Habitat heterogeneity as a mechanism determining plant species diversity:

A combined experimental and observational study

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Also lieg ich im Gras. Und bleibe grad da, wo ich bin.
Und mitternachtsblau weht der Himmel über mich hin.
Und ich tu so als ob, bis ich irgendwann
merke, dass es gelingt und dass ich mir selber genügen kann.

- Sommer für Sommer, Dota

Abstract

Biodiversity is a major topic for humanity as it maintains important ecosystem services. However, biodiversity is declining dramatically. Plant species diversity is a crucial component of overall biodiversity because it determines also biodiversity at higher trophic levels. For the maintenance of biodiversity, it is important to understand the mechanisms driving plant community structure and dynamics, especially those mechanisms that affect plant species coexistence. Heterogeneity of the habitat is a key mechanism affecting biodiversity and it is generally assumed to increase plant species diversity. Because habitat heterogeneity is influenced by land use both on a local and a landscape scale, land use may influence diversity directly and indirectly via habitat heterogeneity. Understanding these impacts is important because it would enable us to manage biodiversity via land use. Grasslands are an ideal model system to experimentally test the mechanisms by which land use affects diversity, because on the one hand, we know quite a lot about direct land use effects, e.g. of mowing or fertilization, on biodiversity and on the other hand, indirect effects on biodiversity that are mediated by homogenization of habitat conditions are likely.

Here, I investigated both experimentally and in an observational study the effects of land use on habitat heterogeneity and the mechanisms by which heterogeneity influences plant species diversity. For the experimental part, I created grassland microcosms in which two different types of heterogeneity were manipulated. In a first experiment five levels of compositional heterogeneity, i.e. increasing numbers of patches of different habitat types were created, ranging from a single to 16 habitat types per plot. In a second experiment, I manipulated the configurational heterogeneity by creating increasingly fragmented plots composed of two habitat types each. The habitat types in both experiments were created by imitating common land-use practices (mowing/ grazing, trampling, fertilization) as well as soil depth. The observational study looked at how land use intensity affects habitat heterogeneity across several scales. It took place in two regions of Germany along local gradients of the intensity of three common land-use practices mowing, fertilization, and grazing.

The experiments showed no change in diversity with heterogeneity. A switch from deterministic extinction in homogeneous habitats to stochastic extinction in heterogeneous habitats where habitat patches were smaller was observed. This is in line with recent theory. In the second experiment, fragmentation effects interacted with the contrast between habitat types and suggest that spatial mass effects are most important for increasing diversity at high fragmentation and intermediate habitat contrasts. In the observational study, I showed homogenizing effects of fertilization and mowing on habitat characteristics, as well as an increase of habitat heterogeneity due to grazing, as suggested by previous studies. Overall, plant species diversity was more directly affected by the mean of the habitat parameters than by indirect effects caused by changes in small-scale habitat heterogeneity.

Kurzfassung

Biodiversität ist ein entscheidendes Thema für die Menschheit, da sie wichtige Ökosystemleistungen aufrechterhält. Allerdings nimmt die Biodiversität dramatisch ab. Die Vielfalt der Pflanzenarten ist eine entscheidende Komponente der Biodiversität, da sie die Biodiversität auf höheren trophischen Ebenen mitbestimmt. Für die Erhaltung der Biodiversität ist es wichtig, die Mechanismen zu verstehen, die die Struktur und Dynamik von Pflanzengemeinschaften bestimmen, insbesondere jene Mechanismen, die die Koexistenz von Pflanzenarten beeinflussen. Die Heterogenität des Lebensraums ist ein Schlüsselmechanismus, der die Biodiversität beeinflusst, und es wird allgemein angenommen, dass sie die Vielfalt der Pflanzenarten erhöht. Da die Heterogenität des Lebensraums sowohl auf lokaler als auch auf landschaftlicher Ebene von der Landnutzung beeinflusst wird, kann die Landnutzung die Vielfalt direkt und indirekt über die Heterogenität des Lebensraums beeinflussen. Das Verständnis dieser Auswirkungen ist wichtig, weil es uns ermöglichen würde, die Biodiversität über die Landnutzung zu beeinflussen. Grünland ist ein ideales Modellsystem um die Mechanismen, durch die Landnutzung die Diversität beeinflusst, experimentell zu testen. Wir wissen einerseits recht viel über direkte Landnutzungseffekte auf die Biodiversität, z.B. durch Mahd oder Düngung, und andererseits sind indirekte Effekte auf die Biodiversität wahrscheinlich, z.B. durch die Homogenisierung des Lebensraums.

Ich habe sowohl experimentell als auch in einer Beobachtungsstudie die Auswirkungen der Landnutzung auf die Heterogenität und die Mechanismen, durch die die Heterogenität die Pflanzenartenvielfalt beeinflusst, beobachtet. Für den experimentellen Teil habe ich Grünland-Mikrokosmen angelegt, in denen zwei verschiedene Arten von Heterogenität manipuliert wurden. In einem ersten Experiment wurden in fünf Stufen die Anzahl von unterschiedlichen Habitattypen von einem einzigen bis zu 16 Habitattypen pro Mikrokosmus erhöht. In einem zweiten Experiment wurde in drei Stufen die Fragmentierung von jeweils zwei Habitattypen erhöht. Die Habitattypen in beiden Experimenten wurden durch Imitation gängiger Landnutzungspraktiken (Mahd/Beweidung, Zertrampeln, Düngung) sowie der Bodentiefe geschaffen. Die Beobachtungsstudie untersuchte, wie sich die Intensität der Landnutzung auf die Heterogenität von Lebensräumen auf verschiedenen Skalen auswirkt. Sie fand in zwei Regionen Deutschlands entlang lokaler Gradienten der Intensität von drei gängigen Landnutzungspraktiken Mahd, Düngung und Beweidung statt. Die Experimente zeigten keine Veränderung der Diversität entlang der Heterogenitätsgradienten. Es wurde ein Wechsel von deterministischem Aussterben in homogenen Habitaten zu stochastischem Aussterben in heterogenen Habitaten, in denen die einzelnen Habitate kleiner waren, beobachtet. Dies steht im Einklang mit gängigen Theorien. Im zweiten Experiment interagierten die Effekte der Fragmentierung mit dem Kontrast zwischen den Habitattypen was nahe legt, dass sogenannte Masseeffekte bei hoher Fragmentierung und mittleren Kontrasten zwischen den Habitattypen am wichtigsten für die Erhöhung der Diversität sind. In der Beobachtungsstudie zeigten sich homogenisierende Effekte von Düngung und Mahd auf die Habitatparameter, sowie eine Zunahme der Habitatheterogenität durch Beweidung, wie es frühere Studien nahelegen.

Insgesamt wurde die Pflanzenartenvielfalt eher direkt durch den Mittelwert der Habitatparameter beeinflusst als durch indirekte Effekte, die durch Veränderungen der kleinräumigen Habitatheterogenität verursacht wurden.

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Contents

Declaration of My Own Working Contribution to the Present Thesis

This manuscript is based on the work I did during my PhD. I worked in the field in the regions of Hainich-Dün and the Swabian Alb, as well as at the common garden in Tübingen. The fieldwork and the design of the experiments were conceived by Ronen Kadmon, Katja Tielbörger and myself. During my research I was advised by my supervisors Katja Tielbörger and Yvonne Oelmann.

I carried out all statistical analysis myself, but discussed the approaches with my supervisor Katja Tielbörger and my colleagues Pierre Liancourt and Maria Majekova, as well as with Caterina Penone and Hugo Saiz from the synthesis group from the German Biodiversity Exploratories.

The first draft of the entire thesis was written by myself, but I included the comments and suggestions I received from my supervisors Katja Tielbörger and Yvonne Oelmann.

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General Introduction

Biodiversity, i.e. the diversity of life, has been a major focus of ecological research, but it is also important for mankind (IPBES 2019). For example, it has been shown that biodiversity stabilizes ecosystems and therefore helps to maintain important ecosystem functions and services such as biomass production, pollination, carbon storage, or water regulation, to name a few (e.g. reviewed by Tilman et al. 2014). However, biodiversity is declining dramatically (IPBES 2019), and the recent IPBES report has identified changes in land use as the main driver of this decline (Pereira et al. 2010, IPBES 2019). It is therefore crucial to understand the mechanisms that lead to the maintenance of biodiversity, and especially how these are affected by land use practices. Such a knowledge may help in managing biodiversity in a changing world in a more sustainable manner (Habel et al. 2013).

Plant species diversity is a particularly important component of overall biodiversity because they are the primary producers and thus provide food for higher trophic levels, but they also comprise habitat for many other organisms. Furthermore, plants are the main target organisms for land use decisions, e.g. in forestry or agricultural systems. Research on factors affecting plant species diversity is extremely abundant, and there seems to be a general consensus about the main determinants of plant diversity. Namely, productivity and disturbance have been identified as key drivers for structuring plant communities (e.g. Overton & Levin 2003, Rajaniemi 2003). Therefore, plant communities distributed along productivity and disturbance gradients (both natural or man-made), have been ideal model systems for studying mechanisms determining plant species coexistence. Among the most studied plant communities are grassland systems. Grasslands are one of the two most diverse ecosystems in the world (Habel et al. 2013) and they may even exceed, on a small scale, species richness observed in enigmatic systems such as tropical rainforests (Wilson et al. 2012). Grasslands are widely distributed and common in Europe, but unlike in other regions of the world (e.g. North America), they are mostly semi-natural or entirely man-made, i.e. their existence and structure is dependent on human activities such as mowing, grazing, or fertilization (Ellenberg & Leuschner 2010). These have different, but well-established effects on plant species diversity in grasslands. For example, data from many previous studies have shown that fertilization of grasslands is commonly associated with a loss of species (e.g. Klimek et al. 2007, Blüthgen et al. 2012, Socher et al. 2013), mostly due to increased above-ground competition (Grime 1973, Rajaniemi 2003, Klimek et al. 2007). Mowing and grazing are disturbances whose effect on biodiversity is assumed to be unimodal, i.e. low frequency or intensity of disturbance decreases diversity via competitive exclusion, while high levels of disturbance decrease diversity via direct effects on species mortality (intermediate disturbance hypothesis (Connell 1978)).

Interestingly, increasing land use intensity via fertilization and/or mowing intensity may also be indirect and influence biodiversity by homogenizing habitat conditions (e.g. Tilman 1982, Questad & Foster 2008). However, solid evidence for such indirect effects via homogenization is still missing (Blüthgen et al. 2016). Furthermore, grazing is considered to lead to higher species diversity than mowing, because it may increase the degree of small-scale heterogeneity via trampling or local enrichment of nutrients from livestock feces (e.g. Seifan et al. 2010, Liu et al. 2016). Despite the general assumption that indirect effects of land use activities that operate via habitat heterogeneity are important, there are surprisingly few sound experiments. Namely, for testing the above or related hypotheses one should actively manipulate habitat heterogeneity, or investigate how spatial patterns of land use effects relate to species diversity across scales in the field.

Effects of habitat heterogeneity on diversity

For a long time, habitat heterogeneity has been assumed to always increase diversity, because it enables different species to coexist. I.e. habitat heterogeneity allows to avoid competitive exclusion in space (Whittaker & Levin 1977, Tilman 1982, Gigon & Leutert 1996). The explanation for this positive relationship is based on niche theory (Hutchinson 1957), i.e. habitat heterogeneity creates more opportunities for species (with different niches) to coexist, as habitat filtering leads to different communities in different habitats (Keddy 1992). Up to now there is a lot of support for such a positive heterogeneity-diversity relationship (in the following: HDR) from observational studies (Lundholm & Larson 2003, Tews et al. 2004, Hortal et al. 2009b, Stein et al. 2014, Stein & Kreft 2015). However, the few experimental studies did not unequivocally support this idea (Lundholm 2009, Tamme et al. 2010, Fahrig et al. 2011). Also, despite the wide acceptance of a positive HDR there are studies which show negative or unimodal distributions or no trend at all (Palmer 1992, Lundholm 2009, Tamme et al. 2010, Gazol et al. 2012). One reason for these contradicting results might be an unclear definition of ’heterogeneity’. Within the term ’heterogeneity’ different aspects of habitat characteristics are pooled, most often fragmentation of the habitat and/or combinations of different habitat types (Fahrig et al. 2011, Stein & Kreft 2015). To distinguish between those aspects, the terms configurational heterogeneity (i.e. the spatial pattern, or fragmentation of cover types) and compositional heterogeneity (i.e. the variety of cover types) have been introduced (Fahrig et al. 2011). Compositional and configurational heterogeneity should differ fundamentally in their effect on species richness. Especially in experiments, where the total area or an experimental unit is kept constant, compositional heterogeneity leads to a confounding between area and habitat type, i.e. including more habitat types leads to less area per habitat type. This is a problem, as the positive relationship between area and diversity is another generally accepted law in ecology. This ’area diversity relationship’ is based on neutral theory (Hubbell 2006), i.e. random processes determine the size of the species pool depending on the size of the habitat. Therefore, two processes may act simultaneously and have opposing effects on species richness, which would lead to a unimodal, rather than an increasing HDR (Kadmon & Allouche 2007). This idea has been developed and supported by empirical data as the so-called area-heterogeneity trade-off (AHTO) (Kadmon & Allouche 2007, Allouche et al. 2012). More recently, there has also been an experimental study supporting this model (Ben-Hur & Kadmon 2020a), but the generality of this unimodal relationship has yet to be established.

Experiments using configurational heterogeneity can avoid the confounding by habitat type, i.e. the usage of only two habitat types in different configurations would exclude effects of total area changes of the habitat type. However, the original theory of positive HDRs is based on niche theory (Hutchinson 1957), and therefore on compositional heterogeneity. Thus, testing a HDR using configurational heterogeneity might test different mechanisms creating species richness, and therefore might be the reason for those unequivocal results. Therefore, there is a need to distinguish between the different aspects of heterogeneity and disentangle the mechanisms responsible for shaping the relationship between the different kinds of heterogeneity and diversity.

Scales of habitat heterogeneity

A challenge for defining and quantifying habitat heterogeneity in the field is that it occurs on many different scales. For example, habitat heterogeneity can be locally affected by disturbances such as molehills, voles or grazing, or on a larger scale e.g. by anthropogenic land use such as mowing (Seifan et al. 2012). Likewise, the effects of disturbances on diversity in turn could be positive on one scale and negative on the other. Only few studies to date have focused on quantifying how land use affects habitat heterogeneity across different scales, and especially the mechanistic role of small-scale habitat heterogeneity for determining species richness in grasslands is understudied (Veen et al. 2008, Gazol et al. 2012, Brandt et al. 2013, Hart et al. 2017). This is surprising because the potential role of small-scale habitat heterogeneity for species coexistence has been acknowledged (Tilman & Pacala 1993) and also put forward many times to explain differences in community composition among grasslands under different land use such as grazing *versus* mowing (Questad & Foster 2008, McGranahan et al. 2012).

It should also be noted that habitat heterogeneity is itself a heterogeneous measure and difficult to quantify. For example, it may include abiotic parameters like light availability, soil moisture or nutrition values, or biotic variables like plant cover or plant height. These parameters are generally not independent, as moisture might be influenced by plant cover and light availability by plant height, i.e. heterogeneity is a multivariate measure itself. Additionally, all of those parameters bear the potential to be influenced by at least one of the common land-use practices, such as mowing reduces plant height and therefore increases light availability.

Effects of land use on diversity and habitat heterogeneity

Understanding land-use effects on biodiversity is important as land use is undergoing drastic changes due to new social demands (Habel et al. 2013), and because lad use change is the most important determinant of the current biodiversity crisis (IPBES 2019). The direct relationship between land use and diversity has been extensively studied (Proulx & Mazumder 1998, Austrheim & Eriksson 2001, Socher et al. 2013, Gossner et al. 2016, Chisté et al. 2018) and yielded conflicting results. These inconsistent outcomes might result from confounding effects: Confounding between certain types of land use is very common, e.g. there is a positive association between mowing regime and fertilization (Socher et al. 2013) or between the type of grazing and productivity (e.g. cattle grazing is more common than sheep or goat grazing on fertile grasslands), which generates non-random distributions of different types of grasslands in the landscape (Klimek et al. 2007). Interestingly, despite the multitude of studies and the established relationships between land use practices and biodiversity, the causal links between land use practices and species diversity are still not clear. As explained above, a main reason is that the indirect effects of land use that operate via habitat heterogeneity have been rarely studied and separated from direct effects. With my study, I attempted to fill these gaps.

It is currently unknown, whether land use influences the single parameters of habitat heterogeneity by shifting the **mean** of the measure (e.g. fertilization decreases average light availability) or by affecting the **variance** of the measure and therefore increasing the heterogeneity of the parameter. For example, grazing may increase while mowing may decrease the magnitude of spatial variability in light availability. Practically, this distinction implies that changes in land use may influence both the mean and the variance of ecologically important factors. The changes in mean of ecologically important factors generate differences in species diversity among grasslands subjected to different land use, while the changes in variance of those factors additionally generate differences in species diversity within grasslands subjected to the same land use. Both effects can be expected to determine the diversity of grassland communities (Figure 0.1). A reasonable assumption is that α-diversity is determined predominantly by the mean level of the relevant factors, while β-diversity (the degree of spatial variation in species composition) is determined predominantly by the magnitude of spatial variability (i.e. the variance) in those factors. Thus, species diversity at any scale is determined by both its α-diversity and β-diversity components, where the latter is caused by indirect effects that cause changes in habitat heterogeneity.

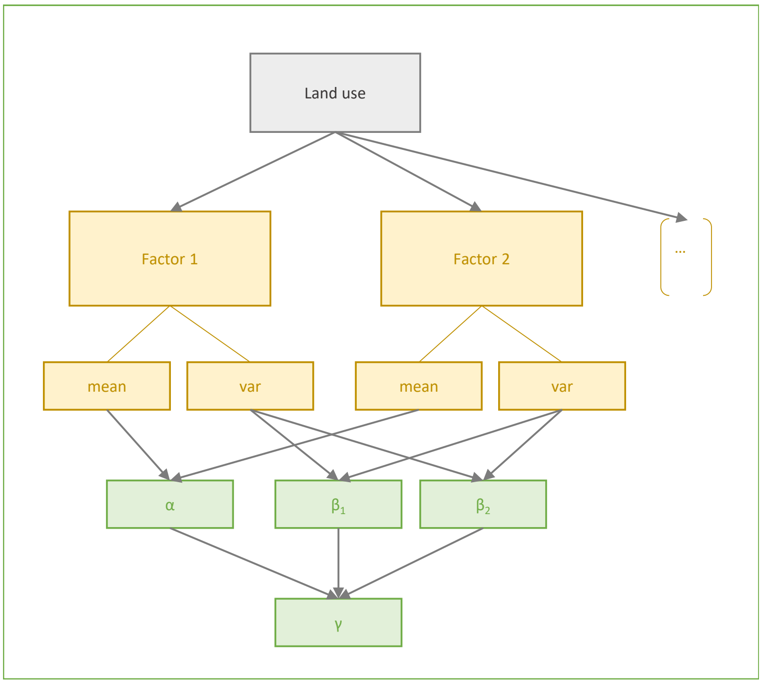


Fig. 0.1 Theoretical model of the pathways by which land use can influence γ-diversity.

Clearly, more theoretical and empirical research is needed to understand the mechanisms by which direct and indirect effects of land use and natural factors affect local diversity. Specifically, a systematic assessment of the relative importance of direct effects on α-diversity *versus* indirect effects, mediated by small-scale habitat heterogeneity, on β-diversity on the total diversity in an area is still missing. Thus, it is not known whether, for example, the well-established negative effect of land use intensity on species richness is the sole result of increasing productivity associated with rapid competitive exclusion.

Thesis objective and outline

The objective of the thesis was to understand causes, patterns, and consequences of small-scale habitat heterogeneity on the diversity of grassland communities. Moreover, I was particularly interested in finding the causal links between land use and small-scale habitat heterogeneity and grassland diversity. By doing both, experiments in a common garden as well as field observations across large ranges of land use, I addressed causal mechanisms as well as 'realistic' patterns in the field.

Specifically, I measured habitat heterogeneity in sites representing different land use types. All measurements were coupled with corresponding measurements of α-, β- and γ-diversity in order to identify key drivers and scales of spatial heterogeneity in species composition.

As land management and habitat conditions are confounded, controlled microcosm experiments were crucial to investigate the link between habitat heterogeneity and species diversity. These experiments provided one of the first experimental tests of the area-heterogeneity trade-off (Schuler et al. 2017, Liu et al. 2019, Ben-Hur & Kadmon 2020a).

The general aim was to understand the mechanisms by which spatial heterogeneity among land-use types and spatial heterogeneity within land-use types affect the diversity of grassland communities. This distinction is based on the assumption that differences in land use generate spatial heterogeneity both among grasslands (by influencing average ecological conditions of grasslands subjected to different management practices) and within grasslands (by modifying the magnitude of spatial variation in ecological conditions within the grassland). My ultimate aim was to understand the extent to which, the mechanisms by which, and the scales over which these two sources of heterogeneity affect the diversity of grassland communities.

More specifically, my work was composed of three interrelated studies which are summarized in three consecutive chapters:

In the first chapter, I analyzed plant species richness along a gradient of five levels of **compositional heterogeneity** to test the ’area-heterogeneity trade-off’ and the underlying mechanisms.

In the second chapter, I analyzed plant species richness along a gradient of **configurational heterogeneity** with respect to contrast and area effects by means of a second microcosm experiment. I hypothesized that the AHTO also applies for configurational habitat heterogeneity, but that the strength of the effect would be mediated by the level of contrast between habitat types.

In the third chapter, I analyzed the impact of land use on habitat heterogeneity in the field, by focusing on different spatial scales across a gradient of land use intensity that was provided by the framework of the Biodiversity Exploratories (Biodiversitäts Exploratorien 2020). The overarching hypothesis was that land use effects on biodiversity are both direct and indirect and operate both via changes in means as well as in the variance in habitat conditions.

In my final chapter, I take an integrated view on the experiments and observations, which addressed both similar scales as well as species, and I outline future research directions.

Chapter 1

Compositional habitat heterogeneity has no effect on species richness in a long-term microcosm experiment

Habitat heterogeneity is expected to have important effects on species diversity. However, underlying mechanisms are still subject to ongoing discussions. Niche theory predicts species richness to increase with more habitats, but species richness should decrease if the habitat fragments are too small, due to stochastic extinction. Therefore, the area-heterogeneity trade-off theory (AHTO) predicts a unimodal relationship between habitat heterogeneity and species richness along a heterogeneity gradient. However, this theory has been rarely tested and the few existing studies show contradicting results. Here, I tested the AHTO in an artificial grassland plant community by means of a common garden experiment. I monitored plant species richness, community structure and extinction probabilities for five years across a habitat heterogeneity gradient. Habitat conditions were manipulated with varying combinations of soil depth, fertilization, mowing and trampling, resulting that were assembled to represent one, two, four, eight or 16 habitats, respectively. The results showed no change in species richness across the five heterogeneity levels, despite the fact that I could show that deterministic extinction dominated under homogeneous conditions, and stochastic extinction under high habitat heterogeneity. However, these two types of extinction appeared to sum up to similar, area-specific, extinction rates across the gradient when all experimental plots are pooled. Overall, I could show that in contrast to the dominating assumption, habitat heterogeneity did not increase species richness.

Introduction

In the current biodiversity crisis, extinction rates have increased to unprecedented levels (IPBES 2019). Besides the ethical aspect of this sixth mass extinction, the loss of biodiversity will also greatly impede human well-being, due to the loss of important ecosystem functions and services (Tilman et al. 2014, IPBES 2019). To maintain species diversity, a deep understanding of the factors and mechanisms shaping it is crucial. A key factor determining species diversity is habitat heterogeneity, which has been long supposed be positively correlated with species richness (e.g. Tews et al. 2004), because more habitats support more species with different niches (Hutchinson 1957). The positive relationship between diversity and heterogeneity ('heterogeneity-diversity relationship', HDR) finds important support in the literature, especially in field studies (Lundholm & Larson 2003, Tews et al. 2004, Hortal et al. 2009a, Stein et al. 2014, Stein & Kreft 2015). Despite the wide acceptance of the positive HDR and the above supportive field observations, few experimental studies exist that have looked at the mechanisms by which habitat heterogeneity affects species diversity. Moreover, the few existing experiments have yielded any possible HDR. These include positive (Lundholm 2009, Tamme et al. 2010, Fahrig et al. 2011, Ben-Hur & Kadmon 2020a), negative (Gazol et al. 2013), unimodal (Liu et al. 2019), and no relationship at all (Lundholm 2009).

One issue that raises when approaching these contradicting results is the usage of the term 'heterogeneity'. When heterogeneity is regarded as the number of habitat types in a fixed area (compositional heterogeneity *sensu* Fahrig et al. 2011), a landscape with more habitats will also contain smaller habitat patches, i.e. habitat heterogeneity has two aspects – the number and size of habitat patches, and these are not independent from each other. Therefore, species richness in heterogeneous landscapes is also affected by area, and area affects diversity via another fundamental law: the species-area relationship (Schoener 1976). Namely, the positive relationship between area and diversity was the base of highly influential work (e.g. equilibrium theory of island biogeography (Wilson & MacArthur 1967)) and could be counted as one of nature's most general pattern (Lomolino & Weiser 2001).

When combining the classical, niche-based view of habitat heterogeneity into a more general framework that incorporates neutral processes (i.e. demographic stochasticity as a function of habitat size), a different prediction for the HDR emerges (Kadmon & Allouche 2007): Namely, the area-heterogeneity trade-off (hereafter: AHTO) implies that habitat heterogeneity has two opposite effects on species richness: it increases opportunities for niche partitioning but at the same time, reduces the amount of suitable area available for individual species, thereby increasing the probability of stochastic extinctions. Together, these mechanisms yield a unimodal relationship between habitat heterogeneity and species diversity. Basically, this framework unifies the main elements of niche theory (Hutchinson 1957) and neutral theory (Hubbell 2006), a major challenge in current research in community ecology (Gravel et al. 2006, Chase & Myers 2011).

The first study designed to test the AHTO using large-scale observational data of birds in Spain supported its predictions (Allouche et al. 2012). Nevertheless, both the theoretical basis of the AHTO and the empirical evidence supporting its predictions were subject to criticism as there is still little empirical data to its support (Hortal et al. 2009a, Carnicer et al. 2013, Hortal et al. 2013). Surprisingly, experiments that actively manipulate habitat heterogeneity are rather rare (Lundholm 2009, Gazol et al. 2013) and there are only few directly testing the AHTO (Schuler et al. 2017, Liu et al. 2019, Ben-Hur & Kadmon 2020a). Interestingly, each of the three studies yielded a different result with either negative (Gazol et al. 2013), positive (Ben-Hur & Kadmon 2020a), or a unimodal HDR like predicted by the AHTO (Liu et al. 2019). However, a common drawback of these experiments is the use of only few levels of heterogeneity (Schuler et al. 2017) or the use of configurational heterogeneity, i.e. an increase in the fragmentation of a constant number of habitat types (*sensu* Fahrig et al. 2011) (Gazol et al. 2013, Liu et al. 2019) instead of adding more habitats to a given area, in order to correctly test the AHTO. Only one experiment was manipulating compositional heterogeneity in an annual plant community (Ben-Hur & Kadmon 2020a). The findings of a positive HDR were interpreted as the result of two opposing types of extinction. Namely, with increasing heterogeneity, and thus a reduced area, not only the probability for stochastic extinction is increasing, but also deterministic competitive exclusion may be less likely when population size of dominant species and thus their competitive advantage is reduced (Ben-Hur & Kadmon 2020a). If the stochastic extinction predicted by the AHTO is not increasing in the same range as the deterministic extinction is reduced, the net effect of the different extinction types on richness can stay positive (Ben-Hur & Kadmon 2020a). In this case replicated heterogeneous plots should differ from each other more than replicated homogeneous ones (Ben-Hur & Kadmon 2020a), i.e. species composition in homogenous communities is more predictable than in heterogeneous habitats. Clearly, a single experiment is insufficient to prove the generality of this idea and more robust experiments are needed, ideally in different study systems.

Further theoretical work extended the idea of the AHTO to a theoretical model including all possible known mechanisms influencing species richness in a heterogeneous landscape (Ben-Hur & Kadmon 2020c). A main conclusion from this recent work was that a reduction in area, seems to explain richness relationships much better than fragmentation of single habitats *per se* (Fahrig 2017, Ben-Hur & Kadmon 2020c). Due to the importance of area, the size of the habitat patches in an experiment is crucial. On the one hand, homogeneous habitats should be at a size where stochastic extinction is unlikely. More important, patch size at the highest heterogeneity levels should be small enough relative to the size of the plants to guarantee stochastic extinction, but it should not be too small to be perceived by the plants. The temporal scale of the experiment is also crucial, i.e. it should be sufficiently long to yield visible extinction events. Finally, there should be many different habitat types to enable the detection of species-specific extinctions in homogeneous habitats. Unfortunately, the few existing experiments have failed on one or more of these prerequisites. Some used only two habitat types where the configuration, but not composition, of habitats was manipulated (Gazol et al. 2013, Liu et al. 2019), others included patch sizes that were so small that the resulting microcosm was actually homogeneous (Liu et al. 2019), and even the most extensive experiment (Ben-Hur & Kadmon 2020a) may have had areas at the heterogeneous end that did not cause a large amount of stochastic extinction events. With this study, I attempted to address these challenges.

Central European grasslands are highly diverse ecosystems (Habel et al. 2013), which are heterogeneous by natural and anthropogenic factors (e.g. soil depth or fertilization, mowing, and trampling). These factors alter productivity and disturbance and could be easily transferred to an experimental system to create heterogeneity. However, productivity and disturbance are also key factors known to have an impact on diversity, and probably interact with the effects of area and heterogeneity influencing in concert processes such as competition and habitat filtering. For example, high productivity levels (e.g. through deep soils or fertilization as a common land-use practice) can eventually limit the availability of soil resources (Pastor et al. 1984), leading to increased competition for light, space, nutrients and water and, therefore, to reduced species richness (Grime 1973, Grime 1977, Rajaniemi 2003, Maurer et al. 2006, Klimek et al. 2007). In contrast, extremely low productivity leads to reduced species richness as only few species can cope with a low nutrient supply (Rajaniemi 2003). As a result, the response of grassland diversity to productivity gradients often follows a unimodal shape. Still, various diversity patterns can be observed, depending on the width of the productivity gradient and interactions with other factors such as disturbance (Rajaniemi 2003). The effects of disturbance on species richness depends on the intensity and frequency of the disturbance, with higher species richness at intermediate disturbance levels (intermediate disturbance hypothesis (Connell 1978)). Mowing and grazing are classical land-use practices which create anthropogenic disturbance regimes (Seifan et al. 2010). These practices might have a positive effect on diversity especially in productive grasslands, as they enable competitively inferior species to coexist with fast growing species (Laliberté et al. 2013), however, mowing also might have negative effects on species richness due to homogenizing effects (Chisté et al. 2018).

In this study, I work with regional grasslands communities consisting predominantly out of perennial species to complement an experimental test of the HDR in a system, which was, to the best of my knowledge, not tested over such a long period of time before. I tested whether the switch from deterministic to stochastic extinctions with increasing heterogeneity applies also to long-lived plant communities, and whether this generated a unimodal HDR. Additionally, I expected a more positive effect of heterogeneity on richness in productive than less productive habitats.

Methods

Study system

A common garden experiment in a grassland ecosystem in Tübingen, South-West Germany (48°32'N, 9°02'E, 465 m) was designed, where habitat heterogeneity was manipulated and plant species richness was monitored for five years. In Autumn 2014, boxes (80 × 80 × 40 cm) were embedded in the ground to create independent plots of artificial grasslands (microcosms). Those boxes were made of polyethylene, a heat and frost resistant material (Semadeni AG, Ostermundigens, Switzerland). 16 holes of 1 cm in diameter were centered every 20 × 20 cm on the bottom of the boxes, and a >10 cm thick layer of crushed stones below the boxes ensured drainage of water. The boxes were placed at a distance of 1 m from each other, with the reinforced edge protruding approximately 5 cm from the ground surface. The boxes were filled with a 3:2 mixture of seed-free soil and sand. The substrate was leveled with the surrounding ground surface in order to create more natural conditions (e.g. near-natural freezing regime in winter). To prevent weeds from growing near the microcosms, the ground in between them was covered with a water-permeable fabric.

Each microcosm of 80 × 80 cm area was subdivided into 16 subplots of 20 × 20 cm, that were assigned to one of 16 habitat types. Habitat types were defined by a full factorial combination of the following four treatments which were selected for being main determinants of habitat conditions in (semi-) natural grasslands (Maurer et al. 2006, Ellenberg & Leuschner 2010, Gossner et al. 2016): soil depth (deep: 32 cm/ shallow: 17 cm), mowing (yes/no), trampling (yes/no), and fertilization (yes/no).

The soil depth treatment was installed during the preparation of the microcosms in October to December 2014. Subplots assigned to the shallow soil treatment were manipulated by placing a cube of expanded polystyrene (20 × 20 cm width and 18 cm height, EPS, produced by METZ EPS-Hartschaumzuschnitte, Gemmrigheim, Germany) to the bottom of the box. A canal of 1 cm in diameter in the center of the block was aligned with the respective hole in the bottom of the box to ensure water drainage also in shallow plots. The polystyrene cubes had to be fixed to the bottom of the microcosm to ensure correct soil

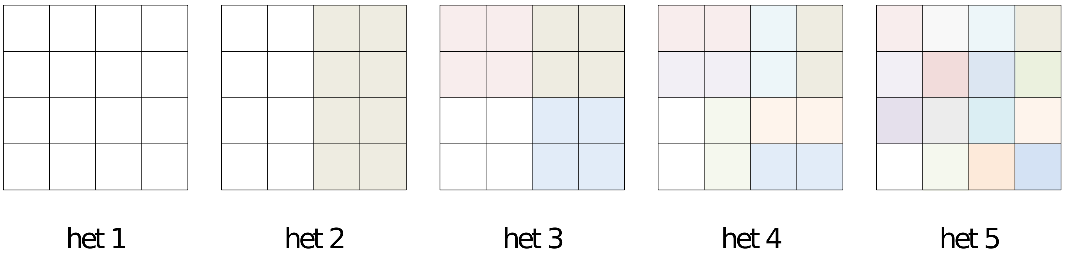


Fig. 1.2 Scheme of the experimental setup of the five heterogeneity levels. Each color represents different habitat conditions.

depth also in the neighboring subplots. For that non-toxic aquarium silicone (to avoid any negative effects of volatiles from the glue) was used.

Fertilization treatments were applied once a year each April adding 3.2g of a solid slow-release N-P-K fertilizer (Osmocote®Exact®High End (12-14 M): 15% N + 9% P+ 11% K; Scotts, Geldermalsen, The Netherlands) to make sure that nutrients stay in the particular subplot. Mowing was performed twice a year in May/June and August/September. Here, the above ground biomass was cut subplot-wise with a pruner 1-2 cm above the soil level. The trampling treatment was performed twice a year after each mowing treatment in May/June and August/September, using a special designed stool with four legs fitting one subplot of 20 × 20 cm, which was pressed once with a weight of roughly 60 kg to each subplot of that treatment (see picture A.1).

To build up the heterogeneity gradient from zero (one habitat type) to high (16 habitat types) heterogeneity levels, all microcosms were assigned to one of five levels of heterogeneity. These defined the number of habitat types within one microcosm (1, 2, 4, 8, 16 habitat types, Figure 1.2). Each level of heterogeneity was replicated 32 times, leading to a total of 160 microcosms which were randomly located over the available area in the common garden. Within a microcosm, habitat types were assigned randomly. To avoid over representation of certain habitat types a given habitat type was only allowed on one cluster per microcosm and the overall area of each habitat type was kept constant.

In December 2014, 40 different common grassland species from South-West Germany were sown to create the artificial grassland communities (see Table A.1 for species list, commercial seed supplier: Rieger-Hofmann GmbH, Blaufelden-Roldshausen, Germany). The species were selected to cover a wide range of different functional types and abundance classes. Furthermore, between-species variation in maximum rooting depth offered the potential for belowground niche partitioning. To evenly distribute the species across habitat types, eight seeds per species were sown into each subplot. To prevent seed dispersal into and out of the microcosms, these were surrounded by 1.5 m high organza tubes supported from wooden sticks, leaving the top open to allow full access of pollinators. The microcosms were weeded regularly, i.e. species dispersing into the microcosms were removed in a seedling stage.

Data sampling

In each subplot, the presence and absence of the initial species was monitored annually at peak season in summer (July/August 2015-2019). The monitoring took place in one event at subplot level after the first regrowth after mowing. All species could be found at this time of the season: the early blooming ones still had their ground leaves intact and the newly emerged seedlings already had a size in which they could be determined.

The presence and absence data obtained at the subplot level was used to calculate species richness (no. spec) per microcosm and per habitat type across the heterogeneity levels for all years of investigation. Additionally, extinction probability between the species initially present (no. spec (present in year 2015)) and the following years (2016-2019) was calculated per microcosm, per habitat type, and for the individual treatments across the heterogeneity levels for all years of investigation as:

$$extinction\ probability = \frac{no.\ spec\ (extinct\ in\ year\ 2015 + x)}{no.\ spec\ (present\ in\ year\ 2015)}$$

All data organization, the following analyses, and the plotting were performed with R (R Core Team 2020). Packages used for data organization were ’dplyr’ (Wickham et al. 2020) and ’tidyr’ (Wickham & Henry 2020).

Statistical analyses

Relationship between habitat heterogeneity and diversity

To find the specific relationship between habitat heterogeneity and diversity (HDR) for this dataset and to test the proposed shape of the area-heterogeneity trade-off (AHTO), I fitted three different models for each year: a linear one (where a positive relationship would reflect the dominance of niche processes and a negative one the dominance of area processes), a quadratic one (which would have the best fit if the shape of the AHTO was true) and a null model (reflecting no relationship between habitat heterogeneity and diversity). For the first two models, I used linear models, using species richness as a response variable and habitat heterogeneity as a fixed factor (linear: richness ~ het level, quadratic: richness ~ het level + het level^2). These models were compared to a null model (richness ~ 1) using the Akaike information criterion for a small number of data points (AICc) from the ’MuMIn’ package (Barton 2020). For each year, the model with the smaller AICc was chosen and the r^2 from the original model was taken to evaluate the absolute fit of the model to the data.

Community composition across habitats

To test if the different habitat types select for different plant communities, i.e. deterministic processes form specific communities in the different habitat types, I performed multiple canonical correspondence analyses (CCA). For each level of heterogeneity and year, species composition was considered as response variable and the sixteen habitat types as explanatory variables. I visually compared the explained variance of the CCAs across years and across heterogeneity levels, to compare over time and along the heterogeneity gradient how much variance was explained by the habitat types. To exclude that the distinction between the communities was mainly affected by one treatment, I repeated the analyses with the four single treatments (i.e. soil depth, fertilization, mowing, and trampling) instead of their combinations as explanatory variables. If the communities do not differ between the different habitat types extinctions are driven by stochastic processes.

Extinction probabilities across heterogeneity levels

Because a HDR is shaped by differences in extinction probabilities across heterogeneity levels, I tested for those differences at the microcosm level by using an ANOVA from the package ’car’ (Fox & Weisberg 2019). The analyses were done for all years (e.g. extinctions from 2015 to 2019).

Additionally, I tested whether habitat types influence extinction probabilities in dependence of patch size, reflecting the decreasing size of habitat patches along the heterogeneity gradient. To do so, I performed an ANOVA for the data of 2019 and plotted the extinction probabilities of the habitat types against the patch size with a smoothened curve. Here, more productive habitats should show a greater decrease in extinction probabilities than less productive ones in case deterministic processes are dominant.

To exclude that effects of extinction probability came from a single treatment (i.e. soil depth, fertilization, mowing, or trampling) and not from the combination of them (i.e. the habitat type), I compared the relationship of heterogeneity and extinction probability within one treatment for both of the specifications (deep/shallow; yes/no). To do so I performed an ANOVA for the linear regression explaining extinction probability by the treatment including the interaction with heterogeneity.

Results

Relationship between habitat heterogeneity and diversity

Species richness at microcosm level was similar across all heterogeneity levels, indicated by the null model which had the best fit, i.e. the smallest AICc (intercept: 12.09, Figure 1.3, Table A.3). This was true for all study years (Table A.3).

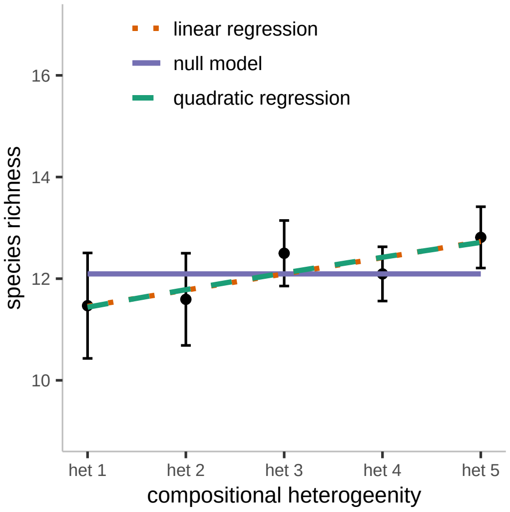


Fig. 1.3 Mean species richness in 2019 across all levels of heterogeneity with linear regression (violet line), quadratic regression (reddish dots), and the null model (greenish dashes).

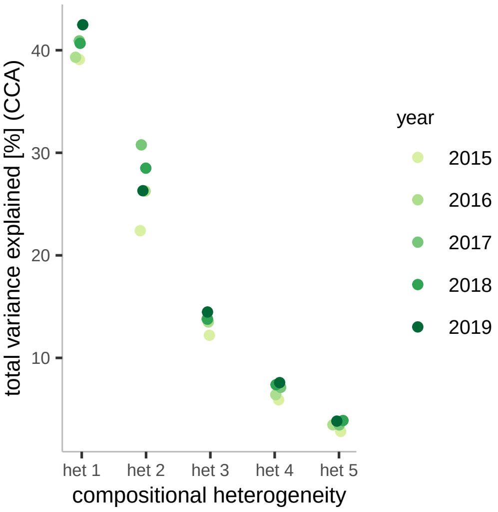


Fig. 1.4 Results of the CCAs with species as response variable and the sixteen habitat types as explanatory variables. The x-axis shows the five levels of heterogeneity. The y-axis describes the total variance explained by the habitat types for the different CCAs.

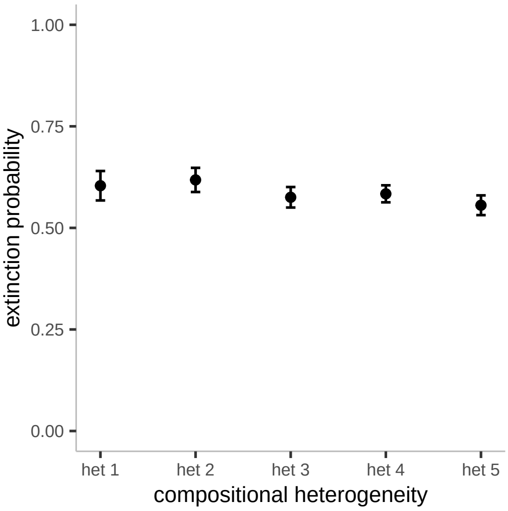


Fig. 1.5 Mean extinction probability +/- SE per microcosm for the time interval 2015 – 2019 across heterogeneity levels.

Community composition across habitats

Different habitat types selected for different communities, but this habitat filtering effect depended on the size of the habitat patches. The results of the Canonical Correspondence Analyses (CCAs) showed that the variance explained by the different habitat types (produced by the combination of the four single treatments) decreased linearly from homogeneous (around 40% variance explained) to the most heterogeneous microcosms (around 3% variance explained) in all years of observation (Figure 1.4). Additionally, within one level of heterogeneity, the variance explained increased with time (Table A.5). CCAs with the four single treatments as explanatory variables generally gave lower values for the explained variance than the CCAs for the different habitat types (around 15% variance explained for the homogeneous microcosms (Table A.5), but show the same trend over time. A great amount of variance between the communities explained by the habitat types indicates that the differences across the habitat types are great and clearly distinct from the community compositions of other habitat types.

Extinction probabilities across heterogeneity levels

There were no significant differences (Chisq: 1.95, df: 4, p: 0.74) in extinction probabilities per microcosm across the heterogeneity levels (Figure 1.5), irrespective of the time interval (1-5 years) considered (Table A.4).

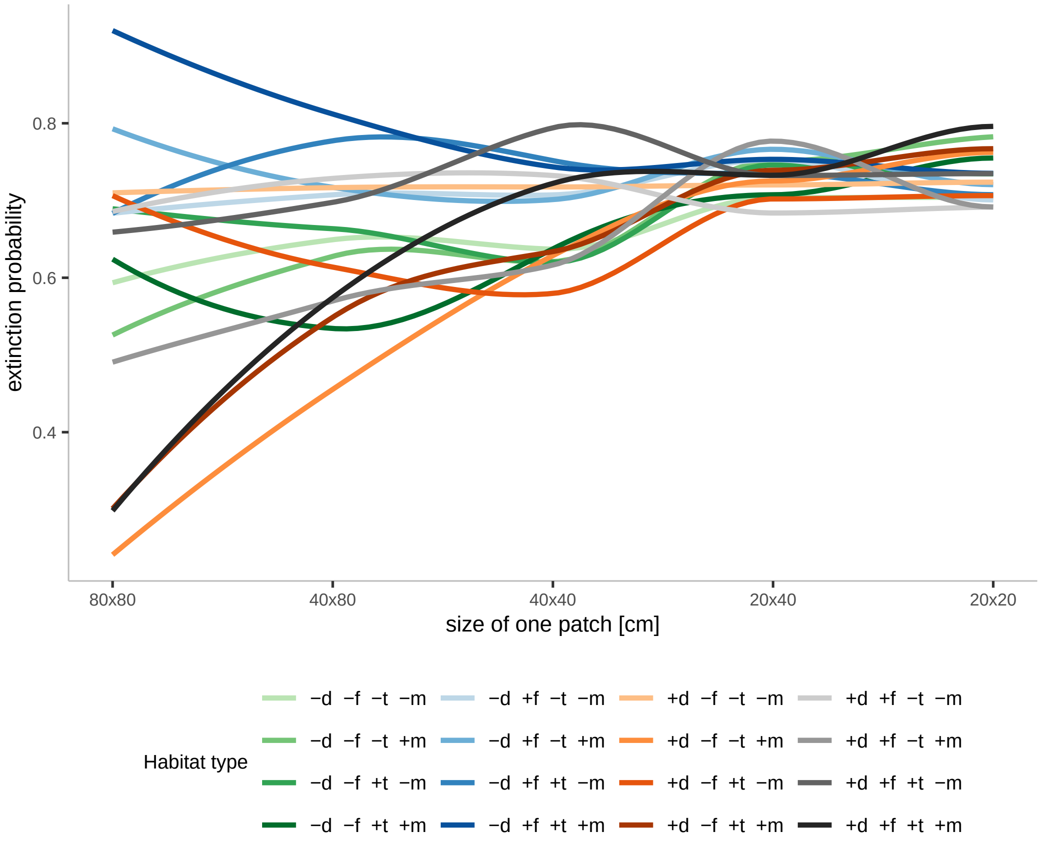


Fig. 1.6 Extinction probabilities (from 2015 to 2019) for the single habitat types against the size of the patches within the heterogeneity levels. Lines were smoothened over the mean extinction probabilities (Wickham 2016). Different colors depict different habitat types, created by the combination of the treatments soil depth (d), fertilization (f), trampling (t) and mowing (m). '-' stands for 'shallow/not treated', and '+' stands for 'deep/treated'.

Habitat specific extinction probabilities from 2015 to 2019 showed an interaction with area (df: 60, F: 1.8206, Pr (>F): < 0.001, Figure 1.6). Specifically, in homogenous microcosms (i.e. largest patch size), the extinction probabilities varied a lot among the 16 habitat types. *Vice-versa*, at high heterogeneity levels (i.e. small patches) the differences among extinction probabilities greatly

Tab. 1.1 Statistical values for the linear models explaining extinction probability as a function of the treatment interacting with heterogeneity level.

treatment	F	df	p
soil depth	6.75	998	0.0095
fertilization	13.62	998	0.0002
mowing	21.78	998	0.0000
trampling	0.06	998	0.8102

decreased and were similar across all habitat types. The highest contrast in extinction probability was between shallow fertilized mown (upper bluish line, Figure 1.6) patches, which had a reduction in extinction probabilities with increasing heterogeneity, and deep mown patches (lower most greyish and brownish lines) which had the greatest increase in extinction probabilities with increasing heterogeneity.

The single treatments soil depth, fertilization and mowing all interacted with the heterogeneity levels in their impact on the extinction probability. Only trampling had no effect on the extinction probability along the heterogeneity gradient (Table 1.1). The more productive version of the treatments fertilization and mowing showed stable extinction probabilities along the heterogeneity gradient, whilst the associated treatment with lower productivity generally exhibited increasing extinction probability along the heterogeneity gradient. In the two highest heterogeneity levels, the extinction probability was similar between treatment pairs, but in homogeneous microcosms the less productive treatments had lower extinction probabilities. Interestingly, for soil depth the effect was reversed, deep soils had lower extinction probabilities at homogeneous microcosms, increasing along the gradient to the same level like shallow soils in heterogeneous microcosms (Figure 1.7).

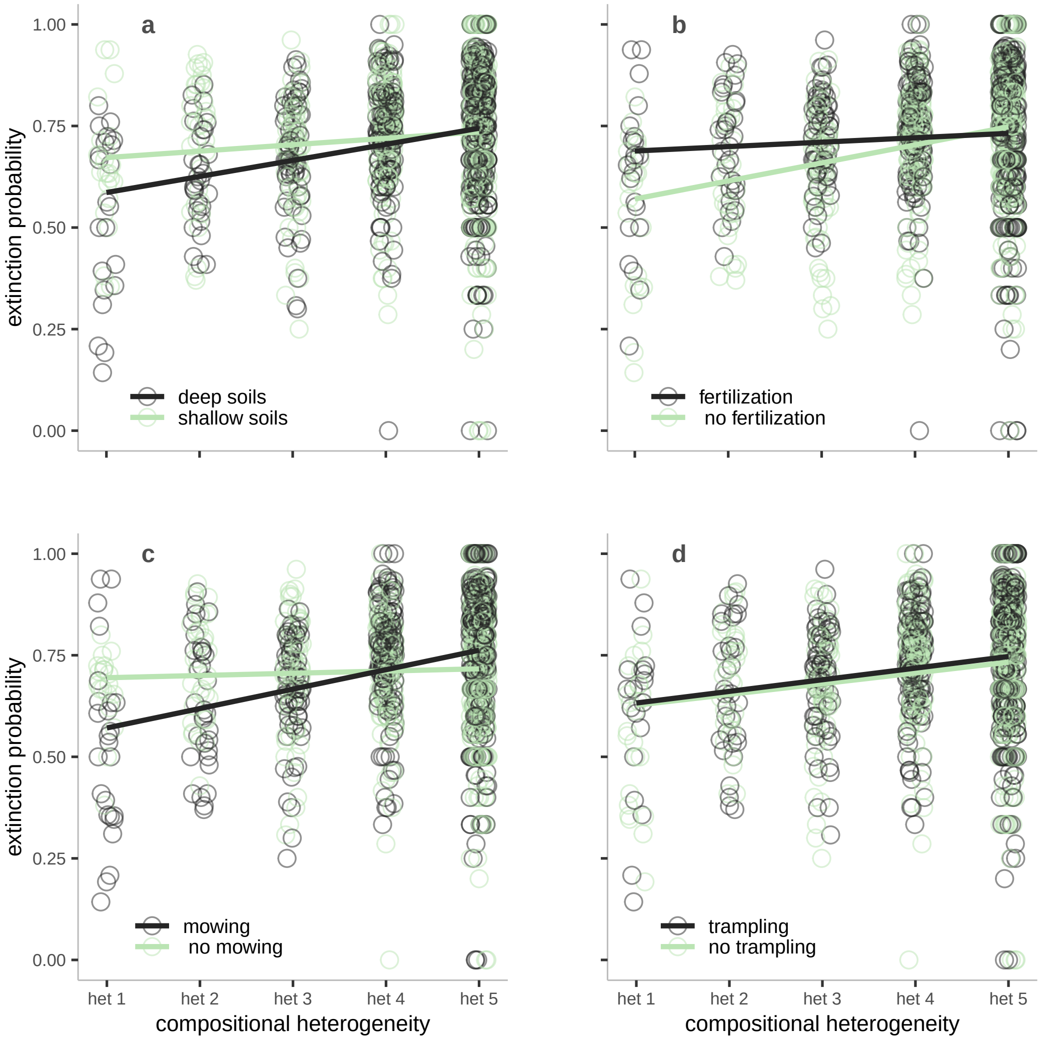


Fig. 1.7 Extinction probabilities split into the single treatments soil depth, fertilization, mowing, and trampling.

Discussion

Neither species richness nor overall extinction probability differed among heterogeneity levels after five years of monitoring. However, the key processes and shifts between deterministic and stochastic extinctions occurred according to theory, as well as area-specific extinction. In the following, I discuss my findings with respect to the initial hypotheses.

In this experiment I tested the effect of compositional heterogeneity, i.e. increasing the amount of habitat types at the expenses of the area of all habitat types, on species richness. I could not find a relationship between heterogeneity and richness, a finding which has been reported before (Lundholm 2009). On a first glance, this contradicts both the common assumption of a positive HDR (e.g. Tews et al. 2004) as well as the refined model of the area-heterogeneity trade-off (AHTO) (Kadmon & Allouche 2007). So taken together, our study joins the few existing experiments looking at HDRs (Gazol et al. 2013, Schuler et al. 2017, Liu et al. 2019, Ben-Hur & Kadmon 2020a), each of which found different patterns. On a first glance, this seems to contradict the AHTO. However, a closer look at the data yields important insights into the mechanisms by which habitat heterogeneity affects the demographic processes determining diversity.

Measuring extinction probability of a system should allow conclusions about the shape of the HDR, as this is the main process shaping richness patterns. The overall data did not indicate any change in extinction probabilities per microcosm along the heterogeneity gradient. The main mechanisms predicted by the AHTO (Kadmon & Allouche 2007) are habitat filtering at homogeneous plots and resulting deterministic extinctions, and stochastic extinctions at heterogeneous plots due to a reduced area. To test the AHTO, ideally the net extinction probability should be separated into its deterministic and stochastic components (Ben-Hur & Kadmon 2020a). Namely, if either of the two dominates at one end of the gradient, this could lead to different shapes of the HDR even though the mechanisms proposed by the AHTO are at work (Ben-Hur & Kadmon 2020a). However, my analyses of the communities and the habitat- and area specific extinction probabilities allowed me to disentangle deterministic and stochastic processes and shed some light onto the reasons for the lack of a unimodal HDR. Interestingly, there was a switch from deterministic to stochastic extinctions, as proposed by the AHTO (Kadmon & Allouche 2007). To the best of my knowledge, this was the first time that this switch from deterministic to stochastic extinctions was clearly demonstrated experimentally. Moreover, it is the first time that an experiment suggested that these two types of extinction are of similar magnitude, which yielded the flat HDR that I found.

I detected deterministic extinctions in homogeneous plots by the combination of two analyses: The results of the CCAs showed a great proportion of variance between the homogeneous plots explained by the identity of the habitat type, and at the same time, there were great differences among habitat specific extinction probabilities. Those results together show that the specific extinction probabilities of the habitat types must be the result of the deterministic process of habitat filtering as the emerging plant communities were very different from each other. The same combination of results allows me to infer stochastic extinction at the most heterogeneous plots (het 4 and het 5), because the CCAs indicated that the communities were essentially the same at the heterogeneous end. I.e. communities became more similar to each other across the heterogeneity levels. Considering that the heterogeneity gradient is inverse to the size of single habitat patches, stochastic extinction is the likely reason for explaining these patterns.

With this data I strongly support area-specific extinction rates, however I found positive as well as negative relationships between habitat type and area. The first study trying to disentangle the mechanisms of the species area relationship found a strong positive dependence of species richness on area (Ben-Hur & Kadmon 2020b). Theoretical considerations lead to the conclusion, that the shape of the AHTO depends on the relative importance of stochastic *versus* deterministic drivers in the specific system (Ben-Hur & Kadmon 2020a). For example, in the latter study, deterministic extinction dominated and thus a positive HDR was found. The differences between that previous experiment and mine were (i)) in the longevity of the study organisms, and (ii) in size and number of habitat patches in the most heterogeneous plots. However, my results support the conclusion of HDRs being dependent on the relative importance of stochastic *versus* deterministic drivers, as the habitat types in my system reacted differently to the reduction of area. Potentially, another set of habitat types would have led to another overall form of the relationship between heterogeneity and (net) extinction. Whilst area effects can be obtained by sampling effects, i.e. simply finding more individuals in greater areas (Ben-Hur & Kadmon 2020b), I can exclude this for my data as the data sampling was always done at subplot level (20 × 20 cm) and not changing along the area gradient, species data for larger areas were calculated out of the subplot data. Additionally, my area gradient included five levels of area and thus gives more solid information about trends than two different areas (Ben-Hur & Kadmon 2020b). For this reasons, my results underpinned the generality of the former study (Ben-Hur & Kadmon 2020b).

The newest theoretical work regarding the relationship between heterogeneity and diversity tried to unify all existing models by including all currently known mechanisms affecting the shape of HDRs into one model (Ben-Hur & Kadmon 2020c). The results could be mainly explained with the mechanisms proposed by the AHTO, deterministic and stochastic extinctions (Kadmon & Allouche 2007). Furthermore, the total amount of effective area of one habitat was found to be more important than the degree of fragmentation, i.e. the level of configurational heterogeneity (*sensu* Fahrig et al. 2011) was less important than the level of compositional heterogeneity (Ben-Hur & Kadmon 2020c). This impact of area was supported by a positive influence of heterogeneity on richness in large but not in small habitats (Schuler et al. 2017). In small habitats with increasing heterogeneity the remaining area was too small to support specialists which would account for an increasing richness at more heterogeneous levels (Schuler et al. 2017). This might also occur in this experiment. The remaining area in the highest heterogeneity level was so small that often only few individuals survived in one subplot. Those might not be specialists and therefore the total richness of the microcosm was not increasing.

The setup of this experiment is quite similar to the most extensive experimental test of the AHTO (Ben-Hur & Kadmon 2020a). While their experiment was performed in an annual system with four years of development, I used primarily perennial species and did the final survey after five years. My results in comparison with the earlier ones could therefore give an idea to the generality of the processes determining richness in those systems. There are other examples of experiments with perennial grassland species (Gazol et al.

2013, Liu et al. 2019), however, those communities were only investigated during their first seasons, i.e. long-term demographic processes could not take place during this period of time. The size of the subplots as well as the size of the microcosms was within the range of the other experiments which had larger (2 m² plot size) (Ben-Hur & Kadmon 2020a) and smaller entities (< 0.5 m² plot size) (Gazol et al. 2013, Liu et al. 2019). The chosen sizes and the length of my gradient increased the number of possible habitat types in the most heterogeneous level compared to the other experiments, additionally more individuals perceive the heterogeneous environment as the subplots are relatively small compared to the potential size of individuals. Contrary to the other long running experiment (Ben-Hur & Kadmon 2020a), I had no physical borders between the subplots to make belowground competition as well as vegetative dispersal possible and thus allow relatively fast extinction events in the perennial communities. On a first glance, one may assume that for perennial species, an experiment has to run quite a long time as community-level responses to manipulations of the environment are driven by demographic processes (birth, death, immigration and dispersal) which operate at longer time scales than in systems of annual species. However, in perennial systems habitat selection can work constantly throughout several years, especially when a plant that establishes early on grows over the years, whereas in annual systems (e.g. Ben-Hur & Kadmon 2020a), there is a possibility for continuous recruitment germination of species from a long lasting seed bank (Cohen 1966), which may retard extinction. In fact, we observed that some individuals in our plots grew to very large sizes over the years and displaced many other species that did not attain large sizes. Through this effect, perennial species may actually not need a longer time span to establish stable communities through effects from habitat manipulations than annual species. Thus overall, I believe that extinction was generally more likely and faster in my system.

The lack of a relationship between heterogeneity and species richness in this dataset might be due to the fact that the extremes of the distinct extinction probabilities of the different habitat types at the homogeneous end of the gradient were similar. While some habitat types (in particular deep soils) had lower species richness with increasing heterogeneity, some (in particular shallow soils) showed the opposite trend. As the changes in extinction probability of the habitat types leveled out each other, the overall species richness for the microcosms stood the same. This results support the ’ecological drift hypothesis’, which states that the process responsible for extinction is changing but not the net extinction (Ben-Hur & Kadmon 2020a). The fact that the shape of the HDR actually differed among habitat types, is particularly interesting, as it connects my work to that of many previous studies that have studied direct habitat (or land use) effects on species diversity. For example, more productive habitats are generally less diverse, making nutrient deposition and fertilization one of the largest threats to biodiversity (Tilman et al. 2014).

The above results regarding habitat-specific extinction probabilities suggest a dominating effect of productivity, as the most prominent difference between habitat types with positive *versus* negative HDRs seems to be soil depth. Soil depth, as well as fertilization, could act as a proxy for productivity as deeper soils could hold more nutrients while fertilization directly adds those. Even though results from experimental tests are not unequivocal, it is theoretically agreed that the productivity-diversity relationship produces a unimodal curve (Fraser et al. 2015). In highly productive systems the diversity is reduced because of competitive exclusion. Introducing a higher heterogeneity those systems would reduce competition and increase diversity and thus highly productive habitats, should profit more from heterogeneity. However, the effects of the single treatments on extinction probabilities showed the expected productivity pattern only for fertilized and unmown treatments, counterintuitively, in homogeneous microcosms deep soils had a lower extinction probability than shallow soils. The important difference leading to this effect between shallow and deep soils in our system might be the greater water storage of deep soils. For homogeneous plots with deep soils increasing heterogeneity reduces the mean soil depth and thus total water storage capacity. During the course of the experiment, generally dry years occurred (316-430 mm/a in 2015-2019 (Tübingen 2021), instead of 932 mm/a (AM Online Projects 2021)). Furthermore, the rainfall in the summer month with the main growing season were below the long-term mean (Table A.2). This dryness probably affected species richness in shallow habitat types stronger than in deep ones. Thus, for homogeneous deep soil microcosms the overall drought stress increased with increasing heterogeneity, and therefore extinction probability might increase as well. These findings are very interesting because they suggest that habitat heterogeneity is not necessarily desirable, and greatly depends on whether the current land use acts on high or low productivity (low or high diversity, respectively) systems, and whether other limiting factors (such as water availability) change the direction of the impact of habitat parameters.

With this experiment I was able to show a change from deterministic dominated extinction events in homogeneous situations to stochastic dominated extinction events in heterogeneous situations. I also found that the habitat type is influencing the strength of the reaction in a way that multiple outcomes of a HDR were possible. So habitat heterogeneity might buffer species richness by combining habitat types reacting differently to heterogeneity.

Chapter 2

Mass effect and area have opposing effects on species richness

The heterogeneity of a habitat is an important driver for the coexistence of plant species but the shape of habitat heterogeneity-diversity relationships (HDRs) remains controversial. One reason may be that the role of different processes such as deterministic *versus* random extinction, habitat identity, and dispersal have not been addressed comprehensively. Here, I use a microcosm experiment in which I manipulated configurational heterogeneity, i.e. the spatial arrangement and size of two habitat types, as well as the contrast between habitats. This experiment also allowed me to test the intermediate difference hypothesis which states that spatial mass effects increase species richness the most when habitats are not too different from each other.

In replicated microcosms simulating temperate grassland communities, I created three levels of heterogeneity, and I added a homogeneous treatment, by means of creating four levels of contrast among habitats. The contrasts were realized by manipulating between one and four habitat conditions (i.e. trampling, mowing, fertilization, soil depth) simultaneously. My experiment was able to dissect the relative role of spatial mass effects, random extinction due to small area, and deterministic extinction due to niche-based processes.

Species richness and extinction probability was constant across heterogeneity levels, irrespective of contras. However, I found support for the 'Intermediate Difference Hypothesis' in that intermediate contrasts between the habitat types lead to the highest richness. Interestingly, this effect interacted with the spatial configuration of habitats and it could only be found at high heterogeneity levels. This indicates that dispersal increased diversity via the spatial mass effect only when distances among habitats were small and habitats differed to some extent, but not too strongly. My experiment showed a strong interaction among habitat quality, scale, and spatial configuration, highlighting the need to include all these aspects of habitat heterogeneity into future experiments.

Introduction

Habitat heterogeneity is thought to be a major determinant of local and regional biodiversity, because it creates opportunities for species with different niches to coexist (Hutchinson 1957, MacArthur & Wilson 2001, Tews et al. 2004). Hence, the positive relationship between species diversity and the heterogeneity of a habitat, is one of the most widely accepted laws in ecology, and many observational studies provide support for this relationship (from now on: heterogeneity-diversity relationship: HDR) (Lundholm & Larson 2003, Hortal et al. 2009b), Stein et al 2014, (Tews et al. 2004, Stein & Kreft 2015)). However, the few experiments testing the effect of habitat heterogeneity on richness revealed contradicting results (Collins & Wein 1998, Gazol et al. 2013, Liu et al. 2019, Ben-Hur & Kadmon 2020a). One reason for these contradicting outcomes may result from ambiguous definitions of the term ’habitat heterogeneity’, including aspects that – if not clearly separated – might obscure important influences on species diversity (Fahrig et al. 2011, Stein & Kreft 2015). Namely, on the one hand, habitat heterogeneity is often referred to as the fragmentation (configurational heterogeneity *sensu* Fahrig et al. 2011), i.e. the spatial pattern of habitat types. On the other hand, habitat heterogeneity can also describe the diversity of different habitat types (compositional heterogeneity *sensu* Fahrig et al. 2011). Both kinds of habitat heterogeneity have been used to experimentally test the HDR hypothesis, but configurational heterogeneity has been studied more often (Collins & Wein 1998, Gazol et al. 2013, Liu et al. 2019). However, the original theory of a positive HDRs is based on the assumption that ’more different habitats support more species’, due to niche theory (Hutchinson 1957), i.e. this hypothesis corresponds to compositional heterogeneity. Therefore, any test of a HDR must clearly define the type of heterogeneity addressed. Furthermore, the mechanisms creating differential diversity patterns could differ between compositional and configurational heterogeneity, which may explain why experiments yielded equivocal results.

A problem with experimental studies of compositional heterogeneity is that they are confounding area with habitat type, i.e. as the number of habitat increases, their size decreases (e.g. Ben-Hur & Kadmon 2020a). This in turn increases the likelihood of stochastic extinction, as postulated in the species-area relationship, another universal law in (MacArthur & Wilson 2001, Hubbell 2006). Experiments using configurational heterogeneity can avoid such confounding when, as is usually the case, two habitat types in varying levels of fragmentation (usually: scales of checkerboard patterns) are created (Collins & Wein 1998, Gazol et al. 2013, Laanisto et al. 2013, Liu et al. 2019) but the area of the habitat types is kept constant. To that end, the analyses should treat (large) homogeneous plots (e.g. Gazol et al. 2013, Liu et al. 2019) separately. Yet, such experiments then have little power of detecting the importance of niche-based processes for diversity, which increase with the number of different habitats. It is therefore desirable to design experiments that manipulate not only the configuration but also the identity of the habitat types used.

Specifically, in addition to the number and spatial configuration of habitat types, the contrast, i.e. the strength of difference between habitat types, determines critically whether or not habitat heterogeneity is actually perceived by an organism, and whether or not it can persist in more than a single habitat. Namely, if two habitat types differ largely, i.e. have a great contrast, then the total number of species in a system should be larger than when differences are small, because the species selected from a species pool would be more different from each other. However, the relationship between contrast and species richness is not only positive. Namely, a species specialized on one habitat and dispersing into a neighboring habitat will have a very low probability of surviving in the other habitat, if the contrast is very large. *Vice-versa*, when the differences among habitats are subtle, i.e. the contrast is low, habitat heterogeneity effects on mortality would be marginal, i.e. fewer species would go extinct. A seminal study illustrating the importance of contrast among habitat types for species richness that was conducted in the Rothamstead Park Grass Experiment has summarized these ideas (Kunin 1998). Namely, the author hypothesized a greater increase in richness at borders between habitats which were not too different than at edges between very different habitats. This hypothesis has led to the so-called ’Intermediate Difference Hypothesis’ which states that species richness in one habitat is highest when adjoining habitat types exhibit an intermediate difference. Specifically, when looking at a gradient of habitat differences, the ’diversity at the edge’ (*sensu* Kunin 1998) will follow an unimodal curve. At the ’high difference’ end, seeds of species dispersing from their preferred habitat into the adjacent one will not survive, while when habitats are extremely similar, their species composition would be very similar (Kunin 1998). Clearly, this hypothesis is highly relevant for studying the effect of habitat heterogeneity on species richness, as it addresses both niche-based processes as well as dispersal among habitats as determinants of species richness. This first test on the one hand supported the existence of mass effects but on the other hand questioned the relevance of it for supporting plant species diversity. Unfortunately, there is virtually no further study that has experimentally tested the intermediate difference hypothesis.

The main mechanism by which dispersal affects species richness is the ’spatial mass effect’ (Shmida & Ellner 1984) which was later coined ’source-sink dynamics’ (Pulliam 1988). It describes the fact that species can exist in ’sink’ sites where they are not self-maintaining because mortality rates exceed birth rates, by continuous immigration from neighboring source habitats. The strength of the mass effect is determined by the properties of the species, for example the amount of seeds and how far those can disperse, as well as by the above-described differences among habitats (Kunin 1998). Yet, dispersal is also affected by the spatial configuration of habitat types, especially by their proximity and the length of the edge, thus linking spatial mass effect theory with the intermediate difference hypothesis and HDRs. Specifically, because with higher (configurational) heterogeneity the amount of borders **between habitats** increases and the connectivity between the patches **within habitats** decreases (Tamme et al. 2010, Laanisto et al. 2013), the importance of mass effects increases accordingly (Smith & Lundholm 2012).

Dispersal is at least partially random and thus unaffected by neighboring habitat conditions (Shmida & Ellner 1984). However, the contrast between two habitat types should have a strong impact on (niche-based) extinction processes, but not on dispersal. I.e. the spatial configuration of habitat types affects dispersal, while the contrast among habitats affects the probability of local extinction. Therefore, experiments combining both different spatial configurations as well as contrasts between habitats could help in dissecting the role of two key processes – dispersal and extinction – on species richness in heterogeneous landscapes: Positive mass effects are caused by increased options for dispersal along the gradient of heterogeneity because the length of the border increases with greater fragmentation. Negative extinction effects increase with increasing intensity of the contrast, because establishment in neighboring plots will become more unlikely with higher contrast even though dispersal will stay the same.

When designing an experiment that manipulates configurational heterogeneity, the scale at which the heterogeneity occurs likely affects the direction of the HDRs as well (Whittaker et al. 2001, Chase & Leibold 2002, Bischoff et al. 2006, Lundholm 2009, Tamme et al. 2010, Gazol et al. 2012). In observational studies, usually a combination of different habitat types is surveyed in a landscape, i.e. at relatively large scales (Fahrig et al. 2011). This is opposed to experiments where the scales of one habitat patch is often reduced down to a few centimeters (Gazol et al. 2013). Clearly, the scale is crucial for the likelihood of dispersal, and inconsistent results from scaling patterns (Lundholm 2009) might be influenced by mass effect. Specifically, at small spatial scales, mass effects play an important role and might obscure HDRs, due to reduced extinctions (i.e. the habitat is actually perceived as homogeneous). At large scales, HDRs might decline, because the sampling units are greater and more distributed at landscape scale and thus, more variance is captured within the sampling units and not among them (Smith & Lundholm 2012). Distinguishing between the effects of area and spatial arrangement thus would help to further understand the processes that structure plant communities and could help to understand scaling patterns as well.

Here, I present the findings of a microcosm experiment that was designed to address the above ideas about heterogeneity, contrast, area, and scale. Namely, by simultaneously manipulating gradients of (i) configurational heterogeneity (with keeping the total area of habitat types constant), and (ii) contrast between the habitat types, I could disentangle the effect of spatial configuration and habitat type on species richness.

I tested the hypothesis that increasing configurational heterogeneity would affect diversity in two opposing manners: larger impacts of mass effects with increasing heterogeneity (Smith & Lundholm 2012) would increase diversity, but larger probability of local extinctions in smaller patches decreases diversity (Kadmon & Allouche 2007). I furthermore hypothesized that the negative effect of area would be strongest when habitat types differ strongly than when differences between habitat types are small. Therefore, the HDR in a system with great contrasts should be less positive than in a system with small contrasts, where configurational heterogeneity may be unimportant.

Microcosm	The microcosm is one artificial grassland of 80 × 80 cm area.
Subplot	The microcosms are divided into 16 regular subplots of 20 × 20 cm area, allowing to arrange habitat types in a regular pattern.
Patch	A single ’chess field’ consisting of one habitat type is called a patch. The number of per habitat type increases with increasing heterogeneity.
Cluster	The overall area of one habitat type within a microcosm is named cluster. It is always half of the area of the microcosm but can be arranged in three different ways along the heterogeneity gradient.
Contrast	The contrast is the strength of difference between two combined habitat types, ranging from low contrast (three of four treatments are identical in the combined habitat types) to very strong contrast (all four treatments are different among the combined habitat types).

Methods

Study system

A long-term experiment was designed in a common garden in Tübingen, south-west Germany (48°32'N, 9°02'E, 465 m a.s.l.) that simulated gradients of (i) configurational heterogeneity, and (ii) contrasts between habitat types at the same time. The microcosms represented temperate European grassland plant communities as these have been studied most intensively and for a long time (e.g. Ellenberg 1954, Grime 1973, Vogt et al. 2019 Rothamsted) they are easy to manipulate, and the species are not too long-lived to exhibit a response to the treatments.

From October to December 2014 the microcosms were established by embedding boxes of 80 × 80 × 40 cm in the ground to create independent artificial grasslands. Within these microcosms, 16 subplots of 20 × 20 cm each were marked to allow regular subsets of different habitat types within one microcosm. The boxes were made of polyethylene, a heat and frost resistant material (Semadeni AG, Ostermundigens, Switzerland). They were placed at a distance of 1 m from each other, with the reinforced edge protruding approximately 5 cm into the ground and were filled with a 3:2 mixture of seed-free soil and sand. The substrate was leveled with the surrounding ground surface in order to create more natural conditions (e.g. near-natural freezing regime in winter). 16 holes of 1 cm in diameter were centered every 20 × 20 cm (i.e. at the center of each subplot), and a >10 cm thick layer of crushed stones below the boxes ensured drainage of water. To prevent weeds from growing near the microcosms, the ground in between them was covered with a water-permeable fabric.

In December 2014, 40 different common grassland species from South-West Germany were sown to create the artificial grassland communities (see Table A.1 for species list, commercial seed supplier: Rieger-Hofmann GmbH, Blaufelden-Roldshausen, Germany). The species were selected to cover a wide range of different functional types and abundance classes. Furthermore, between-species variation in maximum rooting depth offered the potential for below ground niche partitioning. Eight seeds per species were sown into each subplot, ensuring that irrespective of the heterogeneity treatment, the seeds were distributed evenly across all plots. To prevent seed dispersal into and out of the microcosms, they were surrounded by 1.5 m high organza tubes which were open at the top to allow full access for pollinators. Even though seedless soil and organza were used to prevent other species to establish, some entered, therefor the microcosms were weeded regularly, i.e. species emerging the microcosms which were not sown, were removed in a seedling stage.

Design of the gradients

Each microcosm was randomly assigned to one of three configurational heterogeneity levels which were realized in a checkerboard fashion using two different habitat types (Figure 2.8). The checkerboard fields ('patches') differed in size along the heterogeneity gradient and thus were composed out of different numbers of subplots (Figure 2.8). Thus, depending on the heterogeneity level, so called 'clusters' of similar habitat conditions differed in average size of the patches, but the total area of a habitat was kept constant. The levels were named according to the order of the number of homogeneous patches within one microcosm, i.e. one, two, four and sixteen patches, named het 0 – het 3 (Figure 2.8). Note that the level 'one habitat type' was strictly speaking not part of the heterogeneity gradient, but served as baseline information about the maximum number of species within a given habitat type, and for investigating area effects on species richness. Each level of configurational heterogeneity was realized with four different levels of contrast among the two habitat types, i.e. ranging from very subtle contrast (c1) to very strong contrast (c4, Figure 2.9).

The habitat conditions defining the different habitat types were inspired by one natural (soil depth) and three anthropogenic (mowing/grazing, trampling, and fertilization) factors. Previous studies in the same system had indicated that among those factors, soil depth is the most important and trampling has little effect on species richness (Braun et al. unpubl.). Specifically, the habitat types were created as follows: soil depth (deep: 32 cm/ shallow: 17 cm), clipping (yes/ no), trampling (yes/ no), and fertilization (yes/ no).

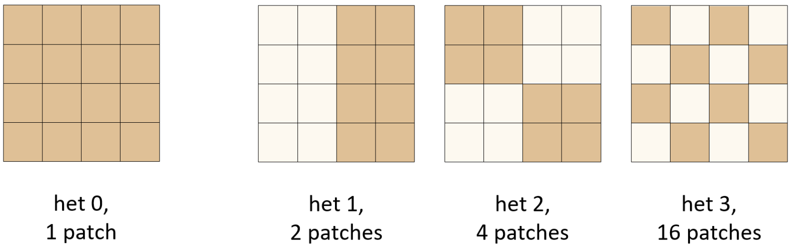


Fig. 2.8 Three levels of configurational heterogeneity (het 1-het 3) combining two habitat types within one microcosm. To control for area homogeneous microcosms only consisting out of one habitat type are included as well (het 0). One microcosm consists of 16 subplots. The habitat types are arranged in a checkerboard pattern and the gradient of configurational heterogeneity is built up by increasing the number of separated habitat patches (2, 4, and 16 patches).

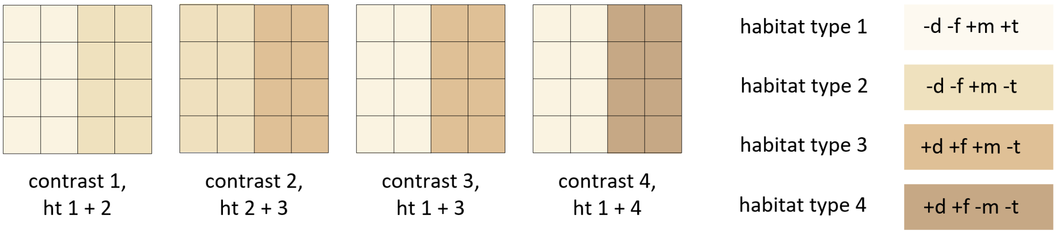


Fig. 2.9 Four levels of contrast combining two out of four habitat types. Habitat types are composed from four different treatments: soil depth (+d = deep soils, -d = shallow soils), fertilization (+f = fertilized, -f = unfertilized), clipping (+m = clipped, -m = unclipped), and trampling (+t = trampled, -t= untrampled). Contrast 1 (c1) is differing in one treatment (t), contrast 2 (c2) in two treatments (d, f), contrast 3 (c3) in three treatments (d, f, t), and contrast 4 (c4) is differing in all four treatments (d, f, m, t).

The soil depth treatment was installed during the preparation of the microcosms in October to December 2014. Subplots assigned to the shallow soil treatment were manipulated by placing a cube of expanded polystyrene (20 × 20 cm width and 18 cm height, EPS, produced by METZ EPS-Hartschaumzuschnitte, Gemmrigheim, Germany) to the bottom of the box. A canal of 1 cm in diameter through the center of the block was aligned with the respective hole in the bottom of the box to ensure water drainage also in shallow subplots. The polystyrene cubes were fixed to the bottom of the microcosm using non-toxic aquarium silicone (Marina Silikonkleber Aquaria, Dow Corning GmbH, D-65201 Wiesbaden) to avoid lateral movement within plots.

The other treatments were applied regularly during the course of the experiment: Fertilization was done with 3.2 g of a slow-release N-P-K fertilizer each year in April (Osmocote® Exact® High End (12-14 M): 15% N + 9% P+ 11% K; Scotts, Geldermalsen, The Netherlands). Clipping was performed twice a year in May/June and August/September. For this treatment the above ground biomass was cut subplot-wise with a pruner 1-2 cm above soil level. The trampling treatment was performed after the clipping treatment twice a year in May/June and August/September. For that a custom made stool was used, which was pressed to each subplot of that treatment (see Figure A.1 for picture).

In order to create the different contrasts, I assumed, based on previous studies (e.g. Blüthgen et al. 2012, Socher et al. 2013) and own data from the microcosms and the field (Chapter 1, Chapter 3), that the treatments differed in the intensity of their impact on plant community structure as follows: soil depth > fertilization >> mowing/clipping >>> trampling. Therefore, the following combinations of treatments were selected to create the four levels of contrast (Figure 2.9): The weakest contrast (c1) only differed in the trampling treatment which is supposed to have the subtlest effect of all treatments, both habitat types had shallow, unfertilized soils and the clipping treatment. The strongest contrast (c4) was realized with the ’most productive’ combination (deep soil, fertilization, no clipping, no trampling) *versus* the ’least productive’ (shallow soil, no fertilization, clipping, trampling), so both habitat types differed in all four single treatments. The second weakest contrast (c2) differed in soil depth and fertilization while both habitat types had the clipping treatment and were not trampled. The first strong contrast (c3) differed in soil depth, fertilization and trampling while both habitat types had the clipping treatment. Each level of configurational heterogeneity and contrast was replicated five times, and 20 homogeneous microcosms, i.e. five replicates per habitat type, were added, which lead to a total of 80 microcosms. The microcosms were allocated randomly over the available area in the common garden.

Data collection

Species richness was scored annually at peak season in summer (July/August 2015-2019) after the first regrowth after mowing. The number of species was determined by recording the presence of the initially sown plant species (which defines the maximum number of 40 species). All investigations took place at the subplot level, irrespective of the heterogeneity level. Therefore, richness could be analyzed at the constant area of the subplot throughout the heterogeneity gradient, additionally to the constant overall area of one habitat type.

Data analysis

For the analysis, only data of the sampling in 2019 was considered, so that the system with perennial plants had a reasonable time to establish. Species richness of the microcosms was calculated based on species data from the subplot level. The subplot level data was pooled along the heterogeneity gradient to calculate the extinction probabilities from the number of species that were extinct from 2015 to 2019, divided by the number of species present in 2015. I used linear models to analyze the influence of the levels of configurational heterogeneity on richness and extinction probability of the microcosm. For extinction probability I differentiated between extinction probability of the microcosm (i.e. the whole system), extinction probability on subplot level (i.e. the smallest investigation unit), and extinction probability of a cluster (the overall area of one habitat type within a microcosm, i.e. half of the area of each microcosm). I also modeled the interaction of levels of configurational heterogeneity with the contrast between the two habitat types. For extinction probability of the clusters I included a second analysis with the interaction of the habitat type. To control for area, I added homogeneous microcosms (het 0) to the analysis and calculated richness and extinction probability of the patches (i.e. a single ’chess field’ consisting of one habitat type). Here I used linear models to test the influence of the patch size (i.e. 80 × 80 cm, 40 × 80 cm, 40 × 40 cm and 20 × 20 cm) interacting with contrast level (c1 – c4) on species richness and extinction probability, respectively. I used model selection to find the most parsimonious model and the Tukey HSD test to identify different groups.

All data analyses were done with R (R Core Team 2020).

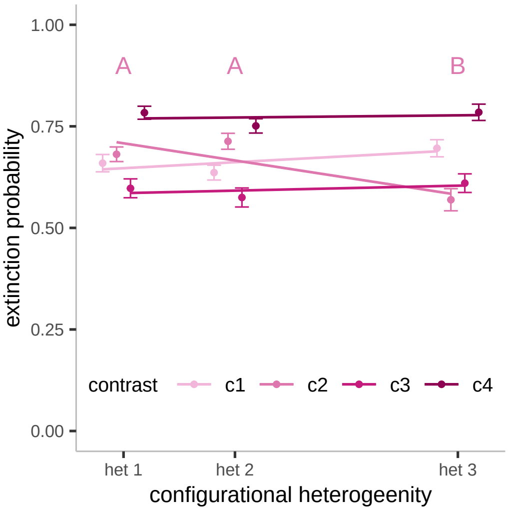


Fig. 2.10 Extinction probability on subplot level differed between the contrasts of the combined habitat types. Contrast c2 was additionally interacting with the level of configurational heterogeneity of the microcosm: with increasing heterogeneity, extinction probability was reduced. Letters A and B displaying significant differences of the means of contrast c2 (Tukey test, $p < 0.05$).

Results

Heterogeneity effect at subplot level

There was a significant interaction between level of configurational heterogeneity and contrast between the habitat types in the effect on extinction probability of the subplots ($p < 2.2e-16$, $F = 20.49$, $df = 888$, adj-$r^2 = 0.13$). The extinction probabilities decreased with heterogeneity when two treatments differed (c2), while no effect of heterogeneity was observed on extinction probabilities within the other contrasts (Figure 2.10). There was no overall effect of the level of configurational heterogeneity on extinction probability of the subplots ($p = 0.37$, $F = 0.81$, $df = 58$, $r^2 < 0.001$, Figure A.2 c).

Species richness at intermediate contrasts

Species richness at microcosm level was neither affected by the level of configurational heterogeneity ($p = 0.80$, $F = 0.068$, $df = 58$, $r^2 = 0.001$, Figure A.2 a), nor by the interaction between level of configurational heterogeneity or con-

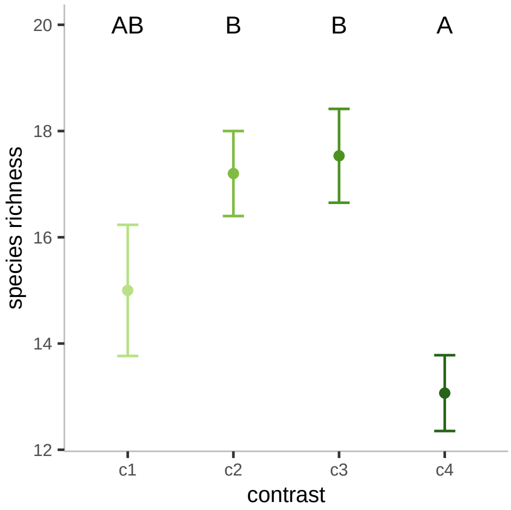


Fig. 2.11 Species richness of the microcosm was dependent on the level of contrast. The intermediate contrast levels have a significant higher richness, than the extreme contrast. Means with common letters do not differ from each other (Tukey test, $p < 0.05$).

trast ($p = 0.04$, $F = 2.319$, $df = 52$, $r^2 = 0.135$). Reducing the model to the most parsimonious one led to a significant difference in contrast between c2 and c3 on one hand and c4 on the other hand ($p < 0.001$, $F = 5.03$, $df = 56$, adj-$r^2 = 0.17$, Figure 2.11) with highest richness at intermediate contrasts. The effect of contrast on species richness was independent of the level of configurational heterogeneity.

Extinction probabilities at intermediate contrasts

There were no effects of the level of configurational heterogeneity on extinction probability at the levels of the microcosm and the cluster (microcosm: $p = 0.98$, $F = 0.001$, $df = 58$, $r^2 = 1.31e\text{-}05$, Figure A.2 b; cluster: $p = 0.5$, $F = 0.45$, $df = 110$, $r^2 = 0.005$, Figure A.2 d). These results for extinction probability mirror the ones of richness (Figure 2.13). Extinction probabilities were highest for the lowest and strongest contrast, and lowest for the intermediate contrasts (microcosm: $p = 0.005$, $F = 4.7$, $df = 56$, adj-$r^2 = 0.16$, Figure 2.12 a; cluster: $p = 1.77e\text{-}06$, $F = 11.24$, $df = 108$, adj-$r^2 = 0.22$, Figure 2.12 b), the level of configurational heterogeneity had no impact.

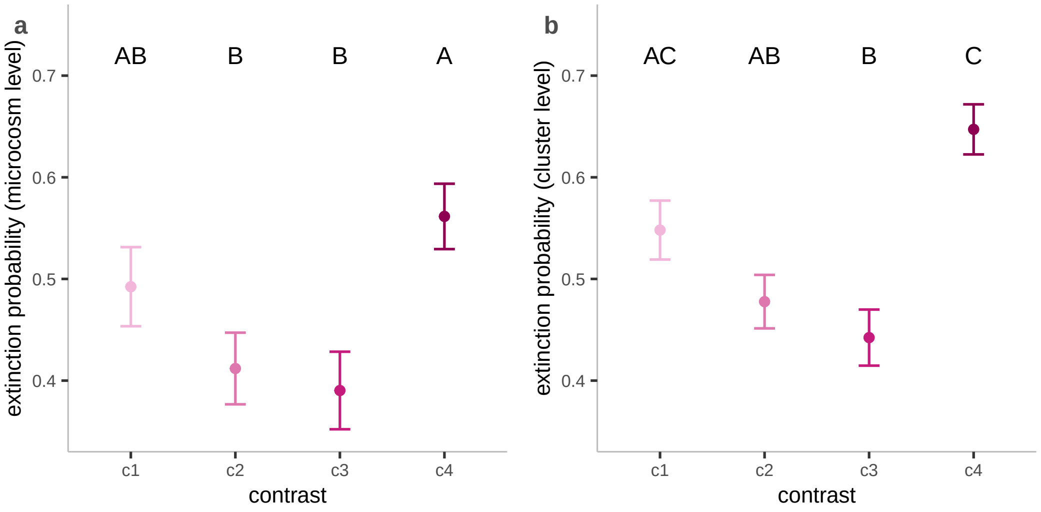


Fig. 2.12 a) Extinction probability of the microcosm as a function of the contrast between the combined habitat types. Means with common letters do not differ from each other (Tukey test, $p < 0.05$). b) Extinction probability of the cluster level as a function of the contrasts between the combined habitat types. Means with common letters do not differ from each other (Tukey test, $p < 0.05$).

Overall, the level of contrast was more important in defining extinction probability than the level of heterogeneity.

Extinction probability for the habitat types

Differences between the habitat types were analyzed at the cluster level, i.e. the overall area of one habitat type. Across the heterogeneity gradient, these differences in habitat types were more important in defining extinction probabilities than the heterogeneity level. On cluster level, habitat type significantly influenced extinction probability ($p = 0.009$, $F = 4.0$, $df = 108$, adj-$r^2 = 0.076$). A Tukey post hoc test showed a significant lower extinction probability for habitat type 3 than for habitat type 4 (p-adj = 0.008), which are both deep soils and fertilized but different in the clipping treatment (Figure 2.13). This means that there was a difference in extinction probabilities of some chosen habitat types, but the spatial arrangement of the habitat types did not influence the extinction probability.

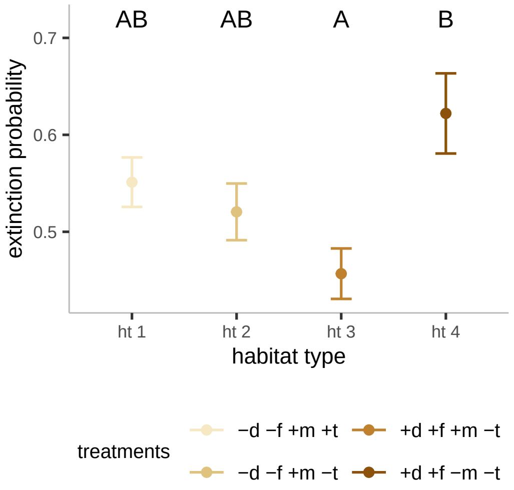


Fig. 2.13 Extinction probability on cluster level as a function of habitat type. Means with common letters do not differ from each other (Tukey test, $p < 0.05$).

Area richness relationship

The area specific analysis showed that richness significantly decreased with a reduction in size of the patches, and that extinction probabilities increased with smaller patches (Table 2.2, Figure 2.14). The least and most productive habitat types in our experiment (ht1 and ht4) had generally lower richness and higher extinction probability than habitats with intermediate productivity.

Tab. 2.2 Species richness and extinction probability for all habitat types as a function of patch size.

Response	F	df	r2	p_{adj}	Combination of treatments	Habitat type
extinction	15.59	168	0.079	0.000	-d -f +m +t	ht1
extinction	7.66	113	0.055	0.007	-d -f +m -t	ht2
extinction	2.13	113	0.010	0.148	+d +f +m -t	ht3
extinction	1.76	58	0.013	0.190	+d +f -m -t	ht4
richness	249.8	168	0.600	0.000	-d -f +m +t	ht1
richness	151.8	113	0.570	0.000	-d -f +m –t	ht2
richness	234.7	113	0.672	0.000	+d +f +m -t	ht3
richness	75.7	58	0.559	0.000	+d +f -m -t	ht4

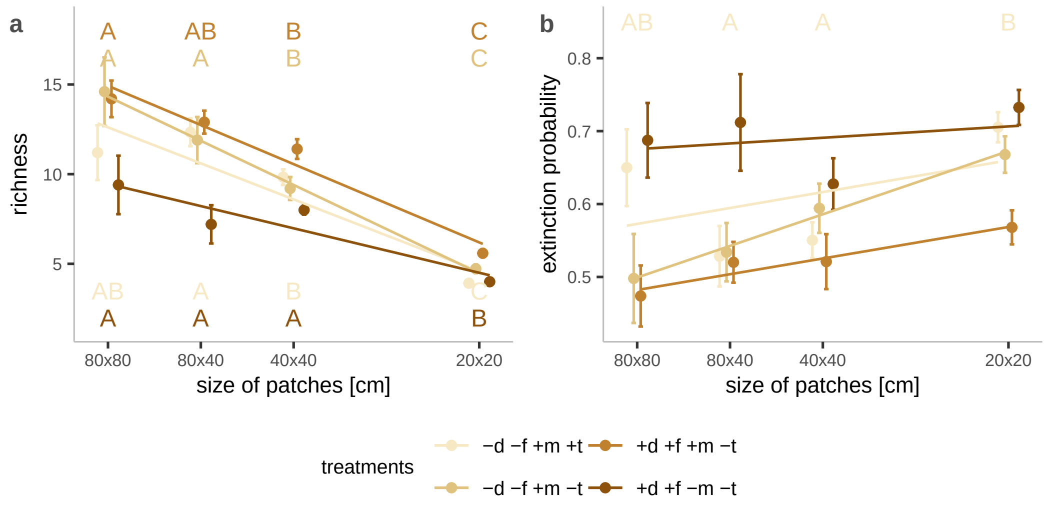


Fig. 2.14 a) Richness and b) extinction probability as functions of patch size. Different letters depict differences between the patch sizes as given from Tukey HSD.

Discussion

Overall, there was no effect of configurational heterogeneity on species richness. However, the level of contrast between habitat types strongly affected richness via extinction and showed that intermediate contrasts supported the highest species numbers. In the following, I discuss these findings with respect to the initial hypotheses.

Configurational heterogeneity is not increasing richness

I found no effect of increasing configurational heterogeneity on richness or extinction probability, irrespective of the scale of observation. Namely, neither between nor within microcosms or subplots. This lack of effect of habitat heterogeneity on extinction and richness was also the same across the four levels of contrast. I therefore conclude, that overall, the spatial arrangement of two habitat types does not influence species richness. This finding contradicts previous studies which found various HDRs. However, I believe that these findings should be interpreted with caution due to several shortcomings of previous experiments. First, most previous experiments which tested the HDR related to configurational heterogeneity instead of compositional heterogeneity (Collins & Wein 1998, Gazol et al. 2013, Liu et al. 2019) and thus looked at only two habitat types. But positive HDRs should result from differences in niches among species and thus require compositional heterogeneity (Hutchinson 1957, MacArthur & Wilson 2001), therefore, a two-habitat gradient of configurational heterogeneity alone may be insufficient to increasing niche space and should thus not produce a positive relationship between heterogeneity and diversity. This may explain the lack of heterogeneity effects in a former study, where two levels of configurational heterogeneity had no statistical differences in richness (Liu et al. 2019). It should be noted though, that these authors concluded a positive heterogeneity effect because the comparison with homogeneous plots of either habitat type showed a greater richness for the heterogeneous plots (Liu et al. 2019). This was despite the confounding with area and is thus strong evidence for niche-based processes (Hutchinson 1957) dominating over area effects (Hubbell 2006).

Influence of chosen habitat combinations on extinction probabilities

Interestingly, the manner in which habitat heterogeneity affected richness (and, mirror-wise, extinction probabilities) differed in a predictable manner among the contrasts between the two habitat types. Namely, at intermediate contrast, species richness was highest, and extinction probability lowest, at subplot level this was true for the highest heterogeneity level. This relationship, which is caused by mass effects (Shmida & Ellner 1984) is the first strong experimental evidence for the suggested ’intermediate difference hypothesis’ (Kunin 1998), but shows at the same time, that contrasts and heterogeneity level interact. I suggest that at low heterogeneity levels, seeds are more likely to disperse within habitats, whereas at the highest heterogeneity level, the closest neighboring subplots belong to a different habitat. Therefore, spatial mass effects should be more prominent at the highest heterogeneity level. They should increase along the gradient of configurational heterogeneity, as the amount of borders, where mass effects happen (Kunin 1998, Metcalfe et al. 2019), increases with higher fragmentation. Overall, my data suggests that the effects of configurational heterogeneity might be strongly dependent on the chosen combination of habitat types. *Vice-versa*, the effect of contrast among habitat types strongly interacts with the scale at which the habitat heterogeneity occurs.

Strength of mass effect

I found that only contrast was responsible for differences in extinction probability and richness at the scales of the microcosm and the cluster. The data supports the ’Intermediate Differences Hypothesis’ as it showed highest richness, and correspondingly lowest extinction rates, at the intermediate contrast levels while significantly differing from the strongest contrast level. This hypothesis proposed strongest mass effects (Shmida & Wilson 1985), and thus a greater increase in richness, in situations where neighboring habitats have intermediate differences in their habitat conditions (Kunin 1998). My results correspond with the results of other studies, even if these did not specifically test the ’Intermediate Difference Hypothesis’ (Hettenbergerová & Hájek 2011, Smith & Lundholm 2012, Metcalfe et al. 2019). Interestingly, my data showed this pattern not only to be true within one habitat type (confirmed by the results at cluster level) similar to previous studies (Kunin 1998, Kadmon & Tielbörger 1999, Hettenbergerová & Hájek 2011, Metcalfe et al. 2019), but also within the whole system (confirmed by the results at microcosm level). This new perspective on the positive results from mass effects on whole systems should be tested further. Since this is the first study of its kind, more experiments are needed to confirm the universality of my findings.

Positive area richness relationship

I found significantly reduced richness with decreasing patch size irrespective of habitat type. This is in line with the species-area relationship which is based on the assumption that smaller areas support smaller populations and thus increase the probability of random extinction (MacArthur & Wilson 2001, Hubbell 2006, Kadmon & Allouche 2007). Interestingly, the area effect on extinction was only significant for the two habitat types created by the less productive treatments. The habitat types created by the more productive treatments (i.e. deep soils and fertilization) exhibited both the lowest and highest extinction rates, irrespective of the heterogeneity gradient. This may be explained by constantly high deterministic extinction in large patches. Here, dominance of habitat specialists and thus competitive exclusion is more likely than in small patches, where stochastic extinction dominates (Ben-Hur & Kadmon 2020b). The relatively low extinction rate along the heterogeneity gradient might be explained by a constant competition release due to the mowing treatment (Socher et al. 2013). Despite the weaker effects on extinction for two habitat types, the trend of increasing extinction probability is significant, supporting the presence of area effects within experiments of configurational heterogeneity even though the overall area of one habitat type stays constant. This area effect could cause negative HDRs found in experiments using configurational heterogeneity (Gazol et al. 2013).

Conclusion

With this experimental setup I was able to separate the mechanisms of dispersal and extinction, which both affect the heterogeneity diversity relationship (HDR). Namely, dispersal is a major determinant of diversity at edges between habitat types, thus when habitat patches are getting small. At the same scale neutral processes that determine stochastic extinction were most important as well. *Vice-versa*, niche-based processes that determine deterministic extinction were mostly important when habitat patches were large. Thus, a most intriguing finding of my study is that spatial configuration, scale, and contrast interact in affecting species diversity. Namely, the importance of spatial mass effects increased with increased configurational heterogeneity, and the strength of the mass effect was dependent on the contrast between to neighboring habitat types in a non-linear way, and that this had been partly predicted by the intermediate difference hypothesis (Kunin 1998). As a consequence, the relationship between configurational heterogeneity and richness can take any shape (positive, neutral, or negative), highlighting the importance of including both contrast, habitat identity and habitat heterogeneity into future experiments related to HDRs.

Chapter 3

Land use structures small-scale habitat heterogeneity but plant species composition reacts on larger scales

Land use changes are the main determinant of the current biodiversity crisis. The direct effects of land use on diversity, like a reduction in diversity with an increase in fertilization, are well known. However, the indirect effects of land use on diversity by shaping habitat heterogeneity are not yet clear, but are vital to obtain a comprehensive picture on impacts of land use changes. Grasslands are the ideal model system for testing effects of land use, as they are a common ecosystem in Europe, display a wide gradient of land use intensity, but are threatened by increased land-use activities. Also, it has been suggested that differences in diversity among differently managed grassland could be mediated by small-scale habitat heterogeneity. Here, I tested this assumption and assessed land use effects on α- and γ-diversity, as well as β-diversity across different scales.

To that end, I investigated the plant species diversity and habitat heterogeneity in grasslands in two regions in Germany along well-described land use intensity gradients. Habitat heterogeneity was inferred from spatial variation in both biotic and abiotic habitat parameters such as plant cover, light availability and soil temperature. Direct land use effects on habitat parameters and species diversity were assessed and compared to indirect effects mediated by habitat heterogeneity.

Land use types markedly affected habitat heterogeneity albeit only at a small spatial scale (<1 m). However, this pattern did not translate into significant effects on plant species diversity. Instead, α- and γ-diversity were consistently and strongly affected by land use. The results suggest a complex picture with scale-dependency of direct and indirect effects, posing the question which mechanisms link increasing habitat heterogeneity to local species diversity across spatial scales.

Introduction

Biodiversity stabilizes ecosystems and therefore supports important ecosystem functions, e.g. biomass production, pollination or carbon storage, (e.g. reviewed by Tilman et al. 2014). However, biodiversity is declining dramatically and the main reason are land use changes (IPBES 2019). For example, agricultural land use is simply increasing in area. Leading to habitat loss for many species, but also the quality, namely intensification of land use via fertilization or excessive use of pesticides is a main cause for species extinctions (Poschlod et al. 2005, Pereira et al. 2010, Habel et al. 2013). Understanding the mechanisms by which land use affects biodiversity is thus important for developing sustainable land management schemes for the future (Habel et al. 2013).

Direct effects of land use on biodiversity have been studied at length, and recommendations for alleviating such effects are in place (e.g. Poschlod et al. 2005, Maurer et al. 2006, Newbold et al. 2015, Chisté et al. 2018). However, land use affects not only specific habitat parameters which in turn relate to species-specific extinction risks, they also affect the spatial arrangement of habitat characteristics. In the following, I will relate to this idea as 'indirect land use effects'.

Spatial community dynamics provide important insights into indirect effects of land-use on biodiversity (Questad et al. 2011). However, those spatial patterns of diversity can be detected on different scales. For example, on a small scale where diversity is measured in one sampling unit, or on a larger scale where it describes the overall diversity of sampling units with greater spatial distances. To distinguish those scale-dependent measurements of diversity, α-, β-, and γ-diversity have been defined (Whittaker 1960, Baselga & Orme 2012). α-diversity reflects the local diversity within one sampling unit (e.g. a small quadrat within a grassland), γ-diversity the overall, ’regional’ diversity of all sampling units within a given larger area (e.g. diversity of an entire grassland), and β-diversity describes the variation in species composition among sampling units. It is a reasonable assumption that α-diversity is determined predominantly by the mean level of ecologically important factors, while β-diversity (the degree of spatial variation in species composition) is determined predominantly by the magnitude of spatial variability (i.e. the variance) in those factors. It is currently unknown, whether land use influences the single parameters of habitat heterogeneity by shifting the mean of the measure (e.g. fertilization decreases average light availability) or by affecting the variance of the measure and therefore the heterogeneity of the parameter (e.g. grazing may increase while mowing may decrease the magnitude of spatial variability in light availability). Practically, this distinction implies that changes in land use may influence both the mean and the variance of ecologically important factors. The changes in mean of ecologically important factors generate differences in species diversity among grasslands subjected to different land use via niche-based deterministic extinction processes (Segre et al. 2014, see also Chapter 1). At the same time, changes in the variance of those factors generate differences in species diversity within grasslands subjected to the same land use and should be directly linked to spatial heterogeneity in habitat conditions (Chisté et al. 2018). In this conceptualization, species γ-diversity is determined by both its α-diversity and β-diversity components.

Temperate grasslands are ideal model systems for looking at direct and indirect land use effects on biodiversity, because they are species-rich, widely distributed, and land-use dependent. In Europe, they are classified as semi-natural systems and under particular pressure of land-use changes (Ellenberg & Leuschner 2010, Pereira et al. 2010, Habel et al. 2013). For all the above reasons, grasslands have been a primary model system for biodiversity research (e.g. Ellenberg 1954, Tilman 1982, Blüthgen et al. 2016).

Classical land-use practices to maintain grasslands are grazing and mowing, but also fertilization to increase the yield. Fertilization of grasslands is commonly associated with a loss of species (e.g. Klimek et al. 2007, Blüthgen et al. 2012, Socher et al. 2013), whilst pastures are generally more species rich than meadows (Bakker et al. 2003). One possible explanation for these relationships between land use and diversity might be the different influences of the land-use practices on habitat heterogeneity which in turn affect diversity.

Habitat heterogeneity is generally assumed to increase plant species diversity, just as direct land use effects, via niche-based processes that reduce the likelihood of competitive exclusion in space (Whittaker & Levin 1977, Tilman 1982, Gigon & Leutert 1996).

Mowing and grazing are disturbances that affect species richness directly, and the best-known hypothesis relating disturbance intensity and frequency to diversity is the 'intermediate disturbance hypothesis' (Connell 1978). However, disturbances also interact with productivity ('dynamic equilibrium model' (Huston 1979)) in that positive disturbance effects dominate especially in productive grasslands where they enable competitively inferior species to coexist with fast growing species (Laliberté et al. 2013). Also, disturbances can have direct effects on species diversity via the removal of individuals, or indirect effects by shaping habitat heterogeneity.

Productivity varies substantially with natural conditions such as local climate (Craine et al. 2012) or soil composition (Pastor et al. 1984), but obviously also with land use such as fertilization (Gough et al. 2000, Laliberté et al. 2013). Similar to the intermediate disturbance hypothesis, the response of grassland diversity to regional gradients of productivity often follows a unimodal shape: at low productivity levels, only few species can survive (Rajaniemi 2003, Fraser et al. 2015), and at high productivity levels competitive exclusion is accelerated and therefore species richness is reduced as well (Grime 1973, Grime 1977, Rajaniemi 2003, Maurer et al. 2006, Klimek et al. 2007, Fraser et al. 2015). Therefore, fertilization of nutrient-poor grasslands is commonly associated with a loss of species (Maurer et al. 2006, Klimek et al. 2007, Kleijn et al. 2009, Socher et al. 2013) as predicted by the productivity-diversity relationship (Tilman & Pacala 1993).

Most importantly, the above land-use practices not only affect the mean values in habitat conditions but also their variance, i.e. they are likely to affect also habitat heterogeneity. For example, grazing is commonly assumed to increase small-scale habitat heterogeneity, i.e. the spatial structure of habitat conditions on a small scale, and create unpredictable disturbances through trampling, deposition of dung and urine, and selective grazing (Bakker et al. 2003, Orwin et al. 2009, Marion et al. 2010). In contrast, mowing is a land-use practice imposing a more homogeneous disturbance regime by affecting all species in the community simultaneously and quite uniformly (Seifan et al. 2012) and reducing species diversity compared to grazing (Gossner et al. 2016). Therefore, the higher richness of grazed *versus* mown grasslands has been attributed to small-scale habitat heterogeneity induced by grazing (Marion et al. 2010, Seifan et al. 2012). However, this assumption has been challenged by studies that found that infertile meadows may have a higher species richness than pastures (Schloz-Murer 2005, Maurer et al. 2006). One explanation may be that grazing represents a less predictable type of disturbance and may thus enable a smaller number of species to adapt to it (Seifan et al. 2012, 2013).

Fertilization is a land use practice that should create indirect effects on diversity, too. Namely, it has been suggested that the observed negative effects of fertilization on species richness could be mediated by a homogenizing effect on resource availability, i.e. it reduces habitat heterogeneity by a reduction of niche dimensions and therefore reduces species diversity as well (Harpole & Tilman 2007). In productive grasslands, the reduced habitat heterogeneity is associated with increased competition for light, space, nutrients and moisture and therefore with reduced species richness (Grime 1973, Grime 1977, Rajaniemi 2003, Maurer et al. 2006, Klimek et al. 2007). However, despite the above previous work, the mechanisms by which fertilization affects diversity via habitat heterogeneity have not been studied in detail (Reynolds & Haubensak 2009).

Overall, the role of small-scale habitat heterogeneity caused by land use for determining species richness in grasslands is understudied (Veen et al. 2008, Gazol et al. 2012, Brandt et al. 2013, Hart et al. 2017). This is surprising because the potential role of small-scale habitat heterogeneity for species coexistence has been acknowledged (Tilman & Pacala 1993) and also put forward many times to explain differences in community composition among grasslands under different land-use practices (Questad & Foster 2008, McGranahan et al. 2012).

Unfortunately, there is yet no systematic study that has compared the attested direct and hypothesized indirect effects on species diversity. One reason may be that habitat heterogeneity is not trivial to measure, and that it occurs across several different spatial scales (Fahrig et al. 2015, Gossner et al. 2016). For example, a straightforward aspect is spatial variation of abiotic habitat parameters such as light availability, soil moisture or nutrition values. However, these affect also biotic measures like plant cover or plant height, which in turn then modify light availability, i.e. heterogeneity is a multivariate measure itself with many non-independent variables. Additionally, all of those parameters bear the potential to be influenced by at least one of the common land-use practices. With my study, I attempted to address this challenge by recording abiotic and biotic aspects of habitat heterogeneity.

Clearly, more theoretical and empirical research is needed to understand the mechanisms by which direct and indirect effects of land use and natural factors affect local diversity. Specifically, a systematic assessment of the relative importance of direct effects on α-diversity *versus* indirect effects, mediated by small-scale habitat heterogeneity, on β-diversity on the total diversity in an area is still missing. Combining direct and indirect effects would help to understand whether, for example, the well-established negative effect of land use intensity on species richness is the sole result of increasing productivity associated with rapid competitive exclusion, or influenced by the homogenization of the habitat.

The goal of this study was to quantify the relative role of direct effects of land use on species diversity, e.g. by the removal of biomass, *versus* indirect effects by shaping habitat heterogeneity at different scales within a grassland. I hypothesize that total richness (γ-diversity) cannot be explained alone by the well-known direct effects of productivity and disturbance on mean habitat conditions (α-diversity), but only by considering their indirect effects on the variance in habitat conditions (β-diversity).

To assess this hypothesis, I studied a system of temperate grasslands in the German Biodiversity Exploratories, a large-scale and long-term study system aiming at investigating land use effects on biodiversity and ecosystem functioning (Fischer et al. 2010). Our study was done in a multi-scale design that encompassed α-, β-, and γ-diversity of species and habitat conditions simultaneously.

I hypothesized that LUI, mowing and fertilization will decrease species diversity via homogenization of habitat conditions across different scales.

Methods

Study system and experimental design

I conducted my fieldwork in two regions: in Hainich-Dün (HAI) in Mai and June 2017 and at the Swabian Alb (ALB) in Mai and June 2018. Both regions are part of the German Biodiversity Exploratories project (Fischer et al. 2010). Within each region, I used 20 plots in differently managed grasslands for my study. The typical land-use practices in Germany are fertilization, mowing and grazing in different intensities and combinations. Our plots covered the entire range of land-use practices in each region in a balanced way. I established the plots of 10 m × 10 m with smaller sampling units in a nested manner: 25 patches of 20 cm × 20 cm were arranged hierarchically within five clusters of 1 m × 1 m (Figure 3.15). This multi-scale design allowed me to test the effects of different land-use practices and their combined effect on α-diversity (the plot mean of species numbers of the patch), γ-diversity (the number of species on plot level), and two levels of β-diversity (dissimilarity in species between patches in one cluster (β_1), and dissimilarity in species between clusters in one plot (β_2)).

Within each patch, I determined the percentage of cover per species. In addition, I recorded the following habitat parameters related to belowground and aboveground resource availability: (i) percentage of cover of dead vegetation, (ii) soil data: depth and temperature difference between 5 cm and 15 cm, soil moisture (mobile TDR (in 2017)/ gravimetric measurement (in 2018)), pH, P, K, and micro nutrients, (iii) plant-related properties: mean plant height (mean of

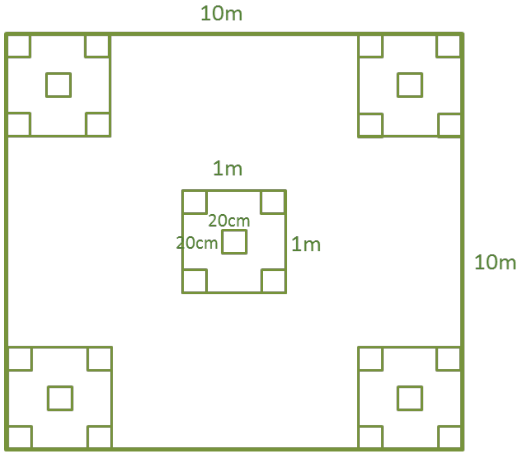


Fig. 3.15 Sampling design in the field: one plot (10 m × 10 m), with five clusters (1 m × 1 m), each with 5 patches (20 cm × 20 cm).

5 points), percentage of light reaching the ground (LI-COR sensor), and above ground biomass. These variables were selected because they were relatively easy to measure in the field, bear the potential to be affected by at least one of the most common land-use practices (fertilization, mowing, grazing), and affect the diversity of species. For detailed parameter sampling see Appendix (page 98).

Calculation of land use intensity

The land-use-intensity index (LUI) was calculated as regional mean of grassland management for the regions ALB and HAI overall for the years of 2006 to 2018 according to Blüthgen et al. (2012), based on information from the land owners on mowing, grazing and fertilization (Vogt et al. 2019) using the LUI calculation tool (Ostrowski et al. 2020) implemented in the Biodiversity Exploratory data base (BExIS, http://doi.org/10.17616/R32P9Q). However, this index has also the disadvantage of combining confounding factors, and thus masking the direct effects of land use practices (e.g. fertilization). Therefore, I also used intensities of the three single land-use practices (fertilization, grazing, mowing) as independent variables.

Calculation of diversities

α-diversity, the 'local' diversity, was calculated as the per-plot average of the species numbers per patch. β-diversity is a measure for the differences in diversity between two or more units of interest and it gives the strength of the spatial differences between communities. I calculated β-diversity for two scales: β_1-diversity on cluster level reflects the small-scale differences between total species richness of the five 20 cm × 20 cm patches within one square meter, and β_2-diversity on plot level reflects the differences in total species numbers between the five 1 m × 1 m clusters within the 10 m × 10 m plot. On both scales β-diversity was calculated using the Bray-Curtis index, a measure of ecological distance for species abundances (Legendre & Anderson 1999) using the package 'betapart' and the function 'beta.multi.abund' (Baselga & Orme 2012). γ-diversity was calculated as the total number of species recorded per plot.

Influence of land use on diversity

To investigate the relationship between land use and diversity (α-, β_1-, β_2-, and γ-diversity), I used linear regressions with the levels of diversity as response variables and mowing, grazing and fertilization, and LUI as discrete predictors.

Influence of land use on habitat parameters

Since the habitat parameters were strongly correlated in some cases, I evaluated the influence of land use on the mean and the variance of the measured habitat parameters by using a redundancy analysis (RDA). First, the mean of each habitat variable was calculated on a plot level using the measured values from the patches (i.e. the mean of the 25 local measurements). The variances were calculated on two levels equivalent to β_1- and β_2-diversity (var1 and var2).

I performed multiple RDAs where the habitat parameters were considered as response variables and each of the single land-use practice as an explanatory variable. They were performed for testing for changes in the mean of habitat parameters, for changes in the variance at the scale of β_1, and for the changes in the variance at the scale of β_2. Additionally, the habitat parameters had to be separated for soil nutrients and other parameters, as the nutrient analysis was only possible for half of the plots. Due to many missing values, the habitat parameter soil moisture was not used for the Hainich-Dün data set. ANOVA like permutation tests were performed for redundancy analysis ('anova.cca') with 999 permutations to obtain significance levels for RDAs. All analysis were performed with the 'vegan' package from R (Oksanen et al. 2019).

Influence of habitat parameters on diversity

To investigate the effects of the changes in the measured habitat parameters, I extracted site scores along the first RDA axis. This axis represents the amount of variation in the measured habitat parameters explained by the single land-use practice (henceforth 'the habitat gradient'). I tested the effect of this habitat gradient on the corresponding diversity measures using linear regression with the site scores as single linear predictors of diversity.

Results

Influence of land use on diversity

γ-diversity (species diversity of the plot) was significantly reduced with increasing intensity of land use. This was true for the gradients of the composite LUI and for the separate effects of mowing and fertilization intensity, but not for grazing intensity (Figure 3.16 a).

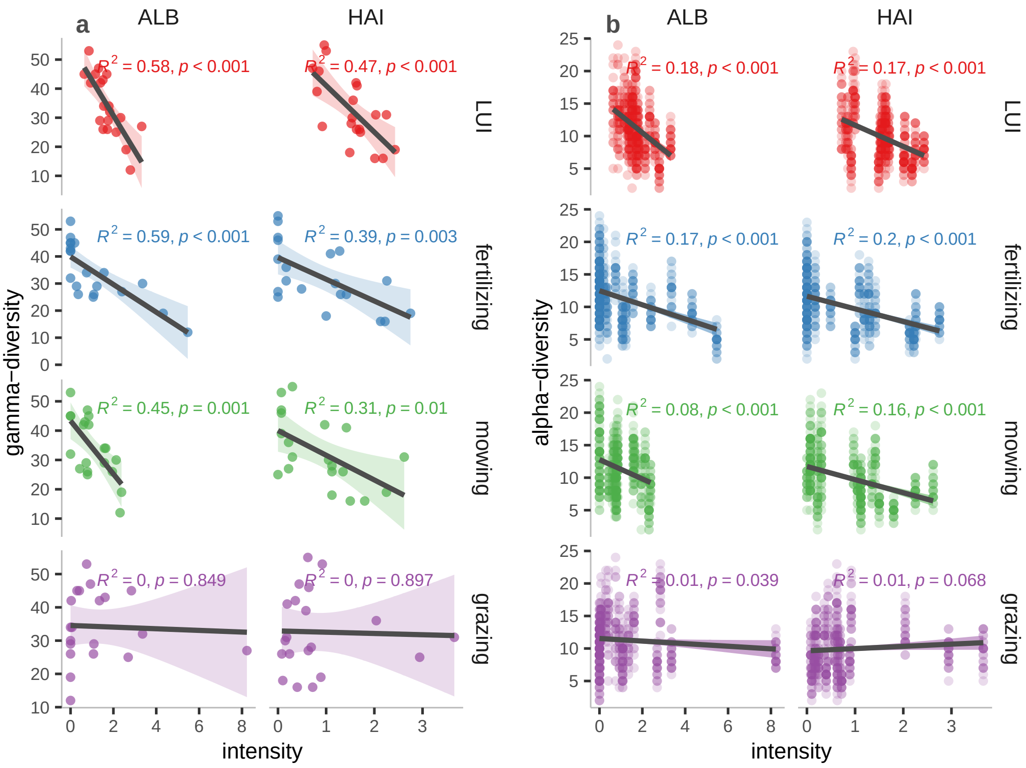


Fig. 3.16 Influence of the different land-use types on a) γ-diversity and b) α-diversity for both Exploratories.

Increasing LUI as well as an increase in either fertilization, grazing and mowing intensity had a consistently negative and a statistically significant effect on α-diversity (mean species diversity of the patches). A single exception occured for grazing in Hainich-Dün (Figure 3.16 b).

LUI had a significant negative effect on β_2-diversity (between clusters in one plot). The same applied to the separate land-use practices grazing in Hainich-Dün, and mowing and fertilization on the Swabian Alb. Even though not significant, fertilizing and mowing in Hainich-Dün had a negative trend on β_2-diversity. Only the trend of grazing on the Swabian Alb was not negative (Figure 3.17 b).

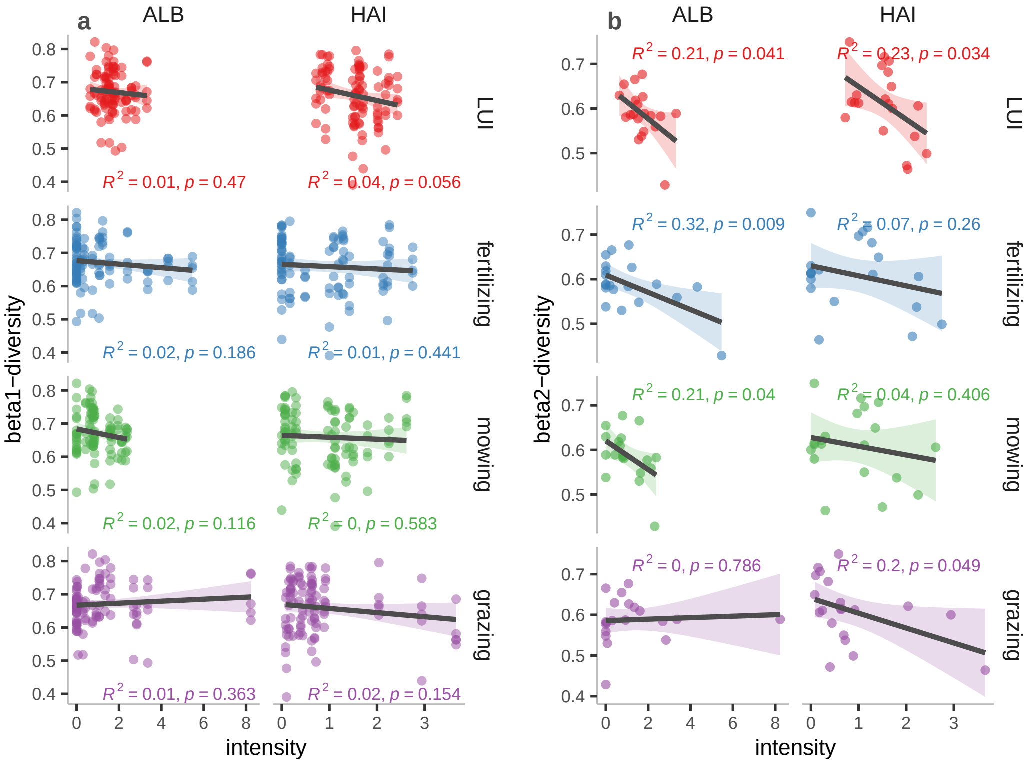
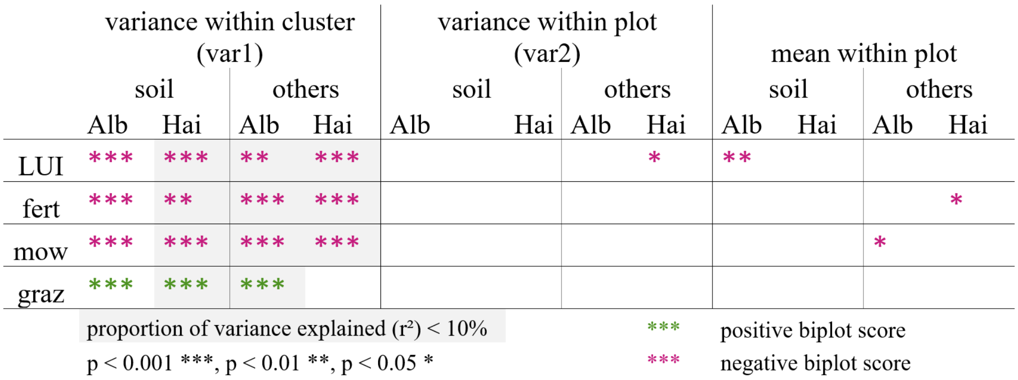


Fig. 3.17 Influence of the different land-use types on a) β_1-diversity, and b) β_2-diversity for both Exploratories.

There was no influence of land use on β_1-diversity (between patches in clusters) in any of the cases, but with a single exception (ALB grazing), the overall effects were consistently negative (Figure 3.17 a).

Influence of land use on habitat parameters

Land use influenced the variance of habitat parameters between patches within one cluster (1 m²) significantly. Similar to the patterns observed for diversity, LUI, fertilization, and mowing affected the variance negatively, while grazing increased habitat heterogeneity. These effects were not detected at larger scales, i.e. between clusters in one plot (100 m²) (Figure 3.18).



	variance within cluster (var1)				variance within plot (var2)				mean within plot			
	soil		others		soil		others		soil		others	
	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai
LUI	***	***	**	***				*	**			
fert	***	**	***	***								*
mow	***	***	***	***							*	
graz	***	***	***									

proportion of variance explained (r²) < 10%

p < 0.001 ***, p < 0.01 **, p < 0.05 *

*** positive biplot score

*** negative biplot score

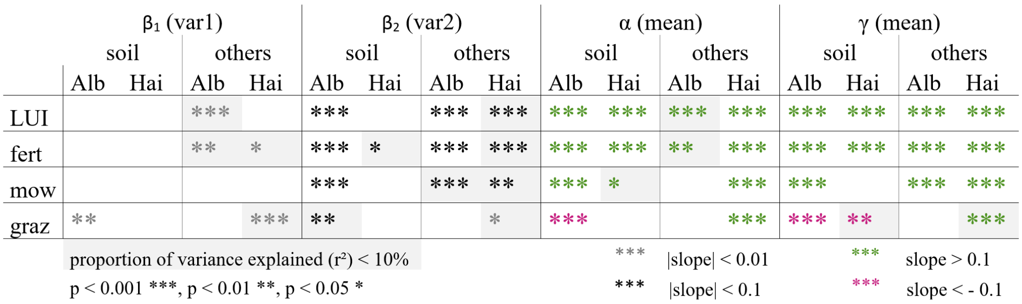
Fig. 3.18 Results of linear models for testing the influence of land use (LUI = land use intensity index; fert = fertilization, mow = mowing, graz = grazing) on habitat parameters. Habitat parameters are classified in two groups: soil related ones (e.g. soil depth and soil temperature) and others (e.g. plant height and cover). The effect of land use on habitat parameters was analyzed at two different scales for the variance of the parameters (i.e. small scale variances within one cluster of 1 m², greater scale variances within one plot of 100 m²), and at the larger scale (plot of 100 m²) also for the mean of the parameters. Only significant values are included in the table, differentiated by the strength of significance. Colors differentiate between positive (green) and negative (pink) relationships. Grey background indicates a low proportion of explained variance.

The variance explained by a single land-use practice (1st RDA axis) was between 5.7% and 27% (mean = 10.5%) for the significant cases of variance within cluster (var1) (Table A.6). Even though the proportion explained dropped in some cases below 10% the overall pattern was highly consistent.

Influence of habitat parameters on diversity

The habitat parameters significantly influenced species diversity at all scales in most of the cases (Figure 3.19). However, the slopes for β-diversity were not much different from zero and the variance explained really low. *Vice-versa*, habitat affected mean values of the habitat parameters on α- and γ-diversity more strongly with larger slopes and r^2 values (Figure 3.19).

The habitat gradients were significant for var1 (on the smallest scale) but generally not for var2 and mean habitat gradients (Table A.6). Additionally, the linear models for the effect of the habitat gradient (site scores along the 1st RDA axis) on β_1-diversity have shown significant relationships only in few cases, in which the r^2 values were very low ($r^2 < 2\%$) as well as the slopes were really small (slope ≤ 0.007) (Table A.7).



	β₁ (var1)				β₂ (var2)				α (mean)				γ (mean)			
	soil		others		soil		others		soil		others		soil		others	
	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai
LUI			***		***		***	***	***	***	***	***	***	***	***	***
fert			**	*	***	*	***	***	***	***	**	***	***	***	***	***
mow					***		***	**	***	*		***	***		***	***
graz	**			***	**			*	***			***	***	**		***

proportion of variance explained (r²) < 10%

p < 0.001 ***, p < 0.01 **, p < 0.05 *

*** |slope| < 0.01

*** |slope| < 0.1

*** slope > 0.1

*** slope < - 0.1

Fig. 3.19 Results of linear models for testing the influence of variance on two levels (cluster (var1) and plot (var2)) or mean (plot level) of habitat parameters on the different types of diversity (β_1, β_2, α, γ) from the same level under the different land use types. Only significant values are included in the table, differentiated by the strength of significance. Small r^2 values are depicted by a greyish background, and greater slopes by green (positive relationship) and pink (negative relationships) colored asterisks).

Discussion

Overall, land-use intensification affected species diversity negatively, and mostly via direct effects on habitat characteristics. However, there were also significant effects of land use on both habitat features as well as on species diversity, indicating a prominent role of indirect land use effects. Interestingly, the manifestation of these effects varies across scales and land use types. In the following, I elaborate on these details.

Influence of land use on diversity

An intensification in mowing, fertilization or LUI lead to a significant reduction in γ-, and α-, diversity. The results that increasing intensities of land use generally reduced γ-diversity, are consistent with previous work, also in the study site (Maurer et al. 2006, Klimek et al. 2007, Blüthgen et al. 2012, Hancock et al. 2013, Socher et al. 2013). The negative effects of mowing and fertilization are not surprising and confirm previous studies (e.g. Maurer et al. 2006, Klimek et al. 2007, Kleijn et al. 2009, Socher et al. 2013, Gossner et al. 2016). Also, in the study system, the two land use intensities are positively correlated, and fertilized grasslands are mown more frequently (Blüthgen et al. 2012). However, for the effects of grazing the picture was not that clear: Generally, grazing is assumed to have positive effects on grassland diversity (Bakker et al. 2003, Orwin et al. 2009, Marion et al. 2010). This positive effect has been mostly shown relative to other land-use practices, e.g. mowing (Socher et al. 2013), or an abandoned treatment (Golodets & Boeken 2006, Speed et al. 2013). In cases where grazing is compared to other land-use practices, it is used as a proxy for low land-use intensity, therefore the positive effect of grazing on diversity is attributed to a low intensity management. Nonetheless, even grazing can arise as a high intensity treatment, which then is supposed to have negative effects on the diversity as well (Klimek et al. 2007, Marion et al. 2010). For this reason, most studies used low to medium stocking rates to infer the effects of grazing on diversity (Bakker et al. 2003, Orwin et al. 2009). In case grazing is assumed to change the effects along an intensity gradient, grazing is regarded as a disturbance and the relationship of the 'Intermediate Disturbance Hypothesis' (IDH) is proposed (Marion et al. 2010). However, along grazing gradients positive relationships with γ-diversity were found (Klimek et al. 2007), as well as a dependence on the nutrient level of the grassland (Proulx & Mazumder 1998, Austrheim & Eriksson 2001). Our results are based on a gradient of grazing intensity which is longer than previous ones and I found no trend in the relationship to γ-diversity. Based on this results I would reject the hypothesis, that grazing per se increases γ-diversity.

With the sampling design I was able to determine α-, and β-diversity, to see if changes in γ-diversity are based on changes in mean diversity of the sampling units (α-diversity) or by changes in the difference between the sampling units (β-diversity). For one case of α-diversity and one case of β_2-diversity we found a negative relationship with grazing intensity. Both were not strong enough to result in an effect of γ-diversity. For LUI, fertilization and mowing α-diversity relationships were as strong as γ-diversity relationships and β_2-diversity relationships much weaker. A homogenization in species diversity was found earlier at a plot scale, comparable to ours (Chisté et al. 2018). Thus I conclude that with increasing intensity of the land use, differences in the vegetation tend to be reduced, even though not on the smallest scale within 1 m^2. The main driver in shaping γ-diversity seems to be the mean species number.

Overall, along the intensity gradient of grazing there is no clear relationship between grazing and diversity. This might be due to counteracting interactions. The effect of land use on plant species diversity was mainly by shaping α-diversity, i.e. the mean species number, than by shaping differences between patches (β-diversity).

Influence of land use on habitat parameters

LUI, fertilization and mowing reduced the variance in habitat parameters between patches of one cluster, grazing increased them. No effect could be found for changes in mean or variance on the greater level.

Indirect effects of land use on diversity are supposed to work via modulations of habitat heterogeneity. Mowing and fertilization are expected to homogenize habitat parameters and thus decrease γ-diversity (Tilman & Pacala 1993, Seifan et al. 2012), whereas grazing is supposed to increase habitat heterogeneity and thus increase γ-diversity (Bakker et al. 2003, Orwin et al. 2009, Marion et al. 2010). I measured a significant homogenization of habitat parameters at the smallest scale indicating that mowing, as well as fertilization, or a general increase in land-use intensity (LUI) have a homogenizing effect on small-scale habitat heterogeneity. Contrary to the other land-use practices grazing should increase habitat heterogeneity, which is proved for the deposition of dung and urine and produced hoof marks (Jaramillo & Detling 1992, Bakker et al. 2003, Orwin et al. 2009, Marion et al. 2010). The idea that grazing increases habitat heterogeneity is supported by my data. I have shown, that grazing leads to a greater variance of the habitat parameters for the smallest scale along the intensity gradient. I therefore could state that grazing leads to greater habitat heterogeneity even at high intensities.

I found the theoretically assumed mechanism of homogenization in habitat parameters by increased LUI, as well as for fertilization and mowing independently, only for the smallest scale between patches (20 cm × 20 cm) in one cluster. This is astonishing in two points: (i) β_1-diversity was neither affected by land-use practices nor by the corresponding habitat gradient, and (ii) β_2-diversity was reduced, i.e. plant species diversity homogenized, even though there was no effect on the corresponding habitat gradient. Overall the direct influence seems to be more important than the indirect one via the structuration of habitat parameters.

Indirect effects of land use on species diversity

Indirect effects of land use are proposed to act via habitat heterogeneity (Maurer et al. 2006). The first step, to show that a modulation of habitat heterogeneity happens along a land-use gradient, was done in the previous section. Those effects on habitat heterogeneity should correlate to the corresponding diversity measure to prove the indirect pathway. However, we did not find this correlation between habitat gradients at the smallest scale (var1) and β_1-diversity. Interestingly, the not significant habitat gradients (var2, mean) did correlate well with the corresponding diversity measures (β_2-diversity, α-, and γ-diversity, Table 2).

Two studies found comparable patterns: while they did find effects of disturbances (grazing and molehills) on habitat structure on the small scale, they did not find corresponding effects on species diversity (Golodets & Boeken 2006, Seifan et al. 2010). Probably, community composition of plants was mainly influenced by environmental gradients, even though the better survival of less well adapted species at disturbed patches might contribute to the maintenance of a higher community diversity (Seifan et al. 2010). However, grazing induced increase in small-scale habitat heterogeneity with the corresponding change in diversity patterns were found as well (Bakker et al. 2003, Deléglise et al.

2011). These contrary results might be due to differences of the mean habitat parameters. We found species α- and γ-diversity to be correlated with the non-significant habitat gradient constitute changes in mean habitat parameters. In productive habitats the effect of light competition seems to be the strongest driver to modulate species richness (Proulx & Mazumder 1998, Bakker et al. 2003). That productivity seems to influence the effect of changes in habitat heterogeneity is not surprising as the effect of disturbances is strongly dependent on productivity and disturbances are the main factor of creating habitat heterogeneity (Jaramillo & Detling 1992, Bakker et al. 2003, Orwin et al. 2009, Marion et al. 2010). Another influence of productivity might be that more productive habitats generally harbor greater plants, which in turn forage greater areas and are thus not dependent on differences in small scales, resulting in no effects of habitat heterogeneity on β_1-diversity (Tilman & Pacala 1993). I could not directly support the idea that land use can indirectly influence species richness via modulation of habitat heterogeneity, but the correlation between species richness and changes in mean habitat parameters hints in the direction of an interaction with productivity. Future studies should thus analyze the influence of habitat heterogeneity in regard to changes in productivity.

Conclusion

Overall, I found neither a positive direct effect of grazing on any diversity measure, nor the correlation of the influenced habitat gradient on the corresponding diversity measure. Reasons for no clear positive relationships might be that grazing is unpredictable in time and therefore a mechanism to which species cannot adapt easily (Seifan et al. 2012), or that the increase in the intensity of grazing (e.g. higher stocking rates, longer duration of grazing, or higher frequency) increases the probability of negative grazing impacts like being eaten or being buried by dung. Additionally, it might be, that effects of habitat heterogeneity are additionally dependent on productivity.

The effect of land use on habitat heterogeneity vanished at the greater scale of the plot (var2). It is known, that high intensity grazing could lead to a homogenization of the vegetation (i.e. reducing β-diversity) and therefore reduce γ-diversity (Austrheim & Eriksson 2001). A certain level of grazing intensity seems to distribute small-scale heterogeneity so evenly over an area, that it vanishes on the greater scale (Austrheim & Eriksson 2001).

Effects of land-use practices on plant species diversity along a gradient of extensive to highly intensive land use are hard to interpret as along this gradient environmental conditions are changing just by the management decision by the farmers, who are always choosing the best suitable practice for the given conditions (Klimek et al. 2007). To choose the management for the highest yield is also the reason, why confounding between certain types of land use is very common, e.g. an association between mowing regime and fertilization (Socher et al. 2013) or between cattle (as opposed to sheep) grazing and grassland productivity leads to non-random distributions of different types of grasslands in the landscape (Klimek et al. 2007). As my data shows that grazing effects on diversity are generally acting in a different direction than mowing and fertilization effects, I recommend to analyze the land-use practices separately in cases were mechanisms are of interest. The combined LUI is masking (positive) effects of grazing on diversity.

I showed for the first time, that grazing is increasing small-scale habitat heterogeneity along a gradient of grazing intensity. However, I found plant species diversity not being affected by land use on this scale. Finding the mechanisms linking this two scales of effects, would help to understand more deeply how land-use practices are affecting biodiversity.

General Discussion

Biodiversity is declining dramatically and the main cause for this decline are changes in land use (Pereira et al. 2010, IPBES 2019). European grasslands, which are dependent on anthropogenic activities (Ellenberg & Leuschner 2010), belong to the most diverse ecosystems in the world (Habel et al. 2013). However, due to changes in land-use, both, single grassland species as well as the habitat type are critically endangered (Finck et al. 2017). The direct effects of land use on diversity have been extensively studied (e.g. (Tilman 1982, Klimek et al. 2007, Questad & Foster 2008, Blüthgen et al. 2012, Socher et al. 2013)). However, land use may also alter habitat heterogeneity (Veen et al. 2008, Gazol et al. 2012, Seifan et al. 2012, Brandt et al. 2013, Hart et al. 2017), which is an important determinant of plant species diversity (Whittaker & Levin 1977, Tilman 1982, Gigon & Leutert 1996). Land-use change may therefore also affect species diversity indirectly through changes in habitat heterogeneity, but such indirect effects are understudied (Blüthgen et al. 2016).

In this thesis, I used an integrative approach combining controlled experiments and extensive sampling of natural grasslands to understand causes, patterns, and consequences of small-scale habitat heterogeneity, caused by land use, on the diversity of grassland communities. This combined approach allowed to extend the mechanistic understanding gained from experiments to the realism of nature.

Short summary of results of the chapters

To investigate the effect of compositional heterogeneity, i.e. the number of different habitats, on species richness, I used a microcosm experiment. This experiment indicated no change of diversity across the heterogeneity gradient, which contradicts all of the existing theories. However, when looking more deeply into the processes determining species diversity, especially extinction, I could find some patterns that were suggested by previous theoretical (Kadmon & Allouche 2007) and experimental (Ben-Hur & Kadmon 2020a) studies. Namely, I showed that deterministic and stochastic extinction acted simultaneously on the plant communities, but that their relative importance varied across the gradient. In fact, deterministic extinction dominated in homogenous habitats, i.e. habitat specialists dominated in such plots. *Vice-versa*, stochastic extinction of all species dominated in the most heterogeneous plots, where habitat patches were small. The contribution of stochastic and deterministic processes to extinction were in line with the predictions of the area-heterogeneity trade-off (AHTO) (Kadmon & Allouche 2007). However, this model predicts a unimodal relationship between richness and habitat heterogeneity. Thus, in contrast to the AHTO, multiple heterogeneity diversity relationships (HDRs) are possible and not only the predicted unimodal one, even when the predicted processes occur.

With my second microcosm experiment, I investigated the effect of configurational heterogeneity on species richness, as well as the effect of contrast between habitat types on diversity. Like in the first experiment, species richness was constant along the gradient of configurational heterogeneity. Interestingly, the contrast between the habitat types influenced species richness. Namely, intermediate contrasts between the habitat types resulted in the highest richness which is in line with the 'Intermediate Differences Hypothesis' (Kunin 1998). Furthermore, the size of the area had a strong effect on the richness, suggesting that confounding between area and heterogeneity might be a common problem in heterogeneity experiments.

The field work in two regions of the German Biodiversity Exploratories was intended to find whether land use affects diversity directly, e.g. by the removal or increase of biomass, or indirectly via the spatial pattern of habitat parameters. I could show that indirect effects indeed occurred but that the direct influence of land use on species richness was more important than the indirect one. However, indirect effects were not unimportant and I found that the land use types structured small-scale heterogeneity of the habitat. Specifically, as predicted by previous studies (Questad & Foster 2008, McGranahan et al. 2012), land-use intensification led to a homogenization of the habitat, and thus amplified the direct effects on diversity. Overall, the mechanisms by which indirect effects occurred remained unclear, and therefore, the question remains which mechanisms shape the interplay of heterogeneity and diversity.

In the following, I elaborate briefly about the implications of my study and directions for further research.

Implications of the results from the experimental chapters (1st and 2nd)

Chapter 1 and 2 show an important positive influence of area on plant species richness (also referred to as ’area effect’) in experimental systems. This positive area effect can affect the results of studies in configurational systems which include homogeneous plots. An increase of species richness from the homogeneous plot to the first level of configurational heterogeneity might be explained by the increase of habitat types (*de facto* compositional heterogeneity). A decrease in richness from the homogeneous plot to the first level of configurational heterogeneity might be explained by the reduction in size of the single habitat types. In the case, that only insignificant differences occur between the following levels of configurational heterogeneity, or that the gradient is very short, the overall trend along the gradient might be driven by the area effect in the first step. This might have happened in some studies (Gazol et al. 2013, Liu et al. 2019). Taking into consideration other experimental studies (Gazol et al. 2013, Laanisto et al. 2013, Ben-Hur & Kadmon 2020a) my results lead to the suggestion that the area effect in configurational systems leads to a negative HDR. For compositional systems the effects of niche and the specific reactions for different habitat types might at the same time result in positive HDRs.

I found the proposed switch from deterministic to stochastic extinctions with decreasing sizes of habitat patches (Kadmon & Allouche 2007, Ben-Hur & Kadmon 2020a). This means the area effect should be considered for the maintenance and protection of valuable habitats. Even though it is necessary to increase the number of habitat types to maximize species diversity, the size of habitat patches should not drop so low that stochastic extinction processes become dominating, or that the habitat is effectively homogeneous (Liu et al. 2019). It is well-known that stochastic extinction especially affects rare species with small populations (Hubbell 2006, IPBES 2019), and these are often those with a particular conservation concern. Yet, for the case of grasslands, I deem it unlikely that specific management practices would lead to a situation where habitat heterogeneity becomes so large that it may threaten rare species. This was also confirmed by the field study which showed some effect of management on habitat heterogeneity, but these effects were rather subtle.

Spatial mass effects are supposed to increase species richness, especially at the borders between different habitat types (Shmida & Wilson 1985, Metcalfe et al. 2019). However, experimental evidence for mass effects is still limited (Kunin 1998, Kadmon & Tielbörger 1999, Metcalfe et al. 2019), and contrasts between habitats, which is important for determining establishment probabilities, have never been manipulated before. Interestingly, I found the properties of the combined habitat types to influence the strength of the mass effect. Specifically, more species were apparently able to establish in a microcosm when contrasts between the habitat types were intermediate, as suggested previously (Kunin 1998, Hettenbergerová & Hájek 2011). In this first experimental study of the ’intermediate difference hypothesis’ (*sensu* Kunin 1998) I confirmed this idea of higher species richness in each of the single habitats due to the mass effect. Furthermore, I found that the overall area of habitats with intermediate differences hold more species in total than the overall area with extremely different habitat types. This could lead to direct implications for general land use practices, especially when very different habitats (e.g. flowering strips and intensive agriculture) are mixed, as is the case in many ’green schemes’ of e.g. the EU CAP (Commission 2021). My work adds to the idea that such a mosaic could result in ecological traps for some species when they have no way of moving out of a habitat without going extinct. My results suggest that buffer areas with habitat conditions intermediate between different kinds of land uses could help in preserving a greater biodiversity on a landscape level.

Implications from the observational study

In the observational study, I showed different land-use practices influencing habitat heterogeneity differently. On the one hand, mowing and fertilization reduced heterogeneity while on the other hand grazing increased heterogeneity within the area of one square meter. This is in line with former hypotheses and studies of the effect of grazing on habitat structure (e.g. (Bakker et al. 2003, Marion et al. 2010, Socher et al. 2013)). However, I found that the direct effects of land use acted on a different scale than the indirect ones via habitat heterogeneity. Namely, α-diversity, i.e. the aspect of diversity which should be influenced by changes in the mean of habitat parameters and not by the heterogeneity, was influenced much stronger by grazing than both scales of β-diversity. Future research should thus focus on how the link between changes in small-scale heterogeneity and species diversity at larger scales could function.

Final Conclusion

Overall, the experimental part was key in disentangling the effects of neutral processes (i.e. area, dispersal, stochastic extinction), and niche-based processes (i.e. habitat identity, habitat contrasts, deterministic extinction) on the manner in which habitat diversity affects species diversity. In future research on HDRs, studies focusing on compositional heterogeneity capture the proposed mechanisms of niche theory (Hutchinson 1957), but may trade-off this advantage against confounding of the above processes. Therefore, experiments manipulating the configuration of habitat types have their merit and help in avoiding area effects, predicted by neutral theory (Hubbell 2006). I could show that in such experiments, the contrast among habitat types as well as the scale of fragmentation may play a key role and interact with the importance of spatial mass effects. Therefore, future studies should include such habitat- and scale-specific effects. Overall, while my experiments and observations showed no evidence for any of the previously suggested HDRs, they provided deep insight into the mechanisms determining species diversity. Finally, the combination of experiments and field observations, as well as the creation of habitat conditions inspired by land use practices helped to ground the theoretical implications in ’real life’, where the goal of land management should be to maximize biodiversity and yield at the same time.

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Appendix

Background information for the experiments (chapter 1 and 2)

Fig. A.1 Picture of the custom made stool to imitate trampling within the subplots of 20 × 20 cm by pressing it to the soil.

Tab. A.1 List of plant species used in the experiments.

species	family
Achillea millefolium L.	Compositae
Agrimonia eupatoria L.	Rosaceae
Anthericum ramosum L.	Asparagaceae
Anthyllis vulneraria L.	Leguminosae
Brachypodium pinnatum (L.) P.Beauv.	Poaceae
Bromus erectus Huds.	Poaceae
Cardamine pratensis L.	Brassicaceae
Centaurea jacea L.	Compositae
Centaurea scabiosa L.	Compositae
Cerastium fontanum Baumg.	Caryophyllaceae
Crepis biennis Lapeyr.	Compositae
Daucus carota L.	Apiaceae
Dianthus carthusianorum L.	Caryophyllaceae
Erophila verna (L.) DC.	Brassicaceae
Euphorbia cyparissias L.	Euphorbiaceae
Festuca pratensis Huds.	Poaceae
Galium verum L.	Rubiaceae
Geranium pretense L.	Geraniaceae
Koeleria pyramidata (Lam.) P.Beauv.	Poaceae
Leucanthemum ircutianum (Turcz.) Turcz. ex DC.	Compositae
Linaria vulgaris Mill.	Plantaginaceae
Lotus corniculatus L.	Leguminosae
Myosotis arvensis (L.) Hill	Boraginaceae
Onobrychis viciifolia Scop.	Leguminosae
Origanum vulgare L.	Lamiaceae
Pilosella officinarum Vaill.	Compositae
Pimpinella saxifrage L.	Apiaceae
Plantago lanceolate L.	Plantaginaceae
Poa annua L.	Poaceae
Prunella grandiflora (L.) Scholler	Lamiaceae
Ranunculus acris L.	Ranunculaceae
Rhinanthus alectorolophus (Scop.) Pollich	Orobanchaceae
Sanguisorba minor Scop.	Rosaceae
Scabiosa columbaria L.	Caprifoliaceae
Sedum acre L.	Crassulaceae
Silene nutans L.	Caryophyllaceae
Stachys recta L.	Lamiaceae
Teucrium chamaedrys L.	Lamiaceae
Tragopogon orientalis L.	Compositae
Trifolium repens L.	Leguminosae

Tab. A.2 Climate data for Tübingen, Germany. Long term means are from AM Online Projects (2021), and values for the duration of the experiment from Tübingen (2021).

time of year	year	amount [mm]
May	2015	36.4
June	2015	50.4
mean	2015	316.4
May	2016	58.4
June	2016	96.6
mean	2016	432.2
May	2017	36.2
June	2017	46.0
mean	2017	421.4
May	2018	55.8
June	2018	26.4
mean	2018	327.6
May	2019	76.6
June	2019	66.0
mean	2019	431.0
May	long term	98
June	long term	86
mean	long term	932

Further results from chapter 1

Tab. A.3 AICc values for models testing mean species richness across all levels of heterogeneity in all years.

model	year	df	AICc	△ AICc
linear model	2015	3	833.27	2.03
quadratic model	2015	4	833.45	2.21
null model	2015	2	831.24	0
linear model	2016	3	861.92	0
quadratic model	2016	4	862.71	0.79
null model	2016	2	862.41	0.49
linear model	2017	3	934.26	0
quadratic model	2017	4	936.26	2
null model	2017	2	937.79	3.53
linear model	2018	3	921.84	1.2
quadratic model	2018	4	923.89	3.25
null model	2018	2	920.64	0
linear model	2019	3	926.47	0.33
quadratic model	2019	4	928.57	2.43
null model	2019	2	926.14	0

Tab. A.4 ANOVA results for extinction probabilities along the heterogeneity gradient (Anova(glm) - function from R package 'car' (Fox & Weisberg 2019)).

extinction probability	LR Chisq	Df	Pr (>Chisq)
2015-2016	6.9604	4	0.1380
2015-2017	6.0520	4	0.1953
2015-2018	1.2328	4	0.8727
2015-2019	1.9543	4	0.7441

Tab. A.5 Total variance explained by the single treatments (mowing, fertilization, trampling and soil depth) as well as the habitat types (i.e. full factorial combinations of the treatments) in the CCA analyses done in separate years (2015-2019).

year	level of heterogeneity	explained variance from CCAs of habitat types	explained variance from CCAs of treatments
2015	1	39.1	13.6
2015	2	22.4	5.4
2015	3	12.2	3.9
2015	4	5.9	2.1
2015	5	2.8	0.9
2016	1	39.3	16.1
2016	2	26.3	9.4
2016	3	13.5	5.1
2016	4	6.4	2.2
2016	5	3.5	1.3
2017	1	40.9	15.8
2017	2	30.9	13
2017	3	13.8	4.8
2017	4	7.1	2.4
2017	5	3.5	1.4
2018	1	40.7	16.1
2018	2	28.5	11.5
2018	3	13.8	5
2018	4	7.4	2.5
2018	5	3.9	1.6
2019	1	42.5	16.5
2019	2	26.3	10.6
2019	3	14.5	5.7
2019	4	7.6	2.9
2019	5	3.8	1.4

Further results from chapter 2

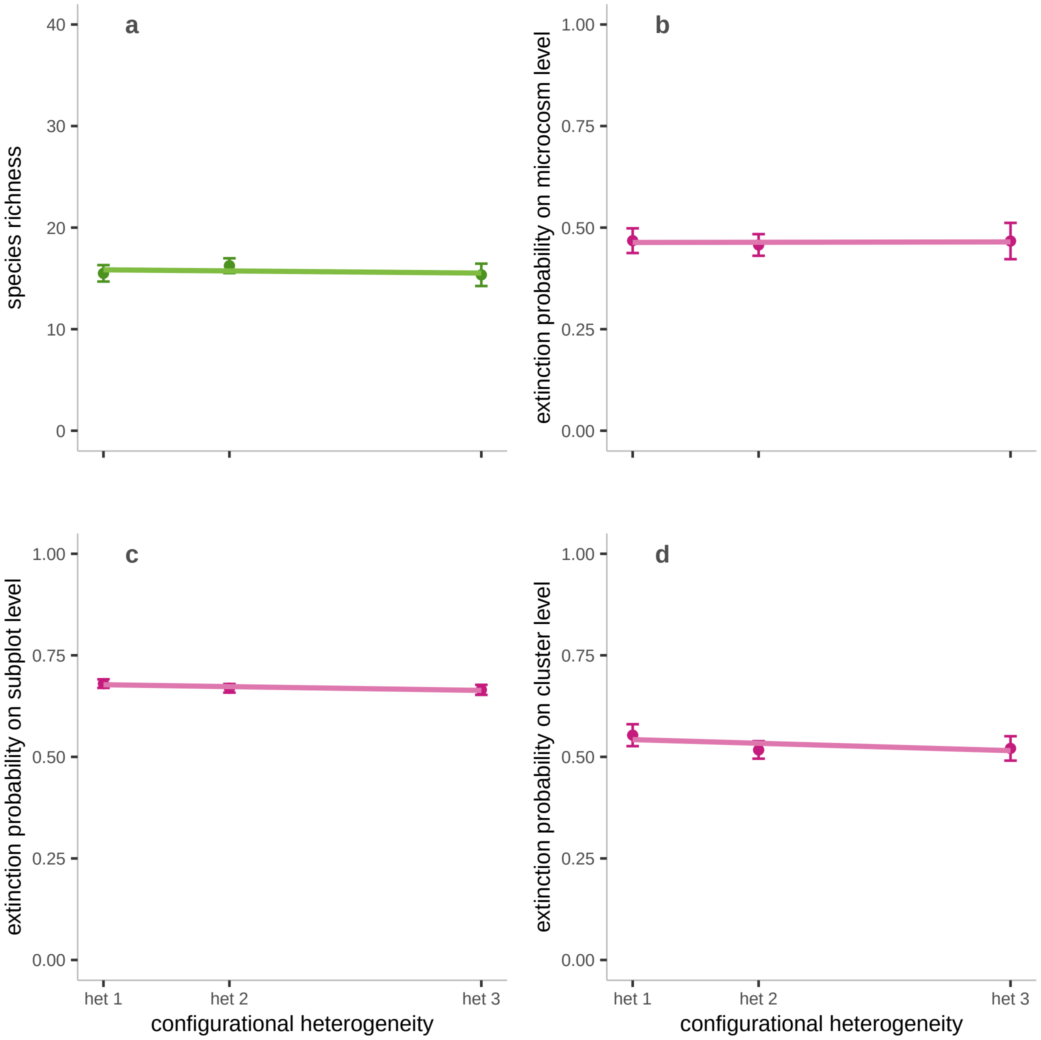


Fig. A.2 a) Mean species richness +/- SE of the microcosm, and b-d) mean extinction probabilities +/- SE of the different levels (b) of the microcosm, c) of the subplot, d) of the cluster) as functions of the level of configurational heterogeneity.

Further results from chapter 3

Tab. A.6 Results of linear models for testing the influence of land use (LUI = land use intensity index; fert = fertilization, mow = mowing, graz = grazing) on habitat parameters. Habitat parameters are classified in two groups: soil related ones (e.g. soil depth and soil temperature) and others (e.g. plant height and cover). The effect of land use on habitat parameters was analyzed at two different scales for the variance of the parameters (i.e. small scale variances within one cluster of 1 m^2, greater scale variances within one plot of 100 m^2), and at the larger scale (plot of 100 m^2) also for the mean of the parameters.

		var1				var2				mean			
		soil		other		soil		other		soil		other	
		Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai
LUI	signif. of model	0.001	0.001	0.002	0.001	0.171	0.114	0.660	0.019	0.003	0.330	0.133	0.023
	explained variance	0.270	0.068	0.063	0.112	0.160	0.106	0.040	0.135	0.296	0.080	0.083	0.151
	biplot score	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
fert	signif. of model	0.001	0.002	0.001	0.001	0.362	0.208	0.299	0.076	0.092	0.419	0.158	0.023
	explained variance	0.185	0.062	0.058	0.098	0.125	0.097	0.063	0.102	0.202	0.071	0.077	0.136
	biplot score	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
mow	signif. of model	0.001	0.001	0.001	0.001	0.418	0.339	0.078	0.125	0.169	0.374	0.011	0.106
	explained variance	0.158	0.065	0.105	0.072	0.112	0.081	0.093	0.089	0.172	0.078	0.129	0.099
	biplot score	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
graz	signif. of model	0.001	0.001	0.001	0.136	0.300	0.105	0.492	0.411	0.398	0.123	0.174	0.826
	explained variance	0.100	0.096	0.057	0.019	0.134	0.121	0.049	0.056	0.109	0.120	0.076	0.026
	biplot score	1	1	1	1	1	1	1	1	1	1	1	1

Tab. A.7 Results of linear models for testing the influence of variance on two levels (cluster (var1) and plot (var2)) or mean (plot level) of habitat parameters on the different types of diversity (β_1, β_2, α, γ) from the same level under the different land-use types.

		beta1 (var1)				beta2 (var2)				alpha (mean)				gamma (mean)			
		soil		other		soil		other		soil		other		soil		other	
		Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai	Alb	Hai
LUI	p	0.867	0.194	0.000	0.264	0.000	0.054	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	r^2	0.000	0.002	0.011	0.001	0.115	0.018	0.145	0.054	0.806	0.163	0.092	0.128	0.670	0.255	0.246	0.236
	df	698	1026	1398	1296	138	206	278	260	68	96	138	123	68	102	138	129
	slope	0.000	0.003	0.007	0.003	0.026	0.009	0.021	0.021	3.287	1.138	0.979	1.381	11.628	5.160	5.238	6.300
fert	p	0.302	0.586	0.002	0.048	0.000	0.041	0.000	0.001	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
	r^2	0.002	0.000	0.007	0.003	0.249	0.020	0.142	0.038	0.811	0.122	0.051	0.159	0.686	0.169	0.247	0.241
	df	698	1026	1398	1296	138	206	278	260	68	96	138	123	68	102	138	129
	slope	0.003	-0.001	0.005	0.004	0.034	0.012	0.020	0.014	2.985	1.248	0.699	1.269	10.652	5.287	5.068	5.266
mow	p	0.134	0.565	0.256	0.175	0.000	0.466	0.000	0.004	0.000	0.050	0.358	0.000	0.000	0.051	0.000	0.000
	r^2	0.003	0.000	0.001	0.001	0.280	0.003	0.054	0.031	0.563	0.040	0.006	0.176	0.504	0.037	0.106	0.251
	df	698	1026	1398	1296	138	206	278	260	68	96	138	123	68	102	138	129
	slope	0.004	-0.001	0.002	0.003	0.035	-0.004	0.014	0.012	2.409	0.636	0.270	1.279	8.839	2.226	3.693	5.098
graz	p	0.010	0.092	0.335	0.000	0.003	0.117	0.751	0.026	0.000	0.117	0.128	0.000	0.000	0.013	0.925	0.010
	r^2	0.009	0.003	0.001	0.019	0.063	0.012	0.000	0.019	0.416	0.025	0.017	0.125	0.325	0.059	0.000	0.050
	df	698	1026	1398	1296	138	206	278	260	68	96	138	123	68	102	138	129
	slope	0.005	-0.004	-0.001	0.007	0.013	-0.008	0.001	-0.007	-1.631	-0.486	-0.343	0.764	-5.597	-2.695	0.070	1.648

Detailed data sampling in the field

Sampling took place in Mai and June 2017 in Hainich-Dün, and Mai and June 2018 at the Swabian Alb. The main investigator always was Ronja Ratzbor with one main scientific assistant per season (Ronja Wedegärtner in Hainich-Dün and Florian Hofmann on the Swabian Alb) as well as one further helping person at single days. Measurements were always done by the same person to minimize the variance between samples.

Species composition, species cover, and cover of dead vegetation

On each plot I determined species composition within each of the 25 sampling units of 20 × 20 cm by visual detection (Figure A.3a). Species were recorded by the second person. After naming the last species no longer than two further minutes were used to look for missed individuals. Directly after that from above the total cover in % of the area of each species was recorded. The recording clerk ensured to mention every species and also the cover of dead plant material.

(a) Determination of plant species diversity by RR.

(b) Determination of plant height by RW.

(c) Determination of light availability by FH.

Fig. A.3 Plant related data sampling

Plant-related properties

To measure the mean plant height a folding rule was held to each patch in the corners and the middle (5 points) and the height of the closest individuals was measured (Figure A.3b). The mean was built out of this points.

To get the percentage of light reaching the ground a LI-COR sensor (LI-250A + Quantum-Sensor LI 190, LI-Cor Inc. Lincoln, Nebraska, USA, sales partner Germany: LI-COR GmbH, Bad Homburg) was placed at least 20 cm above the highest vegetation and directly afterwards on ground level, each at the middle of the patch (Figure A.3c). The light availability on top was set to 100 % and using the light availability on the ground the percentage reaching there was calculated.

After this measurements of the plant related properties the above ground biomass was removed with an edge trimmer (Bosch Isio) at 2 cm above soil surface (Figure A.4a). The biomass was filled patch wise into paper bags and stored aerial in batch boxes until further drying. Weighting of the biomass was done with a fine scale (Kern Präzionswaage EW820-2NM KERN & SOHN GmbH, D-72336 Balingen, precision of 0.01g) after 72 hours at 70 °C in the direr (memmert Reinraum-Trockenschrank UF750, Memmert GmbH + Co. KG, D-91126 Schwabach, and Laboratory drying oven T20 Thermo electron LED GmbH, D-63505 Langenselbold).

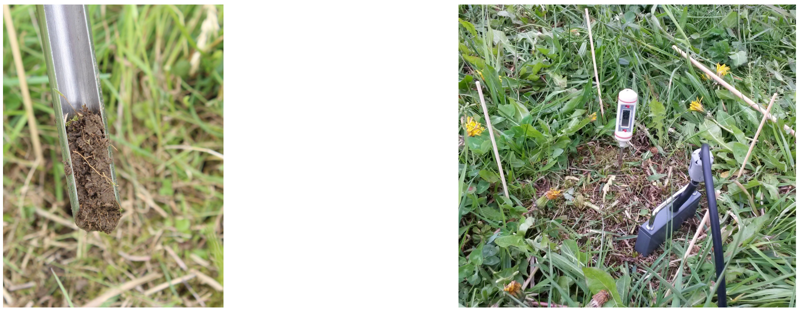
Soil data

Soil depth was revealed by hammering a special nail to the ground as far as possible but to maximum of 40 cm. The scale enabled me to get the local soil depth with an accuracy of 1 cm. It was always taken from the middle part of the patch (Figure A.4b).

Temperature difference was calculated from the measured temperatures in 5 cm and 15 cm depth. The measurement was done with a roasting thermometer (Topelek Digital Cooking Thermometer, model: TECP098AB) which measured the temperature (-50 °C to +300 °C) only in the tip of the nail to an accuracy of 0.1 °C. The thermometer was put to the wanted depth and left for a short time period until the temperature was not changing anymore. Both measurements were done next to each other (Figure A.4b, Figure A.4c).

(a) Subplot with removed biomass

(b) Determination of soil depth and soil temperature



(c) Soil core freshly taken

(d) Determination of soil moisture and soil temperature

Fig. A.4 Soil related data sampling

To measure the soil nutrients soil samples were taken from each patch with a standard soil corer (Ø 22 mm). Due to regulations at the Exploratories only soil samples from the upper 5 cm were allowed (Figure A.4c). As the soil core only had the small diameter 5 samples per patch were taken randomly, removed from mosses, roots, or stones and put into a paper bag into a closable plastic bag to keep the moisture until opened.

For soil moisture different kinds of measurements were taken in both years. In Hainich-Dün a mobile TDR (TRIME-FM, IMKO Micromodultechnik GmbH) was used (Figure A.4d). But as soils were often hard and stony the device was hard to use and broken at some working days. Therefore, a gravimetric measurement was chosen for the Swabian Alb were soils were expected to be even more stony. For the gravimetric measurement soil samples for the nutrient analyses were reused. Each evening they were weighted with a fine scale (Kern Präzionswaage EW820-2NM KERN & SOHN GmbH, D-72336 Balingen, precision of 0.01g) and then air dried. After the field period all soil samples went to the drier at 40 °C for 72 hours and were weighted afterwards with the fine scale again. The water content then was calculated from the difference of both measurements.

After the weighting, soil samples were grinded with mortar and pistil and sieved to < 2 mm grain size. Small roots were removed. Then they were prepared for measuring the pH and the following soil nutrients: phosphorus (P), potassium (K), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), zinc (Zn).

The soil pH was measured using a pH-meter (Mikroprozessor pH-Meter 537, WTW, Xylem Analytics Germany Sales GmbH & Co. KG., 82362 Weilheim, Germany) in a 1:2.5 (w/w) soil to 0.01 M CaCl2-solution. Samples were shaken horizontally for 2 hours at 200 epm. After the soil settled the measurement was done with the electrode in the clear solution for 2 min until the pH-meter showed a stable value.

The extraction of the nutrients was done using Mehlich 3 extraction (Pierzynski 2000). Analysis of the extracts was done by ‘Inductively Coupled Plasma Emission Spectroscopy’ (Perkin Elmer Optical Emission Spectrometer, Optima 5300 DV) using blanc samples, multielement standard solutions, and blind samples of extracting solution. The calculation of nutrient concentration in the samples followed again the Mehlich 3 procedure (Pierzynski 2000).