(Intercept)	-0.1588	0.0518	-3.07	0.0030
fall	0.0008	0.0002	3.45	0.0009
Fall precipitation, **quadratic** — AIC: -169.41, R^2: 0.292				
(Intercept)	0.6864	0.2203	3.12	**0.0026**
fall	-0.0080	0.0023	-3.54	**0.0007**
fall^2	0.0000	0.0000	3.93	**0.0002**

Table S4.5: Linear and quadratic generalised linear models (GLM) results for species' niche as a function of climatic variables (total annual precipitation, and precipitation in every season: winter, spring, summer, and fall, across five years (2015-2019).

	Estimate	Std. Error	t value	Pr(>|t|)
Total precipitation, **linear** — AIC: 97.78, R^2: 0.003				
(Intercept)	1.7260	0.2937	5.88	**0.0000**
prec	0.0003	0.0006	0.52	0.6055
Total precipitation, **quadratic** — AIC: 98.17, R^2: 0.025				
(Intercept)	-0.9183	2.1501	-0.43	0.6706
prec	0.0111	0.0087	1.27	0.2068
prec^2	-0.0000	0.0000	-1.24	0.2185
Winter precipitation, **linear** — AIC: 81.28, R^2: 0.200				
(Intercept)	1.3911	0.1227	11.34	**0.0000**
winter	0.0039	0.0009	4.27	**0.0001**
Winter precipitation, **quadratic** — AIC: 54.09, R^2: 0.458				
(Intercept)	3.0163	0.2957	10.20	**0.0000**
winter	-0.0231	0.0047	-4.94	**0.0000**
winter^2	0.0001	0.0000	5.85	**0.0000**
Spring precipitation, **linear** — AIC: 97.28, R^2: 0.010				
(Intercept)	1.7975	0.1048	17.15	**0.0000**
spring	0.0006	0.0007	0.86	0.3917
Spring precipitation, **quadratic** — AIC: 89.62, R^2: 0.130				
(Intercept)	1.0831	0.2478	4.37	**0.0000**
spring	0.0130	0.0040	3.25	**0.0017**
spring^2	-0.0000	0.0000	-3.14	**0.0024**
Summer precipitation, **linear** — AIC: 82.12, R^2: 0.191				
(Intercept)	2.0766	0.0674	30.80	**0.0000**
summer	-0.0115	0.0028	-4.15	**0.0001**
Summer precipitation, **quadratic** — AIC: 70.83, R^2: 0.323				
(Intercept)	2.2602	0.0792	28.53	**0.0000**
summer	-0.0468	0.0098	-4.78	**0.0000**
summer^2	0.0007	0.0002	3.74	**0.0004**
Fall precipitation, **linear** — AIC: 81.41, R^2: 0.199				
(Intercept)	2.9361	0.2534	11.58	**0.0000**
fall	-0.0050	0.0012	-4.26	**0.0001**
Fall precipitation, **quadratic** — AIC: 56.73, R^2: 0.439				
(Intercept)	8.3261	0.9950	8.37	**0.0000**
fall	-0.0612	0.0102	-6.01	**0.0000**
fall^2	0.0001	0.0000	5.55	**0.0000**

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"Perhaps it's impossible to wear an identity without becoming what you pretend to be."
——Valentine Wiggin in Orson Scott Card's *Ender's Game*, 1985