ZENTRUM
FÜR BIODIVERSITÄT UND NACHHALTIGE LANDNUTZUNG
SEKTION
BIODIVERSITÄT, ÖKOLOGIE UND NATURSCHUTZ

– Centre of Biodiversity and Sustainable Land Use –
Section: Biodiversity, Ecology and Nature Conservation

Effects of land-use intensity in grasslands on diversity, life-history traits and multitrophic interactions

Dissertation zur Erlangung des Doktorgrades der
Mathematisch-Naturwissenschaftlichen Fakultäten der
Georg-August-Universität Göttingen

vorgelegt von
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Lübeck

Göttingen, Februar 2012

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CHAPTER I

General introduction

EFFECTS OF LAND-USE INTENSITY IN GRASSLANDS

Most European grasslands developed through anthropogenic traditional farming (WallisDeVries et al. 2002) and management of grasslands by grazing and mowing prevent successive conversion from grasslands to forests (Balmer & Erhardt 2000). Nutrient poor grasslands developed to an important habitat for many insect taxa, but became very rare in the last decades (van Swaay 2002). Grasslands have undergone severe changes after the Second World War when land-use intensity started to increase due to (a) application of chemical fertiliser, (b) higher numbers of livestock and longer grazing periods, and (c) frequent mowing with machinery (Vickery et al. 2001). Chemical fertiliser application increases the biomass and allows a higher yield (Tilman et al. 2002), but enhances the growth of only few competitive plant species and inhibit slow growing specialised plants, which affects many herbivores and pollinators (Vickery et al. 2001; Kleijn et al. 2009). High grazing intensity and mowing frequency destroy individuals of insect taxa with low mobility and the habitat and food resources for most herbivorous insects and consequently reduce insect species richness and abundance (Vickery et al. 2001; Kruess & Tscharntke 2002; Dennis et al. 2008; Marini et al. 2009). Increased land-use intensity is identified as one of the major drivers for biodiversity loss worldwide and led to a rising number of threatened species (van Swaay et al. 2006; Kleijn et al. 2011), which is predicted to increase in the future (Biesmeijer et al. 2006; Krauss et al. 2010). But not all insect species are similarly affected by increasing land-use intensity, while many species are disadvantaged few species seem to benefit (McKinney & Lockwood 1999). Due to different ecological and life-history trait characteristics species react differently to increasing land-use intensity. Insects on extensively managed grasslands have to deal with low resource availability, while insects on intensively managed grasslands have to cope with a high habitat disturbance and a recurrent food resource removal. Habitat stability allows the specialisation of species in space and time, while habitats with frequent disturbances only allow generalists to survive (Clavel et al. 2010), which can use a broad range of habitats or food plants. Therefore increasing land-use should benefit—or at least harm to a lesser extent—species with traits characteristics specific to generalists (e.g. broad food niche, high reproductive potential) (Clavel et al. 2010; Kleijn et al. 2011).

As species are interacting with each other, different levels of land-use intensity can impact such interactions among species. Effects of different abiotic conditions in environments of distinct regions or different management intensities can change the impact of a single species on another species. This change can cascade up the food chain and thus influence multitrophic interactions (Tylianakis et al. 2008).

I asked in my study, how land-use intensity in grasslands in different regions affects species richness and species abundance and the occurrence of endangered species of butterflies and day-active moths (chapter II), ecological and life-history traits of butterfly communities (chapter III) and multitrophic interactions with focus on an endophytic fungi-grass symbiosis, plant sucking aphids and their natural enemies (chapter IV).

STUDY DESIGN AND ORGANISMS

Biodiversity exploratories and land-use intensity

The “biodiversity exploratories” is a research project funded by the DFG and aims to understand the role of land-use intensity and management for biodiversity and ecosystem processes (Fischer et al. 2010). In three regions, observations and experiments are conducted on a total of 150 grasslands of different land-use intensity, which allows uncovering different effects of land-use intensity and regional dependency.

The three regions are as follows: (1) The Biosphere Reserve Schorfheide-Chorin is located in the lowlands (altitude: 3-140 m) of North-eastern Germany. It is glacially formed with many moors and fens. The mean annual precipitation is low (500-600 mm), which makes it one of the driest areas in Germany. The region was formerly intensively managed, but has the status of an UNESCO Biosphere Reserve since 1990. (2) The hilly national nark Hainich (UNESCO Natural Heritage since 2011) and its surrounding areas are situated in Central Germany with an altitude of 285-550 m. Apart from the national park the surrounding region is intensively managed, but has also some extensively managed calcareous grasslands. The annual precipitation is 500-800 mm. (3) The UNESCO Biosphere Reserve Schwäbische Alb (since 2009) is located in the Swabian Jura low mountain range in South-western Germany on an altitude of 460-860 m with a mean annual precipitation of 700-1000 mm. This region is very heterogeneous, dominated by grasslands with little forest patches and small arable fields. Some calcareous grassland is still grazed by traditional sheep herding. Mean annual temperatures do not differ greatly between the region (Schorfheide: 8-8.5°C, Hainich: 6.5–8°C, Alb: 6-7°C).

In each region 50 selected grasslands managed by farmers cover a gradient from very extensive (Fig. 1.1a) to very intensive land-use intensity (Fig. 1.1b) representative for Central European land-use practices. The grasslands are either mown (meadows), grazed by different livestock mostly cattle, sheep and in the Alb rarely by horses (pastures). Some grasslands are also grazed and mown (mown pastures). The grasslands are either unfertilised or are treated with a different amount of fertiliser.

Figure 1.1 Two representative grassland sites of contrasting land-use intensity. **(a)** Extensive and **(b)** intensive land-use intensity

The land-use intensity is assessed by using a land-use intensity index (LUI) (Blüthgen et al., unpublished manuscript). The LUI combines the amount of fertiliser (F), mowing frequency (M) and grazing intensity (G), based on detailed information of yearly repeated questionnaires of all land-owners and land-users. Amount of fertilizer F_i is calculated by the kg nitrogen N per hectare per year. Mowing M_i is the number of cuts per year. Grazing intensity G_i represents livestock units differently weighted for sheep, cattle and horse per hectare multiplied by the grazing duration in days per year. For each study site *i* each individual LUI component (F_i, M_i, G_i) was standardised relative to its mean of the corresponding region *R* (F_R, M_R, G_R)

$$LUI_i = \frac{F_i}{F_R} + \frac{M_i}{M_R} + \frac{G_i}{G_R}$$

I used the LUI for calculations in chapter II and chapter III and conducted observations on 137 grasslands sites. For the study on multitrophic interactions in chapter IV I chose 40 grasslands in the region Hainich and Alb, in each region ten very intensively and ten very extensively managed ones. The size of the experiment did not allow the exploitation of the whole gradient.

Lepidoptera

Lepidoptera are one of the widest recognized and best studied insect groups worldwide and classically grouped in “butterflies” (Fig. 1.2a) and “moths” (Fig. 1.2b). I surveyed all day-active Lepidoptera, which includes all butterflies and some moth species, because most moths are night-active. Most adult butterflies and day-active moths are specialised flower visitors (Weiner et al. 2011). Larvae feed on plants and some are highly specialised herbivores (Ehrlich & Raven 1964), which makes plant species rich calcareous grasslands one of the most important habitat for Lepidoptera (van Swaay 2002). Lepidoptera are a good target group for investigations about land-use intensity effects on species richness and abundance (chapter II), because they react sensitively on environmental changes even faster than plant species (Biesmeijer et al. 2006; Krauss et al. 2010) and are considered being a good indicator group (Thomas 2005). For day-active Lepidoptera a good knowledge about their species threat status is available in the Red Data Lists of Germany, which enabled me to investigate land-use intensity effects on the occurrence of endangered species (chapter II). Data on ecological and life-history traits are better available for butterflies than for any other insect group (Thomas et al. 2004), which makes butterflies a good group for investigating the effects of increasing land-use intensity on adaption strategies of communities (chapter III).

Figure 1.2 Two day-active Lepidoptera species. **(a)** The butterfly *Melanargia galathea* and **(b)** three individuals of the diurnal moths *Adscita geryon*, both species preferring extensively managed grassland sites.

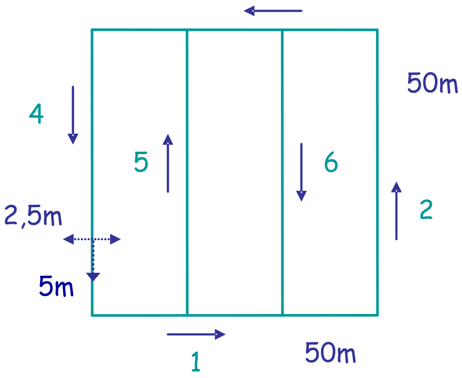

Figure 1.3 Design of a transect on a grassland site. Lepidoptera were caught on 300 m within 30 min. Transects were divided in 6 parts each 50 m of 5 min.

I conducted butterfly and day-active moth surveys on 137 grasslands in the three regions from May to August 2008 with three repetitions. I walked transects of 300 m length within 30 min and caught butterflies and day-active moths by sweep netting 2.5 m each side and 5 m in front of me (Fig. 1.3).

Endophytic fungi

In chapter IV I investigated a multitrophic system with endophytic fungi growing in grasses, grass sucking aphids and their natural enemies. Microorganisms such as endophytic fungi make up an additional trophic level and can have severe effects on herbivores via the host plant quality (Hartley & Gange 2009). I was especially interested, if different land-use intensity, regional effects or predator preferences alter the effects of endophytes on herbivores.

Many cool-season grass species are infected with endophytic fungi, which are referred in the literature to fungal endophytes or simply endophytes. Endophytes are microorganisms growing systemically in the intercellular spaces of above-ground tissues of their host plants. In my study, I investigated the asexual endophyte genus *Neotyphodium* (Schardl et al. 2004). *Neotyphodium* species are highly specialised and live in symbiosis with one host grass species. Due to the asexual life cycle of *Neotyphodium*, endophytes reproduce via the seeds of the host and depend on the reproductive success of the host grass (Schardl et al. 2004). Consequently, it is expected that the symbiosis has a mutualistic character (Saikkonen et al. 2010). Endophytes produce alkaloids toxic to most herbivores feeding on infected grasses, but not harmful to all herbivores (e.g. grasshoppers) (Hartley & Gange 2009). Endophytes enhance growth and biomass gain of the infected plants under water or nutrient stress conditions (Malinowski & Belesky 2000). Results are relatively consistent in laboratory studies, but former field studies on endophyte effects on herbivores lead to contrasting conclusions on the endophyte-grass symbiosis ranging from mutualistic to parasitic ones and are still discussed (Faeth 2009; Rudgers et al. 2010). Some studies showed decreasing herbivore numbers on grasses with an endophyte (in an agricultural used grass species) (Rudgers & Clay 2008), while other showed increasing herbivore numbers in the presence of an endophyte (in a native grass species) (Jani et al. 2010). This indicates that the endophyte effect depends strongly on the environmental context (Vesterlund et al. 2011).

In laboratory studies, predators are shown to be negatively affected when feeding exclusively on herbivores from endophyte infected grass plants (de Sassi et al. 2006).

Consequently, avoiding consumption of herbivores from infected grasses should benefit predators. Some predator communities might be able to distinguish between prey fed on infected grass while others are not (Härri et al. 2008). Therefore, different effects of endophytes on herbivores could also be mediated through different predator communities on grasslands of different land-use intensity or in different regions.

In chapter IV I asked if abiotic (land-use intensity, region) and biotic (predators) constraints alter effects of endophytes on herbivores. Therefore, I conducted the experiment in two different regions and on grasslands with different land-use intensity and manipulated predator access to the herbivores.

I conducted the multitrophic experiment in the region Hainich and Alb on 40 grassland sites in total. I selected ten intensively and ten extensively managed grasslands in each region. I cultivated grass in pots (*Lolium perenne*) which was either infected with an endophyte (*Neotyphodium lolii*) or not infected. I placed four pots on each grassland site, two with *Neotyphodium* and two without. Predators were excluded via a gauze cage on one grass pot with and one without *Neotyphodium*, while predators had free access on the remaining two grass pots. I put 50 aphids (*Rhopalosiphum padi*) on the grass of each pot and assessed the population size regularly (Fig. 1.4).

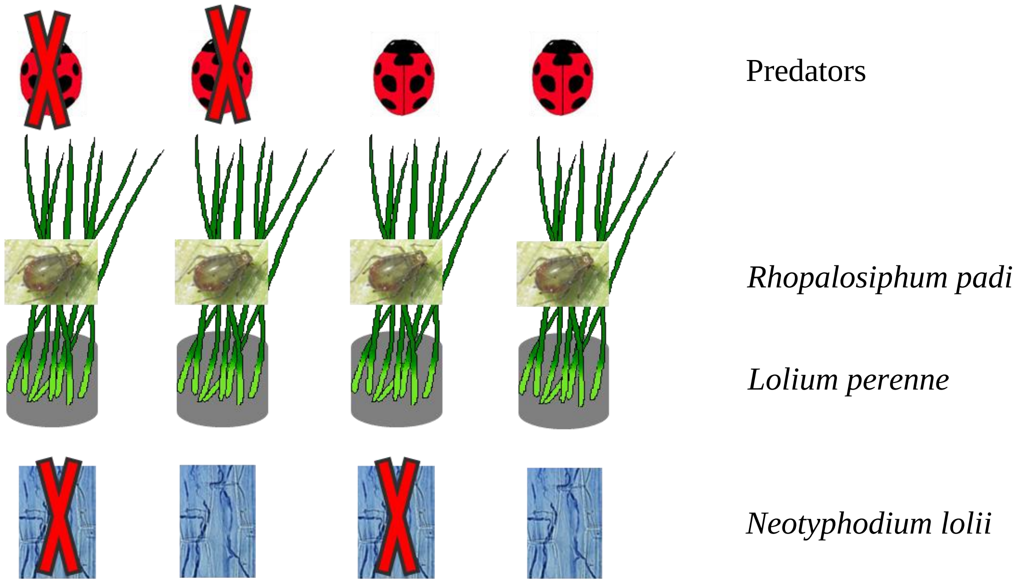

Figure 1.4 Study design of the multitrophic experiment in chapter IV on a grassland site.

CHAPTER OUTLINE AND MAIN HYPOTHESIS

Chapter II

I studied the effect of land-use intensity on Lepidoptera species richness and abundance and the occurrence of endangered species in three different regions using the LUI. I also tested the effects of the single components of the LUI—amount of fertiliser, mowing frequency and grazing intensity—on Lepidoptera species richness and abundance.

My main hypotheses were:

- Lepidoptera species richness and abundance and the occurrence of endangered species decrease with increasing land-use intensity. The strength of the land-use intensity effect may depend on the region.
- All three components of the LUI—Increasing amount of fertiliser, mowing frequency and grazing intensity—affect Lepidoptera species richness and abundance negatively. The effects may depend on the study region.

Chapter III

I tested how ecological and life-history traits of butterfly communities in three regions changed with land-use intensity and whether species with trait characteristics specific to extensively managed grasslands are endangered.

My hypotheses on this topic were:

- Ecological and life-history traits of butterfly communities shift with increasing land-use intensity from characteristics associated with specialists to those associated with generalists.
- Species with a combination of several traits characteristics associated with extensively managed grasslands are endangered.

Chapter IV

I investigated if land-use intensity, the study region—offering different environmental contexts—and predators can alter effects of endophytes (*Neotyphodium*) on aphid abundance feeding on the endophyte infected host grass.

Here I had the hypotheses:

- Study region, management intensity, and predators modify the effects of endophyte infection on aphid abundance.

- The influence of study region, management intensity and predation on aphid abundance is more important than the toxic effect of endophytes.

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CHAPTER II

Management intensity and region predict species richness and the occurrence of endangered day-active Lepidoptera in grasslands

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(to be submitted to *Biodiversity and Conservation* or *Insect Conservation and Diversity*)

ABSTRACT

1. Most European grasslands developed under human activity. However, increased land-use intensity in the last decades caused a reduction in butterfly and day-active moth (hereafter Lepidoptera) diversity, leading to an increasing number of threatened species in Red Data Lists.

2. We asked how strong increasing land-use intensity on grasslands affects Lepidoptera species richness, abundance and the occurrence of endangered species. We also asked how consistent the effect of land-use intensity is between different regions.

3. We conducted surveys of Lepidoptera on 137 grasslands along a land-use intensity gradient in three regions of Germany and tested how land-use intensity affects Lepidoptera species richness, abundance and the occurrence of endangered species. We assessed land-use intensity using a continuous land-use intensity index (LUI) comprising amount of fertiliser, mowing frequency and grazing intensity. We then tested also the effects of the single components of the LUI—amount of fertiliser, mowing frequency and grazing intensity—on Lepidoptera species richness and abundance.

4. Land-use intensity decreased species richness and abundance of Lepidoptera significantly in two regions but not the third. The occurrence of threatened species decreased with increasing land-use intensity independent of region. The regional dependent land-use intensity effect was driven by different regional species pools and the related number of endangered species. In a region with a low species pool and few endangered species, the effect of land-use intensity is less pronounced than in species rich regions with many endangered species. Hence increasing land-use intensity has severe effects in species rich regions. Fertilisation and intensive grazing significantly reduced Lepidoptera species richness, while mowing frequency had no significant effect.

5. Incentives in species poor regions might be less effective and should be offered in regions with still a reasonable large species pool and a high number of endangered species. We recommend protecting grasslands of lowest land-use intensity (calcareous grasslands), which show high species richness. Managers should avoid grassland fertilisation and reduce grazing intensity.

Keywords

butterflies, calcareous grassland, grazing, fertiliser, land-use intensity, mowing, nitrogen, moths

INTRODUCTION

Anthropogenic traditional farming in Europe shaped the landscapes over centuries and nutrient poor grasslands developed to the most important habitats of insect and plant diversity in Central and Northern Europe (van Swaay 2002; WallisDeVries et al. 2002). The last decades were characterised by agricultural intensification, but most studies concentrate on arable land and pay little attention to grasslands (Vickery et al. 2001). Many extensively managed grasslands (low intensively) were turned by increasing land-use intensity into species poor, high biomass productive grasslands, which lead to a rising number of endangered species (WallisDeVries et al. 2002; van Swaay et al. 2006). Grasslands changed due to application of chemical fertiliser, intensification of animal husbandry with higher stock numbers and longer grazing periods and frequent mowing events with machinery (Vickery et al. 2001; Tilman et al. 2002). Pesticide-use is also a major threat for insects, but pesticides are more frequently applied in arable systems and rarely in grasslands (Vickery et al. 2001). Fertilisation of grasslands reduces species richness of many insect taxa including butterflies and day-active moths (Öckinger et al. 2006). In contrast to fertilisation, mowing and/or grazing are essential for the maintenance of most grassland sites in Central Europe as they otherwise transform by succession into forests (Balmer & Erhardt 2000; WallisDeVries et al. 2002). However, when the mowing frequency or grazing intensity is high, butterflies suffer diversity loss (Kruess & Tscharntke 2002; Marini et al. 2009).

Recent studies usually focus only on one aspect of land-use intensity such as fertilisation (Kleijn et al. 2009) or grazing intensity (Dennis et al. 2008). We combined the three major drivers of land-use intensity in grasslands–amount of fertiliser, mowing frequency and grazing intensity of different stock animals–in a land-use intensity index (LUI). The index is a continuous variable avoiding information loss by categorisation and has the advantage that a large number of different management activities (fertilisation, mowing, grazing with different livestock) are easily comparable.

The aim of many conservation programs for grasslands is to maintain an extensive management with any fertilisation and mowing once per year at the end of the vegetation period or with extensive grazing (Potts et al. 2009). We asked in this study how land-use intensity affects diversity and endangered species. We were interested whether the land-use intensity effect differs between regions, because former studies have shown that effects of agricultural intensification can depend on the region due to different environmental contexts (Tscharntke et al. 2005). In intensive agricultural areas the species richness is lower than in heterogeneous areas. Therefore, we conducted our study in three different regions simultaneously.

We used butterflies and day-active moths (hereafter referred as Lepidoptera) as study organism, because they respond sensitively to increasing land-use intensity and respond to environmental changes before plants (Thomas et al. 2004). We used Red Lists of Germany for butterflies and moths to identify endangered species.

Many vascular plant species are nectar sources for adult Lepidoptera and other pollinators and food resources for herbivorous Lepidoptera larvae (Ehrlich & Raven 1964). Vascular plant species richness is therefore a good predictor for butterfly species richness, even though they react more slowly than day-active Lepidoptera (Erhardt & Erhardt 1985; Krauss et al. 2003). We therefore also consider vascular plant species richness in our analyses.

In this study we addressed the following hypotheses:

1. Lepidoptera species richness and abundance and the occurrence of endangered species decreases with increasing land-use intensity. The effect of land-use intensity may depend on the study region.
2. An increasing amount of fertiliser, high mowing frequency and high grazing intensity decrease Lepidoptera species richness and abundance. The effects may depend on the study region

After testing these hypotheses, we will provide management recommendations to conserve butterfly diversity in grasslands of Germany.

MATERIALS AND METHODS

Study regions

We conducted butterfly and day-active moth (Lepidoptera) surveys on 137 grassland study sites in three regions of Germany ranging from north to south separated by a distance of minimum 350 km from each other. The regions differ in climate, soil type, geological and agricultural structure. Historical land-use intensity was lowest in the southern region and increased to the north.

The region Schorfheide-Chorin (hereafter named Schorfheide) is an UNESCO biosphere reserve in north-east Germany. The landscape is characterised by numerous lakes, moors, fens and arable fields and grasslands. This region with an annual precipitation about 520-580 mm is one of the driest in Germany. The second study region Hainich-Dün (hereafter named Hainich) is located in the centre of Germany. This area includes the national park and UNESCO World Natural Heritage (since 2011) site Hainich one of Germany’s largest closed beech forest areas and its surroundings. This region is dominated outside the national park by intensively managed grasslands and agricultural fields, but also has several protected extensively managed grasslands. The third study region is the UNESCO biosphere reserve Schwäbische Alb (hereafter named Alb), located in the low-mountain ranges of the Schwäbische Alb in south-west Germany. The Alb is heterogeneous with a relatively high proportion of grasslands, many of them managed by century-long tradition of sheep herding, interspersed with few agricultural fields and forests. The regions Hainich and Alb contain calcareous bedrocks, while the Schorfheide is a young glacially formed region. We conducted our study within the framework of the project “biodiversity exploratories” in Germany (for further information see: www.biodiversity-exploratories.de, Fischer et al. 2010).

Land-use intensity gradient

We selected altogether 137 grassland sites in the three study regions covering in each region the full gradient from extensively managed to intensively managed grasslands. The grassland sites differed in management type. Some were either mown one to three times per year (meadows) or grazed by different livestock (sheep, cattle or horse) with different stock densities and grazing durations (pastures). Other grassland sites were both grazed and mown (mown pastures). Within all three management types some grassland sites were fertilised with different amounts of fertiliser and some were not fertilised (Appendix 2.1).

We used a land-use intensity index (LUI) combining the amount of fertiliser (F), mowing frequency (M) and grazing intensity (G), based on detailed information of a questionnaire of all land-owners and land-users (Blüthgen et al., unpublished manuscript). Fertilisation intensity F_i represents the kg nitrogen per hectare per year, whereas moving M_i was calculated as the number of cuts per year. Grazing intensity G_i represents livestock units differently weighted for sheep, cattle and horse per hectare multiplied by the grazing duration in days per year. For each study site *i* each individual LUI component (F_i, M_i, G_i) was standardised relative to its mean of the corresponding region R (F_R, M_R, G_R).

$$LUI_i = \frac{F_i}{F_R} + \frac{M_i}{M_R} + \frac{G_i}{G_R}$$

We calculated the average LUI for each grassland study site based on land-use information from 2006 to 2008 to accommodate possible land-use changes for some grassland sites within this time period (Blüthgen et al. unpublished manuscript, Appendix 2.1). Most extensively managed grasslands in the regions Hainich and especially in the Alb were calcareous grasslands mainly grazed by sheep. In the region Schorfheide numerous grasslands were extensively managed, but due to geological appearance it has no calcareous grasslands.

Plant survey

From May to June 2008 vegetation was recorded in all grasslands on 4 x 4 m next to the butterfly transects. We identified every vascular plant species following the nomenclature of Wisskirchen & Häupler (1998) and calculated plant species richness for each grassland site.

Lepidoptera survey

We conducted surveys of butterflies and day-active moths (hereafter termed as Lepidoptera) from beginning of May to mid August in the year 2008. We sampled Lepidoptera on fixed transects on 137 grassland sites in the three regions repeating the sampling three times in a randomised sequence within each region. Each transect had a length of 300 m and we recorded all Lepidoptera within 30 min per site within a five m corridor. We only conducted surveys when weather conditions allowed Lepidoptera activity (Pollard 1977). We caught Lepidoptera with a net and either released them after identification or if necessary collected them for gender preparation for proper determination. For identification and nomenclature we used for butterflies (Hesperiidae, Papilionidae, Pieridae, Lycaenidae, Nymphalidae) Settele et al (2008) and for moths (Zygaenidae, Geometridae, Noctuidae, Arctiidae) Koch (1984) and Ebert et al. (1994-2005). We excluded from our statistical analyses exclusively night-active moth species (according to Ebert et al. 1994-2005) which were incidentally recorded during transect walks. We considered all species as endangered when they were listed in the Red List of Germany as critically endangered, endangered, vulnerable or near threatened. We used for butterflies the Red List (2008) in Settele et al. (2008) and the Red List from 1998 for moths at www.schmetterlinge-deutschlands (Appendix 2.2).

Statistical analyses

We calculated beta-diversity between the grassland sites within each region, using the additive partitioning approach (alpha + beta = gamma) (Gering et al. 2003). As species richness differed between the regions, we calculated beta diversity in percentage. Gamma diversity (100%) was defined as the total number of species within a studied region and alpha diversity as the mean species richness per grassland site observed in a region.

We tested the effects of land-use intensity on the response variables species richness and abundance of 1) butterflies, 2) day-active moths and 3) both combined (Lepidoptera). The species richness data were pooled, while the abundance data were summed up for the three surveys per grassland. Explanatory variables entered the statistical models in the sequence region (Alb, Hainich, Schorfheide) followed by the land-use intensity index (LUI) and the interaction between region and land-use intensity. We applied generalised linear models with the family distribution poisson or quasipoisson (when overdispersion occurred) testing species richness data or negative binomial family distribution (high overdispersion) testing abundance data (Zuur et al. 2009).

We tested the probability on the occurrence of endangered species along the land-use gradient in the three regions using a generalised linear model with binomial distribution family (presence, absence) and cloglog-link (Zuur et al. 2009).

We tested in further statistical models the three components amount of fertiliser, mowing frequency and grazing intensity separately on Lepidoptera species richness and abundance. We tested 1) on a subset of fertilised grassland sites the effect of the amount of fertiliser, 2) on the subset of meadows (only mown) the effect of mowing frequency and 3) on subset of pastures (only grazed) the effect of grazing intensity on species richness and abundance. We excluded mown pastures, because it was not possible to disentangle mowing from grazing effects. We log-transformed the explanatory variables amount of fertiliser and grazing intensity to meet the assumption of normality. We also tested for a significant effect between unfertilised and fertilised grassland sites on Lepidoptera species richness and abundance and if the effect differed between regions. We used generalised linear models with quasipoisson distribution.

We tested also the effect of region, plant species richness and their interaction on Lepidoptera species richness in a generalised linear model with quasipoisson distribution.

We calculated correlations between the explanatory variables LUI and plant species richness, plant species richness and fertiliser (fertilised vs. unfertilised) and LUI and fertiliser and also between the response variables Lepidoptera species richness and Lepidoptera abundance, butterfly species richness and moth species richness and moth abundance and butterfly abundance as Pearson correlations.

In the text we only present the full models, but simplification of the models by backward elimination of the non-significant interactions and variables did not change the results qualitatively. We performed all statistical analyses using the software R (R Development Core Team 2011, version 2.13.1).

RESULTS

In total we recorded 6295 individuals of 98 Lepidoptera species (butterflies: 4967 individuals and 59 species; day-active moths: 1328 individuals and 39 species) (Appendix 2.2). The mean number (alpha diversity) of Lepidoptera species per study site (mean ±SE) differed between regions, decreasing from south to north: Alb (12.0 ± 7.1), Hainich (9.8 ± 4.8) and Schorfheide (6.0 ± 2.5). Similarly, the gamma diversity decreased from south to north: Alb (88 species), Hainich (63 species) and Schorfheide (42 species). The relative beta diversity contributes in all three regions similarly to species turnover (Alb = 86.1%, Hainich = 84.1%, Schorfheide = 85.6%).

Lepidoptera species richness was significantly affected by land-use intensity depending on the region (interaction: $F_{2,131} = 6.4$, $P = 0.002$, Fig. 2.1). Butterfly and day-active moth species richness and abundance were affected similarly. All three regions

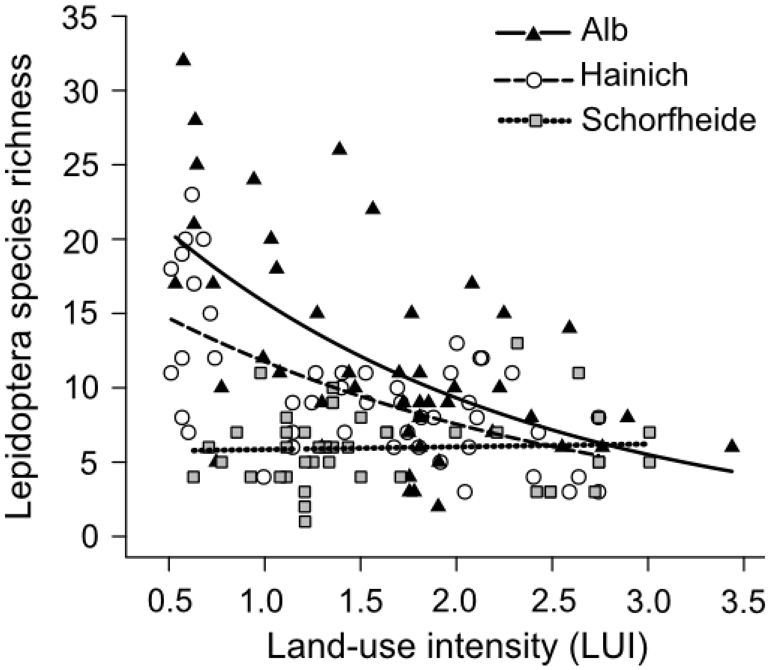

Figure 2.1 Land-use intensity (LUI) effects on Lepidoptera species richness depended on the region (significant interaction between region and LUI). The LUI is a continuous variable measuring the land-use intensity of a grassland study sites comprising amount of fertiliser, mowing frequency and grazing intensity.

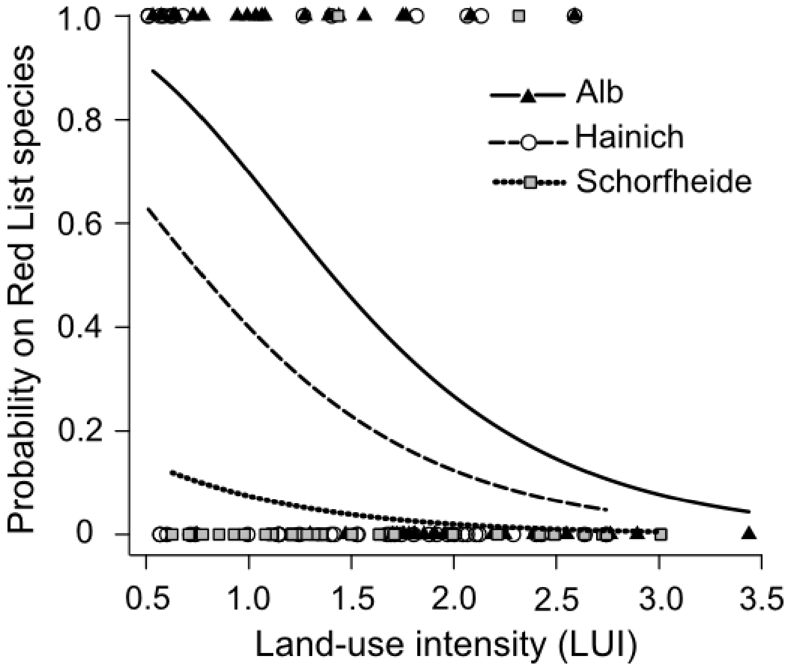

Figure 2.2 The probability on the occurrence of endangered species on grasslands decreased with increasing land-use intensity (LUI) in the three regions Alb, Hainich and Schorfheide, tested in a binomial generalised linear model (absence, presence).

showed a similar low species richness and abundance at low intensively managed grasslands. The species richness in the southern region Alb was very high on extensively managed grasslands and dropped drastically with increasing land-use intensity, while the northern region Schorfheide showed similarly low species richness and abundance on either extensively or intensively managed grasslands.

The probability that endangered species occurred on the grassland sites differed between regions (*P* <0.0001) and decreased with increasing land-use intensity (*P* <0.0001) in all three regions similarly (interaction: *P* = 0.071). Endangered species reached an occurrence probability on extensively managed sites close to 90% in the Alb showing 27 endangered species in total, approximately 60% in the Hainich with 14 endangered species and only around 10% in the Schorfheide with 3 endangered species (Fig. 2.2).

We showed in separate analyses of the three components of the land-use intensity index (LUI) that grazing intensity on pastures affected Lepidoptera species richness negatively ($F_{1,44}$ = 9.2, *P* = 0.004), whereas mowing frequency on meadows ($F_{1,30}$ <0.1, *P* = 0.842) and the amount of fertiliser on the subset of fertilised sites ($F_{1,57}$ <0.1, *P* = 0.959) had no significant effect. These effects were consistent over all three regions (no interactions). Rather more important than the amount of fertiliser on fertilised grasslands was whether grassland sites were fertilised or not (fertilised vs. unfertilised) ($F_{1,133}$ = 57.4, *P* <0.0001). The fertiliser effect was region dependent (interaction: $F_{2,131}$ = 5.4, *P* = 0.006, Fig. 2.3). Lepidoptera species richness was approximately twice as high on unfertilised compared to fertilised grassland sites in the Alb and 50 % higher in the Hainich, while fertilisation had no significant effect in the region Schorfheide.

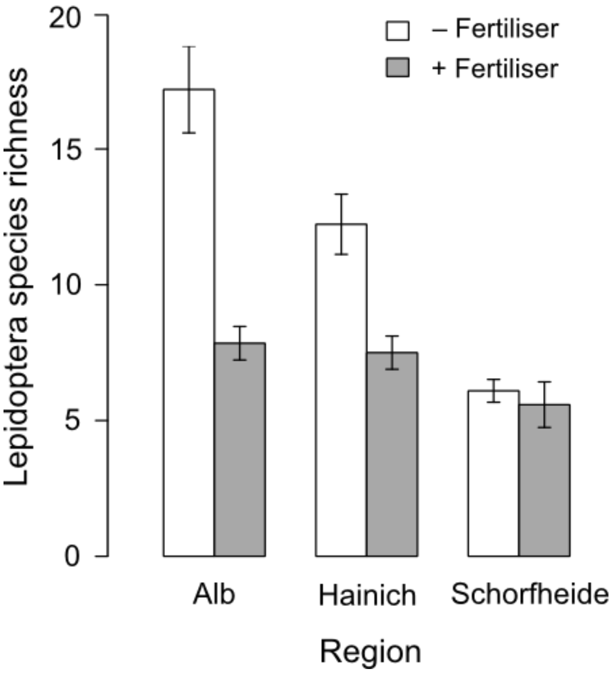

Figure 2.3 The effect of fertilisation (fertilised vs. unfertilised) on Lepidoptera species richness depended on the region (significant interaction between region and fertiliser). While in the regions Alb and Hainich fertilisation affected species richness negatively, it had no effect in the region Schorfheide.

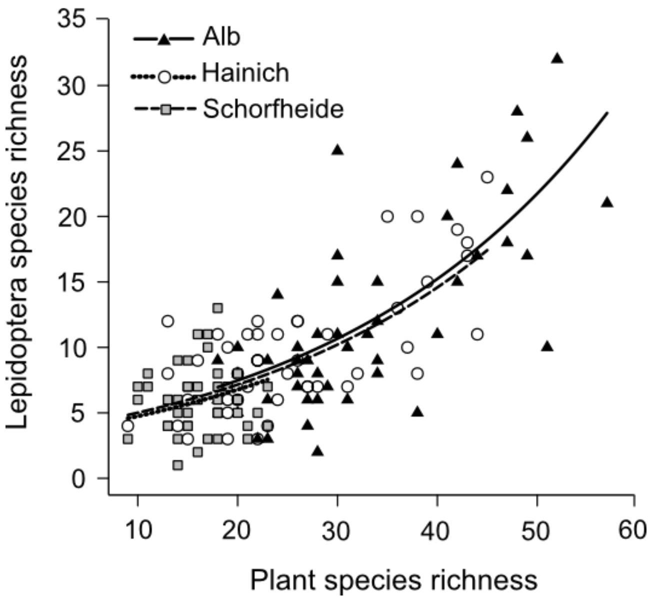

Figure 2.4 Lepidoptera species richness increased with increasing plant species richness, in all three regions Alb, Hainich, Schorfheide similarly (no significant interaction between region and plant species richness).

The species richness of Lepidoptera increased with increasing plant species richness ($F_{1,133}$ = 97.6, *P* <0.0001, Fig. 2.4) consistently in all three regions (interaction: $F_{2,131}$ = 1.3, *P* = 0.268). This was observed in all three regions as indicated by a non-significant interaction of plant species richness with region, despite the obvious absence of a gradient in plant species richness in the Schorfheide.

Correlation analyses showed that plant species richness was negatively correlated with LUI (r = -0.45 *P* <0.0001), and with fertiliser (r = -0.25, *P* = 0.003). LUI and fertiliser were positively correlated (r = 0.73, *P* <0.0001). Lepidoptera species richness was highly positively correlated with Lepidoptera abundance (r = 0.76, *P* <0.0001). Butterfly and moth species richness (r = 0.65, *P* <0.0001) and butterfly and moth abundance (0.63, *P* <0.0001) were also highly positively correlated. Due to the high correlations, the land-use intensity and the regions affected species richness and abundance for butterflies, moths and both combined (Lepidoptera) similarly in all statistical models. We therefore present only results and graphs for Lepidoptera species richness.

DISCUSSION

Agricultural intensification within the last decades led to increasing habitat fragmentation, habitat area loss, and landscape homogenisation, all shown to decrease Lepidoptera species richness and abundance significantly (Steffan-Dewenter & Tscharntke 2002; Krauss et al. 2003; Ekroos et al. 2010). In our study we exclusively focus on effects of increasing land-use intensity, which decreased Lepidoptera species richness and the highly correlated abundance in two of the three regions only and the decline of the two regions was of different magnitude.

Different responses of species richness on land-use intensity in each region might be driven by the respective regional species pool and the number of endangered species. In our study the regional species pool was positively related with the number of endangered species. The region with the highest species pool showed on extensively managed grasslands the highest species richness dropping drastically with increasing land-use intensity, similarly dropped the occurrence of endangered species. The region with the lowest species pool showed a similar low species richness on intensively and extensively managed grasslands and only few endangered species. Regions with few endangered species—most are specialists—have mainly common species in their species pool. Common species are usually only little affected by high land-use intensity, therefore increasing land-use intensity in a species poor region with few endangered specialists does not show such a strong effect compared to a species rich region (Kleijn et al. 2011). Thus increasing land-use intensity has more severe effects in species rich regions with many endangered species.

The declining species pool follows the species richness - latitudinal gradient relationship (Gaston 2000), but species richness differences between our three regions are high compared to a distance of only 700 km between the southern and the northern regions.

The decreasing species pool from south to north relates to the overall increasing land-use intensity from south to north. Endangered specialist species go extinct first when species richness declines due to anthropogenic habitat perturbation (e.g. habitat fragmentation) (Steffan-Dewenter & Tscharntke 2002; Brückmann et al. 2010; Öckinger et al. 2010; Kleijn et al. 2011). When specialised species disappeared from a regional pool only common species remain to colonise potential habitat patches (Zobel et al. 1998; Kleijn et al. 2011).

The low species pool in the northern region could be also explained by the lack of calcareous grasslands in northern Germany. Due to geological appearance calcareous grasslands do not exist in the northern region, while the two more southern regions have many grasslands of this extensively managed grassland type. Several studies have pronounced the importance of calcareous grasslands for endangered species and high Lepidoptera species richness and abundance (van Swaay 2002; WallisDeVries et al. 2002; Polus et al. 2007). Further, calcareous grasslands can function as source habitat for butterflies dispersing into the surrounding, which allows a higher diversity also on more intensively managed grasslands (Öckinger & Smith 2007) and if the sites are large enough, they support the rescue effect for sensitive species on small habitat patches (Hanski et al. 1995).

The three regions differed in their species pool (gamma diversity) and in their average alpha diversity. The species turnover (beta diversity) between regions differed only in absolute numbers, but not in relative proportions. Consequently beta diversity is neglect able to interpret our results.

Butterflies and day-active moths might be not the best target group for conservation issues when comparing the northern region Schorfheide with the southern regions with calcareous grasslands. The extensively managed grasslands in the northern region are important habitats for other taxonomic species groups e.g. endangered amphibians and reptiles (Schneeweiß et al. 2004) and threatened breeding bird species (Ryslavy et al. 2008).

We showed that species richness and abundance of Lepidoptera are strongly correlated with plant species richness, which was previously shown (Erhardt & Erhardt 1985; Krauss et al. 2003), both probably responding to similar environmental factors (Hawkins 2003; Biesmeijer et al. 2006), even though Lepidoptera react faster than plants on habitat perturbation (Thomas et al. 2004; Krauss et al. 2010). Many adult Lepidoptera are specialised flower visitors (Weiner et al. 2011) and larvae of many Lepidoptera species are strict food plant specialists (Ehrlich & Raven 1964). Lepidoptera food plant specialists—many of them are endangered—respond stronger on low plant species richness than food plant generalists (Pöyry et al. 2006). The low plant species richness in the northern region reflects and may contribute to the low Lepidoptera species richness and abundance and the low number of endangered species in the northern region.

The amount of fertiliser on fertilised grassland sites did not play a major role in our study for Lepidoptera species richness and abundance. It was rather more important whether a grassland site was fertilised or unfertilised. This is in line with a study from Kleijn et al. (2009) about nitrogen application effects on plant species richness throughout Europe. Plant species richness was highest on unfertilised grasslands and only little amount of nitrogen decreased species richness exponentially. Above a fertilisation threshold of 30 kg per hectare nitrogen, additional fertiliser did not decrease plant species richness any further. In our study, fertilised grasslands received mostly between 30 and 100 kg N per hectare per year, with very few exceptions. Specialised plants and Lepidoptera species on nutrient poor grassland suffer more from increasing nitrogen in the soil than generalist species (Öckinger et al. 2006; Kleijn et al. 2009; Kleijn et al. 2011), which can explain declining occurrence of endangered species with increasing land-use intensity. In our study the fertilisation effect on Lepidoptera species richness and abundance was region dependent with a negative effect in both southern regions and no effect in the northern region. This supports the overall lack of a land-use intensity effect in the northern region. It may be caused by different soil types between the regions. In the northern region grasslands are mostly on organic soil, which are nutrient rich and more independent of additional fertilisation than the mineral soils in the other two regions.

Mowing frequency (one to three times per year) had no significant effect on Lepidoptera species richness and abundance in our study. Most previous studies show that high mowing frequencies reduce species richness of Lepidoptera (Johst et al. 2006; Marini et al. 2009). Mowing is an abrupt event (Morris 2000) not leaving nectar plants for adults defoliating plants and affecting the larval habitat severely (Johst et al. 2006). One reason that we did not find an effect of mowing frequency could be a long time span of nectar availability on frequently mown meadows. These meadows are not attractive to foraging adult Lepidoptera for short time periods directly after mowing, but plants on frequently mown meadows flower and offer fresh nectar over the whole growing period to complete their life-cycle, while meadows mown once a year show many efflorescent plants, reducing attractiveness for nectar searching Lepidoptera (but not egg-laying butterflies). Also could be a bad timing of the mowing event on rarely mown meadows at our grassland sites one reason for no mowing frequency effects. Recommended is mowing late in the year (autumn) to protect butterflies (Johst et al. 2006; WallisDeVries et al. 2002), which was the case only for few meadows in our study. However, we expected frequent mowing to influence butterfly species richness negatively, but could not observe a significant negative effect.

Increasing grazing intensity decreased Lepidoptera species richness and abundance in our study. This was also shown in previous studies for butterflies (Kruess & Tscharntke 2002) and other taxa such as night-active moths (Littlewood 2008) and beetles and spiders (Dennis et al. 2008), even though not all taxa show a decline in species richness or abundance (Dennis et al. 2008; Sjödin et al. 2008). Low grazing intensity with a low stock number or occasional grazing with high stock numbers leave enough flowers to attract butterflies, but also offer space for low competitive plants, which keeps plant diversity high (Olff & Ritchie 1998) and therefore a broader diversity of food resources to Lepidoptera. Intensive grazing reduces flower and nectar availability for Lepidoptera, and also decreases plant species richness, because only competitive plant species are resistant to trampling and frequent defoliation and are able to persist. Also large herbivores kill larvae of Lepidoptera by trampling or inadvertent ingestion (Vickery et al. 2001). Many Lepidoptera species are attracted to structured habitat with high but light vegetation (Pöyry et al. 2006), which is strongly reduced by intensive grazing (Vickery et al. 2001). Hence, extensive grazing prevents grasslands from successive transformation to forests and keeps diversity high, but intensive grazing has adverse effects and reduces species richness and abundance.

CONCLUSIONS

Grasslands of low land-use intensity are of major importance to maintain a high species richness and abundance of Lepidoptera and to protect endangered Lepidoptera species; especially calcareous grasslands are important to maintain a high diversity of Lepidoptera. But extensive management of grassland has stronger effects in species rich regions, why incentives are therefore most affective in areas with a large regional species pool and many endangered species. In our study the amount of fertiliser did not play a major role, it was more important that grasslands remained unfertilised. Therefore we recommend the abandonment of fertilisation, because only little amounts of fertiliser show strong negative effects on Lepidoptera and thereby especially on endangered species. Mowing did not play a major role, but grazing intensity. To protect Lepidoptera, grazing intensity should be below economical thresholds.

ACKNOWLEDGEMENTS

We thank Elisabeth K. V. Kalko, Eduard K. Linsenmair, François Buscot, Dominik Hessenmöller, Jens Nieschulze, Ingo Schöning, Ernst-Detlef Schulze and Wolfgang W. Weisser for their contribution in setting up the Biodiversity Exploratories project and Swen Renner, Sonja Gockel, Andreas Hemp, Martin Gorke and Simone Pfeiffer for maintaining plot and project infrastructure and also all farmers for permitting us to conduct our surveys on their grasslands. The work has been funded by the DFG Priority Program 1374 "Infrastructure-Biodiversity-Exploratories" (KL 1849/3-1). Field work permits were given

by the responsible state environmental offices of Baden-Württemberg, Thüringen and Brandenburg (according to § 72 BbgNatSchG).

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APPENDIX 2.1

Table A2.1 Grassland study sites in the three regions Alb, Hainich and Schorfheide.

LUI = Land-use intensity Index

Plot ID	Easting	Northing	Region	Grassland	Fertiliser	LUI (06-08)	LUI (2008)
AEG01	3525400	5362300	Alb	meadow	yes	1.8091	1.7639
AEG02	3535100	5360000	Alb	meadow	yes	2.5517	2.5815
AEG03	3539490	5363590	Alb	meadow	yes	2.1881	2.2694
AEG04	3531105	5360425	Alb	mownpasture	yes	1.8208	1.7593
AEG05	3532600	5362100	Alb	mownpasture	yes	1.9548	1.9630
AEG06	3532780	5362700	Alb	mownpasture	yes	2.7728	2.8465
AEG07	3527985	5361580	Alb	pasture	no	0.4994	0.4459
AEG08	3536500	5365100	Alb	mownpasture	no	1.2195	1.2655
AEG09	3537310	5361995	Alb	pasture	no	0.5386	0.5404
AEG10	3519900	5361800	Alb	meadow	no	0.9180	0.9054
AEG11	3525780	5372020	Alb	meadow	yes	1.9053	1.9826
AEG12	3526015	5361315	Alb	meadow	yes	1.8091	1.7639
AEG13	3526900	5361600	Alb	meadow	yes	1.8091	1.7639
AEG14	3538500	5359900	Alb	meadow	yes	1.7232	1.5749
AEG15	3533240	5372265	Alb	meadow	yes	2.7616	2.8003
AEG17	3538460	5362520	Alb	meadow	yes	2.3905	2.4131
AEG18	3538700	5360700	Alb	meadow	yes	1.9548	1.8166
AEG19	3533200	5362300	Alb	mownpasture	yes	2.9572	3.2345
AEG20	3526420	5372480	Alb	pasture	yes	2.1420	2.1185
AEG21	3526505	5367220	Alb	pasture	yes	3.2560	3.2528
AEG22	3538090	5363080	Alb	mownpasture	no	0.9403	0.9054
AEG23	3537990	5364710	Alb	meadow	no	1.2982	1.2804
AEG24	3536620	5362190	Alb	mownpasture	yes	1.7365	1.7012
AEG25	3519300	5361995	Alb	pasture	no	0.6698	0.6420
AEG26	3529985	5362000	Alb	pasture	no	1.3016	1.3923
AEG27	3535600	5364705	Alb	pasture	no	1.2962	1.2796
AEG28	3536485	5369485	Alb	pasture	no	0.6660	0.6684
AEG30	3533900	5368820	Alb	pasture	no	1.0090	0.9672
AEG31	3534100	5369100	Alb	pasture	no	0.6992	0.6702
AEG32	3536000	5369900	Alb	pasture	no	0.5557	0.5577
AEG33	3536400	5368300	Alb	pasture	no	1.3520	1.5614
AEG34	3537100	5368800	Alb	pasture	no	0.8570	0.9853
AEG35	3521400	5371500	Alb	meadow	yes	1.7549	1.7639
AEG36	3522200	5371200	Alb	meadow	yes	1.7549	1.7639
AEG37	3530700	5362120	Alb	meadow	yes	1.8091	1.7639
AEG38	3531540	5366950	Alb	meadow	yes	1.4390	1.2804
AEG39	3531600	5361910	Alb	meadow	yes	1.8091	1.7639
AEG40	3542020	5363710	Alb	meadow	yes	1.4711	1.6389
AEG41	3529600	5359200	Alb	meadow	yes	1.7509	1.6658
AEG42	3527920	5362310	Alb	mownpasture	yes	2.5099	2.4864

Table A2.1 to be continued

Table A2.1 to be continued

Plot ID	Easting	Northing	Region	Grassland	Fertiliser	LUI (06-08)	LUI (2008)
AEG43	3540200	5364100	Alb	mownpasture	yes	2.1939	2.2039
AEG44	3532300	5360110	Alb	pasture	no	1.9492	1.9746
AEG45	3533985	5362715	Alb	meadow	no	1.2982	1.2804
AEG46	3532280	5362220	Alb	pasture	no	1.5932	1.4703
AEG47	3533100	5364700	Alb	pasture	no	0.8702	0.5560
AEG48	3536985	5365015	Alb	pasture	no	0.6841	0.6864
AEG49	3537400	5368900	Alb	pasture	no	0.9113	1.0745
HEG01	4388100	5649700	Hainich	mownpasture	yes	2.6380	2.2293
HEG02	4389900	5652900	Hainich	mownpasture	yes	2.7418	2.3881
HEG03	4390100	5652600	Hainich	mownpasture	yes	2.7418	2.3881
HEG04	4390599	5665418	Hainich	mownpasture	yes	1.9166	2.0724
HEG05	4382901	5676999	Hainich	mownpasture	yes	2.4026	2.4361
HEG06	4387698	5676785	Hainich	mownpasture	yes	2.0656	2.0835
HEG07	4389180	5683280	Hainich	pasture	yes	1.7438	1.5784
HEG08	4389700	5683010	Hainich	pasture	no	1.7111	1.5784
HEG09	4386991	5677798	Hainich	pasture	no	0.7159	0.7356
HEG10	4391720	5683400	Hainich	meadow	yes	1.1454	1.0541
HEG11	4392390	5684000	Hainich	meadow	yes	1.1454	1.0541
HEG12	4400299	5661199	Hainich	mownpasture	yes	2.5896	2.3203
HEG14	4391000	5685520	Hainich	mownpasture	yes	2.1072	2.2047
HEG15	4394000	5660300	Hainich	mownpasture	yes	2.0439	1.9554
HEG16	4392000	5656500	Hainich	pasture	no	0.7397	0.9266
HEG17	4392900	5660600	Hainich	pasture	no	0.5684	0.6027
HEG18	4390200	5684380	Hainich	pasture	no	0.6204	0.8069
HEG19	4393100	5660900	Hainich	pasture	no	0.5684	0.6027
HEG20	4386299	5677498	Hainich	pasture	no	0.5111	0.6446
HEG21	4412899	5673599	Hainich	pasture	no	0.6018	0.5046
HEG23	4383790	5667898	Hainich	mownpasture	no	1.5266	1.5762
HEG24	4384619	5664180	Hainich	mownpasture	no	1.6741	1.6556
HEG26	4386500	5684220	Hainich	meadow	yes	1.1454	1.0541
HEG27	4401802	5662197	Hainich	meadow	yes	1.8805	1.8657
HEG28	4395199	5682299	Hainich	mownpasture	yes	1.9703	2.4442
HEG29	4395300	5682097	Hainich	mownpasture	yes	1.8168	1.6459
HEG30	4385478	5675378	Hainich	mownpasture	yes	2.4277	2.5481
HEG31	4375699	5672100	Hainich	mownpasture	yes	2.0025	1.9971
HEG33	4389879	5665219	Hainich	pasture	yes	1.8041	2.0825
HEG34	4387397	5676720	Hainich	mownpasture	yes	2.0656	2.0835
HEG35	4389100	5677299	Hainich	mownpasture	yes	2.2906	2.1021
HEG36	4395600	5656198	Hainich	mownpasture	yes	2.1237	2.1901
HEG37	4395901	5656297	Hainich	mownpasture	yes	2.1337	2.2033
HEG38	4383979	5665879	Hainich	pasture	no	1.3995	1.4355
HEG39	4384319	5666319	Hainich	pasture	no	1.4037	1.6022
HEG40	4391000	5649100	Hainich	pasture	no	0.9942	0.5690
HEG41	4386015	5677500	Hainich	pasture	no	0.5111	0.6446
HEG42	4392500	5660490	Hainich	pasture	no	0.5684	0.6027
HEG43	4391020	5686400	Hainich	pasture	no	0.6307	0.8147
HEG44	4393508	5658884	Hainich	pasture	no	2.2130	3.0168
HEG45	4395499	5657100	Hainich	mownpasture	yes	1.6915	2.5121
HEG46	4412998	5675399	Hainich	pasture	no	0.5863	0.4058
HEG47	4386520	5684520	Hainich	mownpasture	no	1.4167	1.3733

Table A2.1 to be continued

Table A2.1 to be continued

Plot ID	Easting	Northing	Region	Grassland	Fertiliser	LUI (06-08)	LUI (2008)
HEG48	4387100	5684800	Hainich	mownpasture	no	1.5325	1.7527
HEG49	4387900	5683900	Hainich	mownpasture	no	1.2458	1.1731
HEG50	4389910	5683590	Hainich	mownpasture	no	1.2659	1.0541
SEG01	5431100	5884400	Schorfheide	meadow	yes	3.0078	3.1166
SEG02	5431800	5884600	Schorfheide	mownpasture	yes	2.7228	2.2934
SEG03	5432200	5886100	Schorfheide	mownpasture	yes	2.7431	2.3211
SEG04	5433300	5887300	Schorfheide	mownpasture	no	1.1132	1.0314
SEG05	5433200	5886600	Schorfheide	mownpasture	no	1.1132	1.0314
SEG06	5407899	5886579	Schorfheide	mownpasture	no	1.3091	1.1423
SEG08	5434320	5887310	Schorfheide	mownpasture	no	0.7747	1.4586
SEG09	5407200	5886001	Schorfheide	mownpasture	no	1.2105	1.3637
SEG10	5433000	5886510	Schorfheide	mownpasture	yes	2.7431	2.3211
SEG11	5432691	5886400	Schorfheide	mownpasture	yes	2.7431	2.3211
SEG12	5430800	5884401	Schorfheide	meadow	yes	3.0078	3.1166
SEG13	5420800	5871820	Schorfheide	mownpasture	yes	2.4186	2.8302
SEG14	5431810	5884300	Schorfheide	mownpasture	no	0.9267	1.0314
SEG15	5433780	5886810	Schorfheide	mownpasture	no	1.1132	1.0314
SEG16	5433121	5887501	Schorfheide	mownpasture	no	1.1132	1.0314
SEG17	5408100	5886000	Schorfheide	mownpasture	no	1.3356	1.2433
SEG19	5433800	5888491	Schorfheide	mownpasture	no	0.6280	1.0314
SEG20	5407400	5886591	Schorfheide	pasture	no	1.0801	1.1351
SEG21	5407120	5886900	Schorfheide	pasture	no	1.4348	2.0170
SEG22	5431400	5885889	Schorfheide	pasture	no	0.8536	1.0696
SEG23	5434900	5886600	Schorfheide	meadow	no	1.5019	1.4586
SEG24	5432880	5885000	Schorfheide	meadow	no	1.3552	1.4586
SEG25	5407590	5887379	Schorfheide	meadow	no	1.5019	1.4586
SEG26	5434397	5886403	Schorfheide	meadow	no	1.3552	1.0314
SEG28	5433550	5885000	Schorfheide	meadow	no	1.3552	1.4586
SEG29	5433051	5884700	Schorfheide	meadow	no	0.7080	1.0314
SEG30	5421900	5891300	Schorfheide	meadow	no	1.2086	1.0314
SEG31	5422220	5891400	Schorfheide	meadow	no	1.2086	1.0314
SEG32	5422001	5891720	Schorfheide	meadow	no	1.2086	1.0314
SEG33	5422400	5873220	Schorfheide	mownpasture	yes	1.9948	1.3326
SEG34	5422710	5872920	Schorfheide	mownpasture	yes	2.4907	3.2029
SEG35	5422780	5872600	Schorfheide	mownpasture	yes	2.4907	3.2029
SEG36	5422075	5873350	Schorfheide	pasture	no	1.3555	1.9506
SEG37	5424911	5889781	Schorfheide	pasture	no	1.6393	1.2566
SEG38	5411469	5887874	Schorfheide	mownpasture	no	2.6379	1.8841
SEG39	5421100	5872700	Schorfheide	mownpasture	yes	1.2522	1.0314
SEG40	5422510	5887679	Schorfheide	pasture	no	1.7086	1.7158
SEG41	5423303	5888389	Schorfheide	pasture	no	2.2127	1.7700
SEG42	5430699	5860281	Schorfheide	pasture	yes	2.3175	3.0102
SEG43	5430641	5860939	Schorfheide	pasture	no	1.3186	1.6012
SEG46	5421300	5872420	Schorfheide	pasture	no	1.2829	1.1455
SEG47	5421400	5873401	Schorfheide	pasture	no	0.9785	1.1756
SEG48	5406801	5885900	Schorfheide	mownpasture	no	1.2105	1.3637
SEG49	5423325	5871975	Schorfheide	pasture	no	1.6348	1.5588

APPENDIX 2.2

Table A2.2 Observed Lepidoptera species (butterflies and day-active moths) with their Red List status in the three regions Alb, Hainich and Schorfheide

A = Alb; H = Hainich; S = Schorfheide, RL = Red List status

NT = near threatened, VU = vulnerable, EN = endangered, CR = critically endangered

	number of individuals			
Species	A	H	S	RL
Adscita geryon	7			VU
Adscita sp	2			VU
Aglais io	10		36	
Aglais urticae	5	4		
Agrotis exclamationis	1			
Anthocharis cardamines		1	2	
Apatura iris		1		EN
Aphantopus hyperantus	249	75	4	
Aporia crataegi	1			
Araschnia levana	15	1	1	
Argynnis adippe	5	2		CR
Argynnis aglaja		19		EN
Argynnis paphia			4	
Aricias agestis	2	1		
Aricia eumedon	2			CR
Autographa gamma	130	50	15	
Brenthis ino	1			EN
Callistege mi	17	13	2	
Callophrys rubi	2	1		EN
Camptogramma bilineata	45	159	6	
Carterocephalus palaemon	4			
Celastrina argiolus	1			
Chiasmia clathrata	39	45	9	
Coenonympha arcania	19	2		
Coenonympha glycerion	58		1	NT
Coenonympha pamphilus	73	203	91	
Colias alfacariensis	14			
Colias crocea	1			
Colias hyale	1	13	4	
Cupido minimus	4			EN
Cyaniris semiargus	20			

Table A2.2 to be continued

Table A2.2 to be continued

Species	number of individuals A	H	S	RL
Diacrisia sannio	2			
Ematurga atomaria	13	14	8	
Emmelia trabealis			1	NT
Epirrhoe alternata	31	9	16	
Epirrhoe tristata	22	8		
Erebia aethiops	1			CR
Erebia medusa	80	14		
Erynnis tages	4			
Euclidia glyphica	20	39	4	
Eustrotia olivana			2	
Gonepteryx rhamni	1		16	
Heliothis viriplaca			1	
Hesperia comma	60	1		CR
Idaea ochrata	1	4		
Idaea serpentata	38	33		NT
Issoria lathonia			6	
Leptidea sinapis/reali	5	2		
Lycaena hippothoe	3	2		CR
Lycaena phlaeas	2		4	
Lycaena tityrus	9			
Lythria cruentaria			7	
Lythria purpuraria			18	EN
Maniola jurtina	628	676	204	
Melanargia galathea	335	133	3	
Melitaea athalia-Komplex	2			CR
Melitaea aurelia	12			EN
Melitaea britomartis	6			EN
Minoa murinata	2	7		
Ochlodes sylvanus	5			
Odezia atrata	30		12	
Panemeria tenebrata	19	1		
Papilio machaon	26	19	3	
Phengaris arion	4	1		CR
Pieris brassicae	12	14	10	
Pieris napi	11	15	215	
Pieris rapae	22	95	26	
Plebeius argus	28	22		
Polyommatus bellargus		4		CR
Polyommatus coridon	398	27		
Polyommatus icarus	28	342	122	
Polyommatus semiargus	20			
Polyommatus thersites	1			CR

Table A2.2 to be continued

Table A2.2 to be continued

	number of individuals			
Species	A	H	H	RL
Pontia edusa			1	
Pyrgus alveus	1			VU
Pyrgus alveus Komplex	3			VU
Pyrgus malvae	6	4		EN
Rhodostrophia vibicaria	9			
Rivula sericealis	1	3	3	
Scopula immorata	5	4		
Scopula ornata	8			
Scopula rubiginata		3		
Scotopteryx chenopodiata	33	20		
Siona lineata	1	12		
Spialia sertorius	3	1		NT
Spiris striata	13			VU
Thymelicus acteon	1			CR
Thymelicus lineola	25	59	5	
Thymelicus sylvestris	114	127	1	
Timandra griseata			5	
Tyta luctuosa		7		
Vanessa cardui	2	3		
Xanthorhoe spadicearia	45	11	3	
Zygaena carniolica	67	7		VU
Zygaena filipendulae	18	52		
Zygaena loti	24			VU
Zygaena minos	33			VU
Zygaena purpuralis	8	10		VU
Zygaena viciae	1	22		NT

CHAPTER III

Traits of butterfly communities change from specialist to generalist characteristics with increasing land-use intensity

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(submitted to *Ecography*)

ABSTRACT

1. Land-use intensification leads to species loss and shifts in community composition, but only few studies consider ecological and life-history traits. We asked if certain ecological and life-history trait characteristics are more common on extensively compared to intensively managed grasslands and if a combination of trait characteristics specific to extensively managed grasslands is related to the species threat status.

2. We conducted butterfly transect surveys on 137 grassland sites along a land-use intensity gradient across three regions of Germany in 2008. From literatures we compiled ten ecological and life-history traits associated with the feeding niche, dispersal, distribution, reproduction and development. We then analysed the changes of trait characteristics of butterfly communities from extensively to intensively managed grasslands.

3. Trait characteristics in the butterfly communities changed with increasing land-use intensity in all three regions similarly, while the species diversity decreased only in two regions and showed no response in the third. Adaption strategies of butterfly communities to intensively managed grasslands were characterised by a broad feeding niche, high dispersal ability, high migration propensity, high distribution range, low population density, brood of more than one generation per year, hibernation in a more advanced stage and a long flight period. These trait characteristics are generally associated with generalists.

4. A comparison of the species threat status in the Red List of Germany indicated that species with a composition of opposite adaption strategies—trait characteristics of specialists that are common on extensively managed grasslands—are indeed endangered.

5. In conclusion, analysing ecological and life-history traits allows the identification of the most threatened species before species disappear from ecosystems and should be used in common monitoring programs. More efforts should be spend on gathering highly valuable life-history traits of different taxa.

Keywords

development, dispersal, distribution, endangered species, flight period, grassland, life-history traits, population density, reproduction, voltinism

INTRODUCTION

Today, almost 40% of the terrestrial ice-free land is covered by agroecosystems (Ellis et al. 2010) and their increasing management intensity is known to be one of the main drivers of biodiversity loss (Sala et al. 2000). Due to different adaption strategies, not all species are similarly affected by land-use intensity (Kotiaho et al. 2005). Ecological and life-history traits of species (hereafter termed traits) evolved with environmental conditions and while some traits disadvantage species (Kotiaho et al. 2005; Öckinger et al. 2010) others seem to benefit species with increasing land-use intensity (McKinney & Lockwood 1999; Van der Veken et al. 2004). Therefore, it is crucial to understand how community trait composition changes with land-use intensity to identify the adaption strategies and mechanisms behind species loss and community shifts. The identification of disadvantageous traits allows predictions about community responses to future intensification and helps land-use planners to decide which species are at highest risk of extinction (Kotiaho et al. 2005). Several recent studies have addressed species traits as adaptation strategies mainly in the context of habitat fragmentation, isolation and habitat area loss (Bommarco et al. 2010; Brückmann et al. 2010; Öckinger et al. 2010), but how traits of butterfly communities are changing along a land-use intensity gradient in grasslands across different regions was not yet investigated. Grasslands are one of the most important habitats for many insects. Insects on extensively managed grasslands (low intensively) live in stable habitats with low perturbation, but they have to deal with a shortage of resources. Insects on intensively managed grasslands gain temporarily access to unlimited resources due to fertilization, but they have to cope with high habitat disturbance frequency due to mowing or grazing. Consequently, insect communities evolved contrasting adaption strategies to persist in grasslands of different land-use intensity. Habitat stability in space and time allows specialisation of species, while habitats with high disturbances favour generalist strategies (Clavel et al. 2010). We therefore assume that several traits of the butterfly communities are affected by land-use intensity, shifting from characteristics associated with specialists (e.g. narrow feeding niche, sedentary behaviour) to such associated with generalists (e.g. broad feeding niche, migratory behaviour). We tested traits available in the literature that can be linked to the feeding niche, dispersal, distribution, reproduction and development. We chose butterflies as the target taxonomic group, because they have been shown to be good indicators of environmental change and overall biodiversity (Thomas 2005; van

Swaay et al. 2006) and comprehensive information about butterfly species traits is available for European butterflies (Settele et al. 1999; Thomas 2005).

We expect butterfly communities on intensively managed grasslands to respond in general with broader feeding niches than communities on extensively managed grasslands. Species on intensively managed grasslands may compensate for frequent food removal due to grazing or mowing by utilisation a higher diversity of food plant species (Dennis et al. 2004). High habitat disturbance requires high mobility to locate new habitats; hence species mobility in butterfly communities may increase with increasing land-use intensity. Good dispersal abilities and an increasing amount of intensified grasslands should result in a wide distribution range of butterfly communities on intensively managed grasslands. Furthermore, frequent grazing and mowing on intensively managed grasslands should keep butterfly communities from achieving high population densities. In habitats with a high disturbance frequency and food unreliability, species with high reproductive potential and a fast development should increase their survival rate; e.g. a high number of eggs laid by females and more generations per year enhance the chance of a decent number of offspring surviving to adulthood. An advanced developmental stage close to hibernation is essential to obtain the capacity to fly at the time of spring grazing to avoid the risk of low mobility in egg, larval or pupae stage. In disturbed habitats a long flight period could be an adaptation for species responding to perturbations.

Hence, beside (1) a decline of species diversity with increasing land-use intensity, we expect that (2) traits of butterfly communities shift with increasing land-use intensity from characteristics associated with specialists to those associated with generalists. Traits should change to: a broader feeding niche, higher dispersal ability, higher migration propensity, higher distribution range, lower population density, brood of more than one generation per year, hibernation in a more advanced stage and a longer flight period.

After identifying how trait characteristics change from extensively to intensively managed grasslands (e.g. from narrow feeding niche to broad feeding niche), we compared the composition of trait characteristics of single species with their threat status in the Red List of Germany 2008. (3) We expect that species with a combination of several trait characteristics of extensively managed grassland communities have a higher risk of extinction, which should be reflected in the Red List status of a species.

MATERIALS AND METHODS

Study regions

The effect of land-use intensity on butterfly community traits was studied in three regions in Germany within the framework of the project “biodiversity exploratories” (Fischer et al. 2010) in the year 2008. The regions range from north to south with a minimum distance of 350 km apart from each other. The regions differ in climate and agricultural structure, but every region shows a range of grasslands with a wide land-use intensity gradient.

The region Schorfheide-Chorin (hereafter named Schorfheide) is located in the north-east of Germany in young glacially formed lowlands, characterised by numerous lakes, moors and fens. Grasslands of different land-use intensity are very abundant. The region Hainich-Dün (hereafter named Hainich) is located in the centre of Germany. This region is characterised by high land-use intensity, mainly grasslands and arable fields, but has also several extensively managed grasslands. The Schwäbische Alb (hereafter named Alb) is located in the low-mountain ranges of south-west Germany. The Alb is a heterogeneous region with a relatively high proportion of grasslands; many of them are calcareous grasslands with low land-use intensity, interspersed with few arable and perennial agricultural fields and forests.

Land-use intensity gradient

We chose grasslands of different management types (meadows, pastures, mown pastures) covering a gradient from extensive to intensive land-use intensity within each region. Meadows were mown one to three times per year, pastures were grazed by sheep, cattle or horse, and mown pastures, presenting a mixture of meadows and pastures, were mostly grazed in spring and summer and mown once at the end of the growing period. The grasslands were either unfertilised or different amounts of fertiliser were applied.

We used a land-use intensity index (LUI), a continuous variable, which quantifies variation in land-use intensity (Blüthgen et al. under revision). The index does not suffer information loss due to categorisation and therefore makes different management regimes, like meadows or pastures comparable. A similar index was used in another study (Herzog et al. 2006), but applicable for agricultural crop fields. We received detailed information on the land-use practises from a questionnaire given to all land-owners and land-users

(Blüthgen et al. under revision). The LUI integrates amount of fertilizer (F), mowing frequency (M) and intensity of grazing (G). Grazing livestock was translated into livestock units weighted for their impact on grasslands. For each study site i an individual LUI component (F_i, M_i, G_i) was standardised relative to its mean within the corresponding region R (F_R, M_R, G_R)

$$LUI_i = \frac{F_i}{F_R} + \frac{M_i}{M_R} + \frac{G_i}{G_R}$$

We calculated the LUI for each grassland study site for the study year 2008 and the previous two years 2006 and 2007 and took the average of these three years to get a better estimate of land-use intensity, because land-use changed slightly for some grassland sites within this time period.

Butterfly survey

We recorded butterflies from 7-May to 17-Aug-2008 along fixed transects randomly chosen on each of the 137 grassland study sites in the three regions. We recorded all species and their individual numbers within 2.5 m either side and 5 m in front of the recorder on transects of 300 m length within 30 min. We caught butterflies by sweep net and either released them after identification or collected them when necessary for further identification by gender preparation in the laboratory. We sampled all study sites three times in a randomised sequence within the regions about once a month, but only when weather conditions were suitable for butterfly activity (Pollard 1977). For butterfly identification and nomenclature we followed Settele et al. (2008).

Ecological and life-history traits of butterflies

We compiled ten ecological and life-history traits of butterflies within three ecologically relevant groups from various published literatures – 1. feeding niche, 2. dispersal and distribution, 3. reproduction and development (Appendix 3.1). One trait characterises the feeding niche of the butterfly species. Concerning their plant preferences as larvae species were characterised either as monophagous (feeding on plants of one genus), oligophagous (one family) or polyphagous (more than one family) (data from Settele et al. 1999). We selected four traits related to dispersal and distribution (wing length, migration propensity, distribution index, population density). Male forewing length (data from Higgins & Riley

1978) is a good estimator for dispersal abilities (Cowley et al. 2001; Öckinger et al. 2010), because larger wings enables individuals to fly longer distances. The migration propensity of a species categorises species from extremely sedentary to highly migratory (1-9) based on expert opinions (data from Settele et al. 1999). This categorisation is subjective, but commonly used in the literature (Cowley et al. 2001; Kotiaho et al. 2005). A distribution index developed by Kudnera (2002) indicates the relative size of the European distribution or range of a butterfly species in percentages. We obtained data on population densities from Settele et al. (1999). Two traits relate to reproduction and three to development. Reproductive potential can be measured by the number of eggs, present in a female butterfly at the beginning of the egg laying period, and the number of generations per year (voltinism) (data from Settele et al. 1999). Egg maturation time, hibernation stage and flight period length are linked to development (data from Settele et al. 1999). The egg maturation time is the time a female butterfly needs to produce eggs for the first time after hatching (data from Settele et al. 1999). We achieved data on the developmental stage for hibernation from Settele et al. 1999. The flight period is the time butterflies spend as adults. For multivoltine species we summed the flight periods and for species overwintering as adults we included in the flight period only the warm months (April – September), which are relevant for reproduction and dispersal (data from Settele et al. 2008).

Statistical analyses

We calculated species diversity using the Shannon Index, which combines the number of species with the relative abundance within a site (Hillebrand et al. 2008). We used a linear model to test if butterfly diversity differs between regions and changes with land-use intensity (LUI) and tested also the interaction between regions and LUI.

We tested ten traits in ten separate models for effects of the two explanatory variables region and LUI and the interaction between both. The analysed traits were: Feeding niche, wing length (dispersal ability), migration propensity, distribution index, population density, egg number, generations per year, egg maturation time, hibernation stage and flight period. We calculated the mean for each trait per study site using two approaches. In one approach, we summed the values for a trait of every species present at a study site and divided the sum by the number of species per site; consequently, every occurring species was equally weighted. In the other approach, we weighted the species by their abundance. We summed the values of a trait for each species times the number of individuals of a species per site and divided the sum by the number of all individuals per site. Trait variables without natural numbers as the hibernation stage were given values, from one to seven (egg, three larval stages, pupae, adult, overwintering in warmer regions) (see Stevens et al. 2012 and Appendix 3.1). We applied linear models or generalised least square models with different weight options to deal with variance heterogeneity in our data (Zuur et al. 2007). We used Spearman rank correlation to test for correlation between traits. We conducted all statistical analyses using the software R (R Development Core Team 2011, version 2.13.1).

Trait characteristics composition and the Red List status

After identifying how trait characteristics changed with land-use intensity we tested, if species with a composition of traits characterising butterfly communities of extensively managed grasslands are more endangered due to land-use intensity than others. For this purpose we selected six out of the ten analysed traits: feeding niche, migration propensity, distribution index, generations per year, hibernation stage and flight period. We excluded wing length, because it is similar to the trait migration scores. Egg number and egg maturation time were not significantly affected by land-use intensity. For the population density it is unclear whether large or small densities characterise specialisation (see discussion). Based on our results each trait was classified in one of three categories–extensively, intermediate or intensively managed grassland characteristics–and was ranked with one (extensive), two (intermediate) or three (intensive) (see Appendix 3.2). For example, our results showed that butterflies on extensively managed grasslands had on average a lower distribution range in Europe than on intensively managed grasslands; hence species with a low distribution index (DI) (1-15) were ranked with one, with a medium DI (>15-30) with two, and with a high DI (>30) with three (see Appendix 3.2). We summed the ranks of the selected six traits for each species according to the information available in the published literatures (see Appendix 3.1); a low rank indicates species with a composition of trait characteristics of extensively managed grasslands. We then compared the rank of the species with the species threat status in the Red List of Germany (2008) (*in*: Settele et al. 2008) to identify whether species with low trait ranks are indeed endangered (Table 3.3).

RESULTS

We recorded 4967 butterfly individuals belonging to 58 species on 137 grasslands (Table 3.3). Butterfly species diversity decreased with increasing land-use intensity (LUI) in the Alb and in the Hainich; only in the Schorfheide the species diversity did not change and remained consistently low over the entire land-use gradient (Fig. 3.1, significant interaction between region and LUI ($F_{1,130} = 9.04$, $P = 0.0002$)).

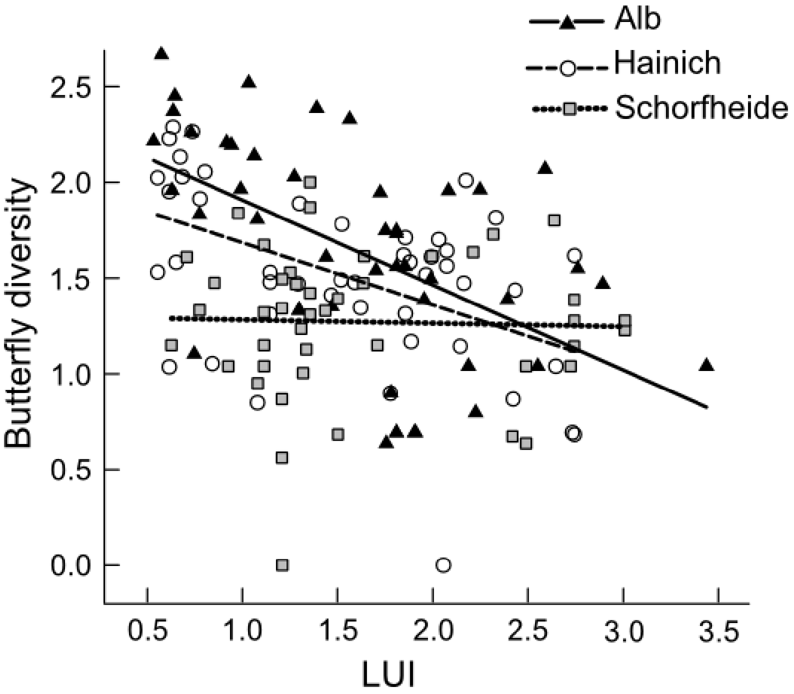

Figure 3.1 Butterfly diversity (Shannon Index) decreased with increasing land-use intensity (LUI) dependent on the study region.

The relationships between the traits and the land-use intensity did not change qualitatively when the mean trait values per site were calculated from species occurrence data alone compared to mean trait values weighted by species abundance. Species caught only once at a site, because e.g. the habitat was not suitable, should be weighted differently than species occurring numerously. We therefore present results only weighting species by their abundance.

Even though species diversity did not change over the land-use intensity gradient in the region Schorfheide (Fig. 3.1), the traits of butterfly communities in the Schorfheide changed with land-use intensity similarly to the other two regions (excepting the distribution index) (Fig. 3.2). The degree of food plant specialisation of butterfly communities decreased on average by 19% towards a broader feeding niche with

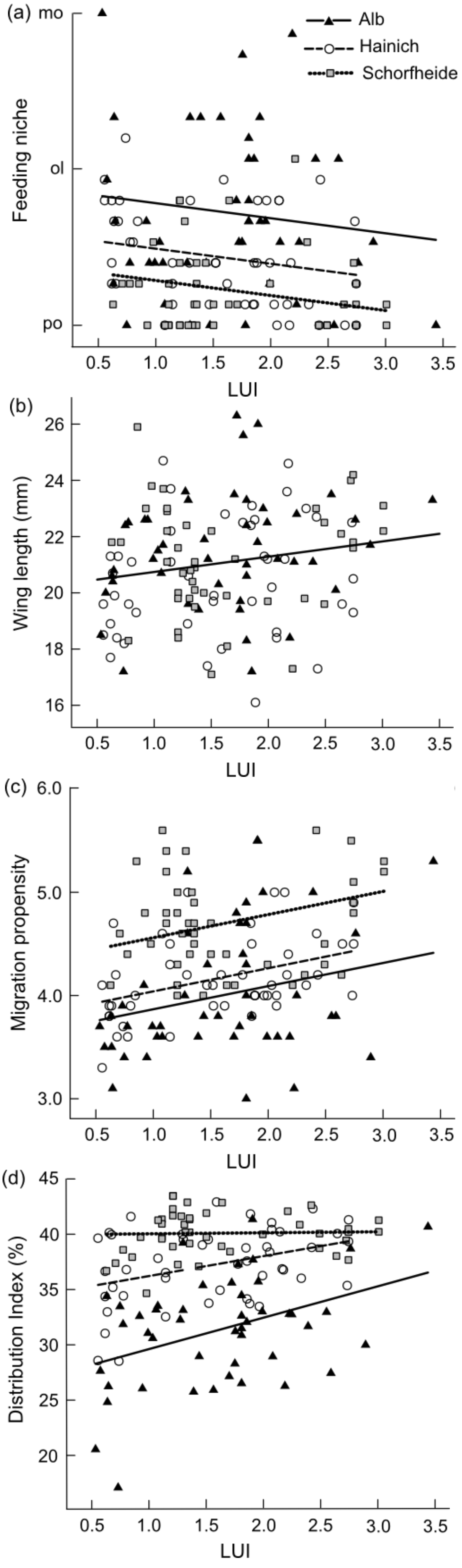

Figure 3.2 continued

increasing land-use intensity (Fig. 3.2a, Table 3.1a). Dispersal ability, migration propensity and distribution of butterfly communities increased with increasing land-use intensity, but population density decreased. The mean wing length of butterfly communities as a measure for dispersal ability increased by 8% (1.6 cm) with increasing land-use intensity (Fig. 3.2b, Table 3.1b) and the mean migration propensity increased by 16% (Fig. 3.2c, Table 3.1c), indicating that overall dispersal abilities and disposition were positively affected by increasing land-use intensity. The effect of land-use intensity on the distribution of butterfly communities depended on the region. The distribution of butterfly communities was not affected by land-use intensity in the Schorfheide, while the distribution increased with increasing land-use intensity in the Hainich and Alb (Fig. 3.2d, Table 3.1d). Butterfly communities on intensively managed grasslands included species, whose population densities were on average 36% lower compared to species on extensively managed grasslands (Fig. 3.2e, Table 3.1e). Three traits related to reproduction and development increased with increasing land-use intensity, while two were not affected significantly. The mean number of eggs produced by females did not respond to land-use intensity

Figure 3.2 continued

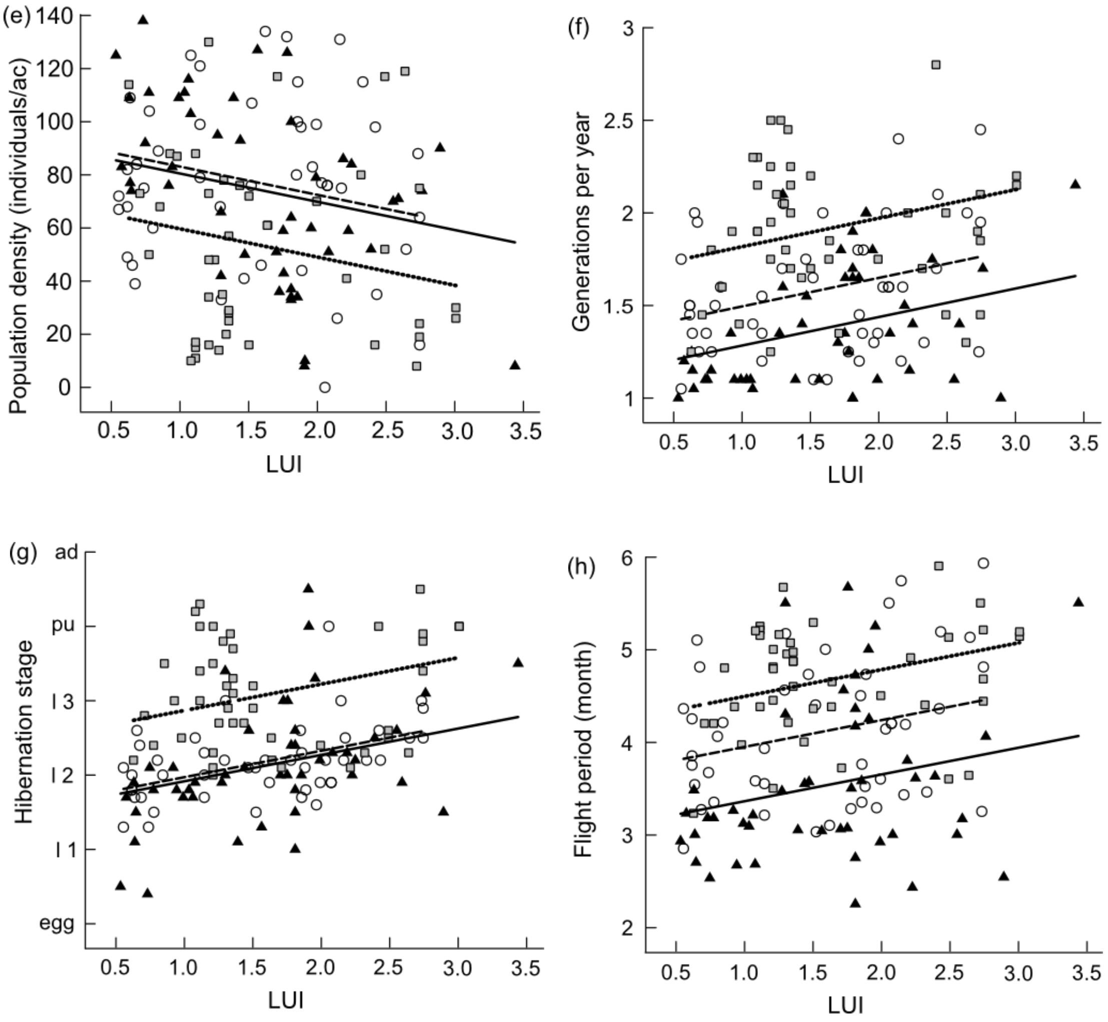

Fig. 3.2 Relations among eight traits of butterfly communities and land-use intensity (LUI) in the three regions Alb, Hainich and Schorfheide. **(a)** The feeding niche (mo = monophagous = one genus, ol = oligophagous = one family, po = polyphagous = more than one family). **(b)** Wing length of butterflies as a measure for dispersal ability. Note that the variable region was not significant; therefore one combined regression line for all three regions is shown. **(c)** Migration propensity on a scale from1 = extremely sedentary species to 9 = highly migratory species based on expert opinion. **(d)** An index for the distribution of butterflies in Europe, larger values indicate a wider distribution. **(e)** The density of butterfly populations. **(f)** The number of generations butterflies produce per year (voltinism). **(g)** The development stage of butterflies during hibernation (l 1, l 2, l 3 = larval stage 1, 2, 3, pu = pupae, ad = adult). **(h)** The flight period is the time span of butterflies as adults.

(Table 3.1f), but butterfly communities produced on average a half generation more per year on intensively compared to extensively managed grasslands (Fig. 3.2f, Table 3.1g). The egg maturation time was not significantly affected by land-use intensity, but differed between regions (Table 3.1h). Butterflies hibernated in an advanced developed larval stage on intensively compared to extensively managed grasslands (Fig. 3.2g, Table 3.1i). The flight period of butterfly communities was extended on average by one month on intensively compared to extensively managed grasslands (Fig. 3.2h, Table 3.1j).

Table 3.1 Effects of region and land-use intensity (LUI) and their interaction on ten ecological and life-history traits (a-j). ANOVA table with *P* and F values of the full models.*

		(a) Feeding niche		(b) Wing length		(c) Migration propensity		(d) Distribution index		(e) Population density	
	df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Region	133,2	**16.75**	**<.0001**	2.67	0.073	**28.07**	**<.0001**	**69.58**	**<.0001**	**7.52**	**<0.001**
LUI	132,1	**5.30**	**0.023**	**4.18**	**0.043**	**15.69**	**0.0001**	**6.30**	**0.013**	**6.89**	**0.009**
Region: LUI	130,2	1.50	0.228	0.18	0.835	2.13	0.123	**5.30**	**0.006**	2.83	0.063

		(f) Egg number		(g) Generations per year		(h) Egg maturation		(i) Hibernation stage		(j) Flight period	
	df	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Region	133,2	0.69	0.505	**31.03**	**<.0001**	**4.71**	**0.011**	**28.99**	**<.0001**	**26.96**	**<.0001**
LUI	132,1	0.99	0.322	**15.20**	**0.0002**	2.72	0.102	**22.61**	**<.0001**	**9.67**	**0.003**
Region: LUI	130,2	2.71	0.071	1.89	0.156	1.64	0.199	1.46	0.236	0.44	0.647

* Significant effects are shown in bold.

The traits used for our analyses were not independent from each other (Table 3.2). Unexpected was the negative correlation between population density and distribution range of butterflies.

Comparing the trait characteristics composition of species with their threat status showed that many species with a low rank value are listed in the Red List of Germany 2008 as endangered. This indicates that species with a composition of trait characteristics associated with extensively managed grasslands are the most threatened species in Germany (Table 3.3).

Table 3.2 Spearman correlation coefficients (*r*) of the relationships between all tested ecological and life-history traits.

	2	3	4	5	6	7	8	9	Flight period
1 Feeding niche	-0.40***	-0.33 ***	-0.59***	0.12 ns	0.005 ns	-0.29***	0.05 ns	-0.48***	-0.23**
2 Wing length		0.33***	0.02 ns	0.003 ns	0.26**	0.03 ns	0.09 ns	0.42***	-0.003 ns
3 Migration propensity			0.64***	-0.61***	0.16 (*)	0.82***	-0.35***	0.87***	0.82***
4 Distribution Index				-0.46***	0.05 ns	0.74***	-0.18*	0.66***	0.74***
5 Population density					0.22**	-0.81***	0.91***	-0.70***	0.74***
6 Egg number						-0.21*	0.40***	0.20*	0.02 ns
7 Generations per year							-0.63***	0.77***	0.93***
8 Egg maturation time								0.50***	-0.51***
9 Hibernation stage									-0.74***

Significance levels are: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, (*) $P < 0.1$, ns = not significant.

Table 3.3 Butterfly species ranked according to the composition of their trait characteristics in comparison with their Red List status (Germany, 2008).

Butterflies were ranked by six ecological and life-history traits (Feeding niche, migration propensity, distribution index, generations per year, hibernation stage, flight period). The lower the rank value, the more traits characteristics associated with extensively grasslands shows a species. RL shows the threat status of a species in the Red List of Germany, 2008.

Species	Rank	RL
Brenthis ino	8	EN
Spialia sertorius	8	
Thymelicus acteon	8	CR
Apatura iris	9	EN
Argynnis adippe	9	CR
Argynnis aglaja	9	EN
Argynnis paphia	9	
Aricia eumedon	9	CR
Erebia aethiops	9	CR
Lycaena hippothoe	9	CR
Melanargia galathea	9	
Melitaea britomartis	9	EN
Polyommatus coridon	9	
Pyrgus alveus	9	VU
Thymelicus lineola	9	
Thymelicus sylvestris	9	
Coenonympha glycerion	10	NT
Cupido minimus	10	EN
Erebia medusa	10	
Hesperia comma	10	CR
Melitaea aurelia	10	EN
Phengaris arion	10	CR
Carterocephalus palaemon	11	
Coenonympha arcania	11	
Colias alfacariensis	11	
Lycaena tityrus	11	
Polyommatus thersites	11	CR
Aporia crataegi	12	
Cyaniris semiargus	12	
Erynnis tages	12	
Melitaea athalia-Komplex	12	CR
Ochlodes sylvanus	12	
Polyommatus bellargus	12	CR
Pyrgus malvae	12	EN
Anthocharis cardamines	13	
Aphantopus hyperantus	13	
Maniola jurtina	13	
Plebeius argus	13	
Araschnia levana	14	
Aricia agestis	14	
Callophrys rubi	14	EN
Issoria lathonia	14	
Leptidea sinapis/reali	14	
Lycaena phlaeas	14	
Polyommatus icarus	14	
Aglais urticae	15	
Coenonympha pamphilus	15	
Colias hyale	15	
Gonepteryx rhamni	15	
Celastrina argiolus	16	
Aglais io	17	
Colias croceus	17	
Papilio machaon	17	
Pieris brassicae	18	
Pieris napi	18	
Pieris rapae	18	
Pontia edusa	18	
Vanessa cardui	18	

EN = endangered, CR = critically endangered, VU = vulnerable, NT = near threatened.

Species names follow Settele et al. (2008).

DISCUSSION

Our results show that overall butterfly diversity decreased with increasing land-use intensity. More importantly, even though in one region species diversity was not affected by land-use intensity, traits of butterfly communities changed in all three regions similarly. This emphasise that even though species richness is not affected by land-use intensity in all regions, there are species with vulnerable adaption strategies. Further our findings indicate that with increasing land-use intensity butterfly communities will be increasingly dominated by species with the following traits: a broad feeding niche, high dispersal ability, high migration propensity, high distribution range, low population density, brood of more than one generation per year, hibernation in a more advanced larval stage and a long flight period. More generally, traits of butterfly communities shift with land-use intensification from trait characteristics associated with specialists to such associated with generalists.

A narrow feeding niche of butterfly larvae characterises a specialised species, while a generalist species has a broad feeding niche (Steffan-Dewenter & Tscharntke 2000). We found proportionally more butterflies with a narrow feeding niche on extensively compared to intensively managed grasslands, presumably owing to a higher plant defence mechanism and a lower mowing and grazing pressure in extensively managed grasslands. Only slow-growing specialised plant species are able to cope with low resource availability on nutrient poor grasslands. Limited resources and a slow gain of biomass demand high herbivore defence, which only enables specialised herbivores to cope with secondary defence compounds of plants (Coley et al. 1985). A high mowing and grazing pressure on intensively managed grasslands can be compensated by utilising a broader range of resources, resulting in a broader feeding niche (Dennis et al. 2004).

We showed that dispersal abilities, migration propensity and distribution of butterfly communities increased with increasing land-use intensity, while population density decreased. Butterfly communities on intensively managed grasslands are faced with frequent habitat disturbances and unreliable food resources, which makes high dispersal ability and high migration propensity a necessary adaptation to be able to (re-) colonize disturbed habitats (Alerstam et al. 2003; Stevens et al. 2010). In contrast, habitat stability of extensively managed grasslands favours communities with low emigration rates, due to high mortality risk of between-habitat dispersal and high costs of dispersal in terms of time and energy which could be allocated to reproduction (Alerstam et al. 2003; Hovestadt & Nieminen 2009).

A large distribution range of butterfly communities on intensively managed grasslands can be explained by several traits of butterflies correlating with distribution. An increased exploitation of resource opportunities, due to a broader feeding niche enables a wider distribution of butterflies in disturbed habitats (Pulliam 2000). High dispersal ability, high migration propensity, many generations per year and a long flight period allow butterflies to explore and colonise distant habitats (Dennis et al. 2004; Lester et al. 2007). Within the last decades habitat area of extensively managed grasslands dropped drastically while the habitat area of intensively managed grasslands increased, which has tremendous effects on the distribution range of specialised butterfly species (van Swaay et al. 2006; Polus et al. 2007).

We found population densities to decrease with land use intensity. On intensively managed grasslands usually the species richness, but also the (relative) abundance is low (Tscharntke et al. 2005). High mowing and grazing frequencies stop butterfly communities from building up dense populations due to unpredictable food resources. In stable habitats with a low perturbation butterflies can complete their life cycle and achieve high densities. Correspondingly Dennis et al. (2004) found low densities of butterfly species feeding on plants characterising intensified habitats and Kotiaho et al. (2005) showed that threatened species, inhabiting usually extensively managed grasslands, show higher population densities than unthreatened species. Contrary to common expectations that species with broad distribution ranges are also locally abundant (Gaston et al. 2000), we found a negative relation between population density and distribution. These findings are in line with some previous findings (Päivinen et al. 2005; Komonen et al. 2009) doubting the generality of the positive density-distribution concept (Johnson 1998). One reason for this discrepancy among studies might be differences in the spatial scale used in the studies (Cowley et al. 2001; Hartley & Kunin 2003) or a bias in sampling efforts (Komonen et al. 2009). Another explanation might be the rarity of extensively managed grassland, because in rare habitats butterflies might achieve maximum population densities, as other suitable habitat patches are difficult to reach (Päivinen et al. 2005). However, interpretations remain speculative and further investigation into the generality of a distribution-density relationship is needed.

A high reproductive potential and fast development with long flight periods can compensate high disturbance frequency and food resource removal in a perturbed habitat.

A high egg load could increase the number of potential offspring surviving and the production of more generations per year may accelerate population growth and also speed up evolution and adaption processes (Cizek et al. 2006). In a previous study a high egg load was observed for butterflies feeding on plants of intensively managed habitats (Dennis et al. 2004). However, our findings show only a tendency toward such pattern. A reason for this might be a potentially high predator and parasitoid pressure on extensively managed grassland demanding also a maximum number of offspring for population maintenance (Steffan-Dewenter & Tscharntke 2002). We found an increased number of generations per year on intensively compared to extensively managed grasslands, which is correlated with the feeding niche of butterflies; this correlation is also found in another study (Altermatt 2010b). A broad feeding niche expands the time span of host plant phenology and enables butterflies to produce more generations per year on intensively managed grasslands. Additionally, butterfly host plants on intensively managed grasslands grow during the whole vegetation period to complete their life cycles after recurrent disturbance events, which is possible due to unlimited nutrient availability.

An on average faster development of butterfly communities on intensively managed grasslands was not achieved by faster egg production after hatching, but by butterflies hibernating on average in a more advanced developmental stage. Immediate egg production after hatching of butterflies might be required also on extensively managed grasslands due to a potentially high predation risk (Steffan-Dewenter & Tscharntke 2002). A more advanced hibernation stage allows an emergence as adult butterfly early in the year, which is a good adaption strategy when the first grazing on intensively managed grasslands occurs in spring; low mobility might then be lethal. We also showed that the length of flight period in butterfly communities increased with land-use intensity. A long flight period enhances the time for populations to react to recurrent perturbation and food resource removal. The prolonged flight period of butterfly communities is highly correlated with a broad feeding niche and high voltinism on intensively managed grasslands, also shown in a study about climate change (Altermatt 2010b). A broad feeding niche enhances the phenological time span of host plants and bi- or multivoltinism enables species to expand their time on wings.

We showed that butterfly communities have fewer specialist and more generalist species with increasing land-use intensity and that highly specialised species showing several identified trait characteristics specific to extensively managed grasslands are endangered. Hence, one can estimate the threat situation of a butterfly species for increasing land-use intensity from the trait characteristics composition. Other factors linked to intensification such as landscape homogenisation, habitat loss and habitat fragmentation were also shown to disadvantage butterflies with specialists´ traits which can intensify the pressure on species of extensively managed grasslands (Steffan-Dewenter & Tscharntke 2000; Ekroos et al. 2010; Öckinger et al. 2010). There is evidence that climate change counteract land-use intensity effects and increase the distribution range of butterfly species (Warren et al. 2001), increase voltinism and induce longer flight periods (Roy & Sparks 2000; Altermatt 2010a), but mainly mobile generalist butterfly species profit (Warren et al. 2001; Pöyry et al. 2009). However, many studies predict, that communities will be increasingly dominated by few butterfly generalist species with further land-use intensity, habitat fragmentation and habitat loss (Warren et al. 2001; Ekroos et al. 2010; Öckinger et al. 2010) which is also expected for other functionally important taxa such as bees (Bommarco et al. 2010). This leads to functional homogenisation of communities which can have severe consequences for ecosystem functioning and services (Olden et al. 2004; Clavel et al. 2010).

CONCLUSIONS

The discrepancy between species richness changes and trait characteristic changes with land-use intensity emphasises the importance of trait analyses to understand mechanisms behind species loss and changes in community composition. Our results show that trait characteristics specific to extensively managed grasslands are associated with specialists and most species showing several of these trait characteristics are threatened reflected by the species status in the Red List of Germany. Consequently the knowledge of a species trait composition can help to identify species at risk of becoming threatened (Kotiaho et al. 2005, William et al. 2010) and conservation and management activities could be implemented before species decline. Species trait data are therefore highly valuable, but unfortunately available only for some indicator species groups like butterflies and birds. More efforts should be spend on gathering data on ecological and life-history traits of different taxa and studies should focus on adaption strategies rather than only species diversity. Only when we know how species are adapted to their environment and how adaption strategies change with changing environment, we can protect species effectively. Our results indicate that conservation programs should protect and restore extensively managed grasslands to protect endangered butterfly species. For example, agrienvironmental schemes can provide incentives for farmers to reduce fertilisation, mowing and grazing intensity to increase grassland quality and quantity.

ACKNOWLEDGEMENTS

We are grateful to Elke Vockenhuber and Vesna Gagic for very helpful comments on a previous version of this manuscript. Laszló Somay kindly identified some butterflies by gender preparation. We thank Elke Vockenhuber, Jochen Fründ and Christoph Scherber for statistical support. We thank Elisabeth K. V. Kalko, Eduard K. Linsenmair, François Buscot, Dominik Hessenmöller, Jens Nieschulze, Ingo Schöning, Ernst-Detlef Schulze, and Wolfgang W. Weisser for setting up the Biodiversity Exploratories project, and Swen Renner, Sonja Gockel, Andreas Hemp, Martin Gorke, and Simone Pfeiffer for maintaining plot and project infrastructure. We also thank the farmers for giving permission to conduct our butterfly transects on their fields. This work has been mainly funded by the Deutsche Forschungsgemeinschaft (DFG) Priority Program 1374 'Infrastructure-Biodiversity-Exploratories' (KL 1849/3-1). Fieldwork permits were given by the responsible state environmental offices of Baden-Württemberg, Thüringen and Brandenburg (according to § 72 BbgNatSchG). J.K. was also funded from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 226852, Scales Project (http://www.scales-project.net).

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APPENDIX 3.1

Table A3.1 Ecological and life-history traits of the identified species from literature sources.

Species	Feeding niche	Wing length	Migration propensity	Distribution index	Population density	Egg number	Generations per year	Egg maturation	Hibernation stage	Flight period
Aglais io	1	28	6	34.54	0.25	445.5	3	7.5	6	6
Aglais urticae	3	23.5	6	39.22	0.6	307	3	7.5	6	5
Anthocharis cardamines	2	21.5	4	39.12	4	33.5	2	3.5	5	3
Apatura iris	3	34	3	16.55	1	48.5	2	7.5	3	2.5
Aphantopus hyperantus	1	22	3	31.19	64	101	2	3.5	3	2
Aporia crataegi	2	31	5	29.39	1	198.5	3	3.5	3	2
Araschnia levana	3	17.5	5	20.1	4	146.5	5	3.5	5	4
Argynnis adippe	3	28	4	28.45	4	48.5	2	10.5	1	3
Argynnis aglaja	3	26.5	3	34.83	4	101	2	7.5	2	2.5
Argynnis paphia	3	31	4	31.67	1	48.5	2	15.5	2	3
Aricia agestis	1	13	4	20.06	64	70	5	3.5	3	4.5
Aricia eumedon	3	15	3	16.42	10	70	2	5.5	3	2
Brenthis ino	2	18.5	2	23.81	40	48.5	2	5.5	1	2
Callophrys rubi	1	14	4	37.32	16	70	2	5.5	5	3
Carterocephalus palaemon	2	14	3	18.71	16	33.5	2	5.5	4	3
Celastrina argiolus	1	15	5	37.38	2.5	48.5	4.5	3.5	5	4
Coenonympha arcania	2	18.8	3	21.77	64	48.5	2	3.5	3	3
Coenonympha glycerion	2	17	2	16.77	64	33.5	2	3.5	3	3
Coenonympha pamphilus	1	15	4	40.74	16	48.5	4	3.5	3	5.5

Table A3.1 to be continued

Table A3.1 to be continued

Species	Feeding niche	Wing length	Migration propensity	Distribution index	Population density	Egg number	Generations per year	Egg maturation	Hibernation stage	Flight period
Colias alfacariensis	2	24	4	12.97	64	101	4	10.5	3	5.5
Colias crocea	2	25	8	30.16	4	146.5	6	10.5	7	5.5
Colias hyale	2	23	5	22.48	10	101	5	7.5	3	5.5
Cupido minimus	3	11	2	23.65	16	48.5	3	3.5	4	3.5
Cyaniris semiargus	3	15.5	4	29.55	64	101	3.5	5.5	3	5.5
Erebia aethiops	2	24	3	9.77	16	48.5	2	5.5	3	2
Erebia medusa	1	22.5	3	9.61	64	48.5	2	3.5	3	2.5
Erynnis tages	2	13.5	3	24.64	16	33.5	3	5.5	4	4
Gonepteryx rhamni	2	28	6	36.25	1	101	2	8	6	6
Hesperia comma	2	14.5	3	24.26	16	33.5	2	5.5	1	3
Issoria lathonia	3	21	5	29.68	4	146.5	6	7.5	3	7
Leptidea sinapis/reali	2	21.5	4	34.03	16	48.5	3	3.5	5	3.5
Lycaena hippothoe	3	16.5	3	21.23	16	70	2	3.5	3	2.5
Lycaena phlaeas	3	13.5	4	40.58	16	70	4.5	5.5	3	5.5
Lycaena tityrus	3	15	3	18.9	64	70	3.5	5.5	3	4
Maniola jurtina	1	23.5	4	36.7	160	146.5	2	15.5	3	3
Melanargia galathea	2	24.5	3	21.84	64	48.5	2	6.5	2	2.5
Melitaea athalia-Komplex	1	17	3	29.97	40	146.5	2	7.5	3	3
Melitaea aurelia	1	15	3	9.23	64	198.5	2	10.5	3	2.5
Melitaea britomartis	1	17.5	2	4.45	64	307	2	5.5	3	2

Table A3.1 to be continued

Table A3.1 to be continued

Species	Feeding niche	Wing length	Migration propensity	Distribution index	Population density	Egg number	Generations per year	Egg maturation	Hibernation stage	Flight period
Ochlodes sylvanus	1	15.5	4	32.22	16	48.5	2	7.5	3	2.5
Papilio machaon	1	35	5	38.38	0.25	48.5	4	3.5	5	5.5
Phengaris arion	2	18	3	16.55	16	198.5	2	1.5.	3	2
Pieris brassicae	1	30.5	7	41.35	4	101	5	7.5	5	5
Pieris napi	1	20	5	43.48	16	48.5	5	3.5	5	5
Pieris rapae	1	25	6	42.06	16	48.5	6	3.5	5	6.5
Plebeius argus	1	13.5	3	31.16	160	33.53	3	5.5	1	3.5
Polyommatus bellargus	2	15.5	3	17.29	4	146.5	4	7.5	3	4.5
Polyommatus coridon	3	16.5	4	16.52	160	70	2	10.5	1	3
Polyommatus icarus	2	16	4	45.45	40	101	4	7.5	3	5
Polyommatus thersites	3	14.5	3	11.06	64	70	4.5	7.5	3	4
Pontia edusa	1	22.5	7	25.00*	4	70	5	5.5	5	5
Pyrgus alveus	3	15	4	15.65	1	48.5	2	10.5	3	2
Pyrgus alveus Komplex	3	15	4	15.65	1	48.5	2	10.5	3	2
Pyrgus malvae	2	12	3	29.45	16	33.5	2	5.5	5	3
Spialia sertorius	3	12	2	9.97	64	33.5	3	5.5	4	2.5
Thymelicus acteon	2	12	3	14.68	260	48.5	2	10.5	2	2.5
Thymelicus lineola	2	13	4	28.54	40	48.5	2	5.5	1	2.5
Thymelicus sylvestris	2	14	3	24.26	40	70	2	5.5	2	3
Vanessa cardui	1	28	8	42.83	0.06	101	5	10.5	7	5.5

- Feeding niche: 1 = polyphagous (more than one family), 2 = oligophagous (one family), 3 = monophagous (feeding on plants of one genus), (Settele et al. 1999).
- Wing length in mm (Higgins & Riley 1978).
- Migration propensity: from 1 = extremely sedentary to 9 = highly migratory. Data are experts' opinion (Settele et al. 1999).
- Distribution index: the relative size of the European distribution or range of a butterfly species in percentages. (Kudnera 2002).
- Population density = individuals per hectare (Settele et al. 1999)
- Egg number: real number (Settele et al. 1999).
- Generations per year: 1 (not in the data set) = a generation every second year, 2 = one generation every year, 3 = sometimes a second generation, 4 = always a second generation, 5 = up to three generations, 6 = up to four generations (Settele et al. 1999).

  In the figure of the main text the axis were back-transformed to actual numbers of generations to make interpretation easier.
- Egg maturation in days (Settele et al. 1999).
- Hibernation stage: 1= egg, 2 = young larvae, 3 = half mature larvae, 4 = mature larvae, 5 = pupae, 6 = adult, 7 = overwintering in warmer regions (Settele et al. 1999).
- Flight period: in months (Settele et al. 2008).

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APPENDIX 3.2

Table A3.2 Values given to the traits for the ranking of species according to their trait characteristics.

In the first row are the ranks 1, 2, 3, we gave to the traits according to the characteristic we identified.

Ranks: 1 = extensively managed grassland character (e. g. monophagous), 2 = intermediate intensively managed grassland character (e. g. oligophagous), 3 = intensively managed grassland character (e. g: = polyphagous)In the rows below are the six selected traits and the trait values for the calculations as obtained from the literature (explanations for the trait values see below and also Appendix 3.1).

Rank	1	2	3
Feeding niche	1	2	3
Migration propensity	2-3	4-5	6-8
Distribution index	1-15	>15-30	>30-45
Generations per year	2	3-4	5-6
Hibernation stage	1-2	3-4	5-6
Flight period	1.5-2.5	>2.5-4.0	>4.0

- Feeding niche:1 = polyphagous (more than one family), 2 = oligophagous (one family), 3 = monophagous (feeding on plants of one genus) (Settele *et al.* 1999).
- Migration propensity: from 1 = extremely sedentary to 9 = highly migratory. Data are expert opinions. In our data set we had no species with a migration propensity value of 1 (extremely sedentary) and 9 (highly migratory) (Settele et al. 1999).
- Distribution index: the relative size of the European distribution or range of a butterfly species in percentages. Distribution range of the identified species ranged from about 4% to 43% (Kudnera 2002).
- Generations per year: 1 = a generation every second year, 2 = one generation every year, 3 = sometimes a second generation, 4 = always a second generation, 5 = up to three generations, 6 = up to four generations (Settele et al. 1999).

  In our data set we had no species with value 1 (a generation every second year).

The axis of the figure in the main text was transformed to actual numbers of generations to make interpretation easier.

- Hibernation stage: 1= egg, 2 = young larvae, 3 = half mature larvae, 4 = mature larvae, 5 = pupae, 6 = adult, 7 = overwintering in warmer regions (Settele et al. 1999).
- Flight period in months (Settele et al. 2008).

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CHAPTER IV

Effects of endophytic fungi on aphid abundance depend on environmental context and are difficult to predict

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(under review in *Oecologia*)

ABSTRACT

1. Fungal endophytes of cool-season grass species produce alkaloids toxic to herbivores, affecting food webs in agricultural and natural ecosystems. Field studies about the effects of endophytes on herbivores are rare and show contradictory results, leading to uncertain conclusions about the nature of endophyte–grass symbiosis. We asked whether the environmental contexts of local and regional scales and predation could modify the effects of endophytes on herbivores.

2. In a full factorial field experiment, we quantified the abundance of the aphid species *Rhopalosiphum padi* on the potted host grass *Lolium perenne*, which was or was not infected with the endophytic fungus *Neotyphodium lolii*. 160 grass pots were located in two regions on altogether 40 grassland sites, 20 intensively managed and 20 extensively managed. We tested the importance of endophyte infection, study region, management of grasslands, predator access, and all two-way interactions on aphid abundance.

3. Endophyte infection reduced aphid abundance significantly in one study region only. In both regions, we found that the impacts of aphidophagous predators and grassland management intensity on aphid abundance were substantially stronger and more consistent than that of endophytes on aphid abundance. In addition, the impact of predators and management on aphid abundance were not modified by the endophyte.

4. We conclude that the impact of endophytes on herbivores can be weak and depends on environmental context at a regional scale. Hence, drawing general conclusions about endophyte–herbivore interactions under field conditions is difficult.

Keywords

bottom-up control, microorganism, multi-trophic interaction, predator exclosure, top-down control

INTRODUCTION

Herbivore abundance can be controlled by predators (top down) and by food plant quality and abundance (bottom up) (Hairston et al. 1960; Price 2002). Furthermore, microorganisms living within food plants can affect herbivores through their influence on food plant quality (Schardl et al. 2004). These microorganisms make up an additional trophic level that can change trophic cascades and food webs (van der Heijden et al. 2008; Hartley & Gange 2009). Fungal endophytes are examples of these symbiotic microorganisms, which were found in all vascular plant species that have been studied (Arnold & Lutzoni 2007). ‘True’ endophytes (Hartley & Gange 2009) of the genus *Neotyphodium* occur only in cool-season grass species; they live hidden within the plant tissue and cause no visible infection. They have an asexual lifecycle and disperse via the seeds of their host (Schardl et al. 2004). The symbiosis between the endophyte and the grass host is mutualistic, parasitic, or a continuum between mutualism and parasitism, and it is still an open topic (Saikkonen et al. 2010). The host benefits from alkaloids produced by the endophyte–grass association, which are toxic to some herbivores studied (Schardl et al. 2004)—e.g. aphid populations exhibit slow growth when feeding on grass infected with *Neotyphodium* endophytes, yet grasshoppers were unaffected (Breen 1994; Meister et al. 2006; Hartley & Gange 2009). Most studies of *Neotyphodium* effects on herbivores and predators have been conducted under laboratory conditions; field studies are less frequent, and the interpretation of their results has been debated (Faeth 2009; Rudgers et al. 2010). Rudgers and Clay (2008) have found that arthropod abundance and diversity decrease in the presence of the endophyte *Neotyphodium coenophialum* in an agronomic grass species. In contrast, Jani et al. (2010) have shown that arthropods not only thrive but also diversify in the presence of an alkaloid-producing endophyte of the genus *Neotyphodium* in a native grass species. An explanation for the contrasting results is the species identity of the host grass and the associated endophyte, which can produce different alkaloids with varying concentrations (Schardl et al. 2004). The herbivore species involved in such experiments might also differ in their response to the various endophyte–grass associations (Breen 1994; Omacini et al. 2001; Hartley & Gange 2009). Another reason for these differing results might be that in some studies, agronomically important and reared grass species were used, whereas in others, native grass species were examined (Saikkonen et al. 2006; Crawford et al. 2010; Faeth & Shochat 2010; Vesterlund et al. 2011). Some authors have also suggested that environmental conditions might influence the effect of endophytes on herbivores (Müller & Krauss 2005; Hartley & Gange 2009; Faeth & Shochat 2010; Saona et al. 2010; Vesterlund et al. 2011), and indeed, field studies in different regions have yielded different results (Omacini et al. 2001; Krauss et al. 2007; Rudgers & Clay 2008; Jani et al. 2010; Saona et al. 2010). We therefore tested whether the environmental contexts of two regions and differently managed grasslands affect the influence of endophytes on herbivores under standardised growing conditions. We chose one abundant aphid species (*Rhopalosiphum padi*) from one breed as an herbivore and one cultivar of the endophyte–grass association (*Neotyphodium lolii–Lolium perenne*). These restrictions were necessary, as it has been shown that intra-specific reactions can vary depending on the genetic background of interacting partners (Faeth et al. 2002; Hesse et al. 2003; Tintjer & Rudgers 2006; Bieri et al. 2009; Bultman et al. 2009).

The toxic effects of endophytes can cascade up the food chain and harm organisms such as predators and parasitoids at higher trophic levels that feed exclusively on herbivores from infected plants (de Sassi et al. 2006; Härri et al. 2008a; Bultman et al. 2009). Even changes in the species composition and food web structure have been documented as effects of these toxins (Omacini et al. 2001; Rudgers & Clay 2008; Jani et al. 2010). Some predators might avoid herbivores feeding on endophyte-infected and alkaloid-producing plants, which could influence interpretations of endophyte symbiosis. Härri et al. (2008a,b) found host selection for parasitoids under laboratory conditions but not in a field study. Their experiment was restricted to one field site, whereas we tested whether different predator communities at differently managed grasslands and regions influence the effects of endophytes on aphid abundance. In the few field studies conducted on the impact of endophytes relative to other regulatory mechanisms such as nutrient availability or predation, the success of endophytes on aphid control has been determined to be relatively low (Krauss et al. 2007; Härri et al. 2008b; Jones et al. 2011; Vesterlund et al. 2011).

Independent of endophyte infection, we expect that aphid control in extensively managed grasslands is better than in intensively managed grasslands, because extensive land-use systems allow better aphid control through increased predator abundance (Roschewitz et al. 2005). Our study design allowed us to test this assumption on a large spatial scale of two distinct regions, in which the full gradients of very extensive to highly intensive grasslands were selected (Fischer et al. 2010).

We studied the effects of endophyte, predation, management intensity, study region, and their unknown but interesting interactions on aphid abundance and formulated the following predictions:

1. Grass infected with the endophyte *N. lolii* reduces aphid abundance.
2. Study region, management intensity, and predation modify the effects of endophyte infection on aphid abundance.
3. The influence of predation, management intensity, and study region on aphid abundance is more important than the toxic effect of endophyte infection.
4. Aphid control by predators in extensively managed grasslands is higher than that in intensively managed grasslands.

MATERIALS AND METHODS

Study species

We selected the agronomically important English ryegrass, *Lolium perenne* L. (Poaceae) as study species and host for the endophytic fungus *Neotyphodium lolii* Glenn, Bacon, and Hanlin (Clavicipitaceae). This grass species is native to Europe and Northern Africa, but many grassland populations in Germany are dominated by reared cultivars that are sown with commonly used seed mixtures (e.g. Klapp & Opitz von Boberfeld 2006). All managed seeds in the experiment belonged to the commercial cultivar Samson, provided by David Hume, AgResearch New Zealand. The grass was either uninfected (E–) or infected (E+) with the wild type of the endophyte, which produces the alkaloids lolitrem B, peramine, and ergovaline (Cheplick & Faeth 2009). The rate for both infections in 100 tested seeds was 92% for E+ seeds and 0% for E– seeds (David Hume, personal communication).

We used bird cherry oat aphids *Rhopalosiphum padi* L. (Aphidae) provided by Katz Biotech AG, Germany, as herbivores on *L. perenne*. *R. padi* is one of the dominant pests in European cereal fields but also feeds on the phloem of various grass species (Blackman & Eastop 2000). *R. padi* is also an abundant aphid species feeding on our host plant, *L. perenne* (Krauss et al. 2007; Jones et al. 2011).

Study regions and sites

We studied two grassland management intensities in two geographically separated regions in Germany to test whether the experimental findings were independent of environmental context, grassland management intensity and the location of the study sites in two distinct landscapes.

The first study region was in southern Germany in the ‘Schwäbische Alb’ (hereafter, Alb); the second region was in central Germany, 350 km further north in the ‘Hainich-Dün’ (hereafter, Hainich). Both regions were dominated by calcareous bedrock. The altitude of the Alb is about 460–860 m above sea level and of the Hainich about 285–550 m above sea level. The Alb (Ulm) showed mean day-temperatures of min. 11.2°C and max. 21.0°C, and had a total precipitation of 327.4 mm during the three-month study period. The Hainich (Eisenach) had mean day-temperatures of min. 10.7°C and max. 19.8°C, and had total precipitation of 281.1 mm (www.wetteronline.de). The Alb is a heterogeneous patchy region comprising intensively managed grasslands interspersed with calcareous grasslands, small agricultural fields, and forests. In contrast to the Alb, the Hainich has a larger proportion of bigger and more intensively managed agricultural fields and grasslands. Both regions are part of a large-scale German project called Biodiversity Exploratories (for details, see www.biodiversity-exploratories.de, accessed 12-August-2011 (Fischer et al. 2010)). In each region, we selected 20 grassland study sites separated by a minimum of 2 km to achieve spatial independence. Grasslands were pastures, meadows, or a mixture of both. Half the sites in each region were intensively managed and half were extensively managed (Appendix 4.1). Intensively managed grasslands were heavily fertilised and mown two or three times per year and/or grazed by livestock—mainly cattle—for several weeks. Extensively managed grasslands were not fertilised and were mown only once per year and/or grazed for short periods. Most extensively managed study sites were calcareous grasslands with sheep grazing that exhibited a significantly higher diversity of vascular plants (mean ± SE, 44.5 ± 13.3) than that in intensively managed grasslands (21.7 ± 4.9) (S. Socher et al., University of Bern, unpublished data).

Experimental set up

We cultivated 160 pots (8 L, Ø 22 cm) with 200 seeds per pot of either *N. lolii*-infected or *N. lolii*-uninfected *L. perenne* grass in commercially available garden compost, which we mixed with soaked water-accumulating granules (Broadleaf P4) to reduce watering efforts in the field. Owing to the high number of seeds, the whole pot was covered with grass even though not all seeds germinated. We placed the plant pots randomly in a climate-controlled greenhouse (16-h light at 19°C, and 8-h darkness at 12°C) and cut the grass twice during its growing period in the greenhouse from mid-March to 01-May-2009, when we started to transfer the grass pots to the study sites within 6 days. At each of the 40 study sites, we installed four experimental pots (2 E+, 2 E–) spaced 3 m apart. We covered all pots completely with a closed Rantai S48 gauze net (Schachtrupp, Germany) with a mesh width of 0.8 × 0.8 mm to exclude parasitoids and prevent predator colonisation before the start of the experiment. We fixed a plastic foil underneath the pots to prevent rainwater from seeping into the ground and the grass roots from growing into the soil (Fig. 4.1). We watered the plants when necessary after counting the aphids but never applied fertiliser. After 2 weeks of acclimatising the plants to outdoor conditions, we cut them to a height of 20 cm and exposed 50 *R. padi* aphids to each grass pot. This activity was carried out within 6 days beginning 18-May-2009. The aphids were reared on barley by the supplier (Katz Biotech AG, Germany), but before use in the experiment, we fed them for several days with uninfected *L. perenne* grass. We used two caged pots at each study site (1 E+, 1 E–) to exclude predators (see Fig. 4.1a); two additional pots (1 E+, 1 E–) on each study site were caged but contained four cut outs (25 × 15 cm) to provide access for aphid predators while ensuring similar microclimatic conditions for the experimental plants (see Fig. 4.1b). We counted the number of aphids in each pot 1 week after exposure; subsequent counts took place every second week, always in the same study site sequence. We conducted six counts of each experimental pot between 25-May-2008 and 6-Aug-2008. One survey of all 160 pots took 6 days because of the large distance between the 40 study sites. We lost two intensively managed study sites in the Alb to vandalism; therefore, 152 experimental grass pots were included in the statistical analyses. A single person conducted all surveys to avoid bias in the searching for and counting of aphids. We counted juvenile, adult, and winged aphids separately and also recorded aphidophagous predators: larvae of hoverflies (Syrphidae), lacewing larvae (Chrysopidae), larval and adult ladybirds (Coccinellidae), earwigs (Dermaptera), spiders (Araneae), carabids (Carabidae), rove beetles (Staphylinidae), and mummies (parasitised aphids). Because earwigs can be aphid predators in other ecosystems (Piñol et al. 2009), we included them as aphid predators. We removed predators that had entered predator-exclusion pots. We did not analyse parasitoids separately because the parasitism rate of aphids was very low (0.07% of all counted aphids; mean ± SE, 0.92 ± 0.14 individuals per pot) during the study year. We counted aphids and predators in each pot for 7 min. When aphids were very abundant, we counted only half of the pot and extrapolated the number. Occasionally other aphid species (mostly *Sitobion avenae* F) colonised the experimental pots in very low numbers, and we, therefore, excluded them from the statistical analyses. We harvested the aboveground plant material 1 week after the final aphid count to measure its biomass (in grams per pot) after 3 days of drying at 80°C.

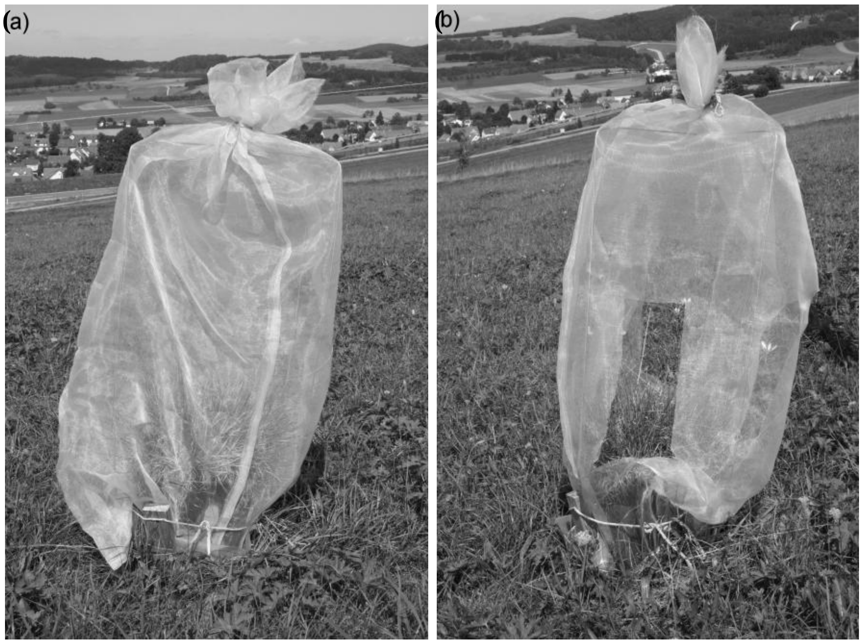

Figure 4.1 The two predation treatments. Experimental pots with the host plant *Lolium perenne* in the field with **(a)** closed gauze net (predator exclusion) and **(b)** open gauze net (predator access).

Statistical analyses

We conducted all statistical analyses using the software R (R Development Core Team 2009), version 2.10.1). We summed the individual numbers of aphids and predators from all six surveys per grass pot and performed analyses for different life stages of aphids as response variables: number of juvenile aphids, number of adult aphids, and winged aphids. Nonetheless, only the total number of aphids is presented hereafter, because results were similar for juvenile and adult aphids, and the number of winged aphids was too low for adequate statistical analyses. Further response variables are the number of predators and plant biomass. We also tested for the total number of aphids for each count survey separately to assess the temporal dynamics of population growth (shown graphically in

Appendix 4.2). We log transformed the data using the equation 'ln[x+1]', when necessary to meet the assumptions of normality and homoscedasticity in the statistical models.

We used linear mixed-effect models with four binary explanatory variables in the sequence: (1) region (Alb, Hainich), (2) management intensity (extensive, intensive), (3) endophyte infection (E+, E–), (4) predation treatment (exclusion, access), and all two-way interactions between these variables. Furthermore, we analysed aphid abundance separately for the two study regions. We used study site as a random factor, as endophyte and predation treatments were nested within that factor. We show results of the full models with all two-way interactions. We also performed model simplifications as proposed by Crawley (2007) for linear mixed-effects models, but the results did not substantially differ from the full models, and therefore, are not shown. We calculated Pearson correlations to identify relationships among plant biomass, total number of aphids, and total number of predators. For aphid abundance as a response variable, we also tested whether the cofactors total number of predators or plant biomass influenced the effect of our explanatory variables. Arithmetic mean and standard error are given throughout the text and shown in the figures.

RESULTS

We counted 198 470 individuals of the aphid species *R. padi* in six counts of 152 experimental pots in 38 grassland sites in the two study regions.

The presence of endophyte infection had no consistent negative impact on aphids (Table 4.1a, Fig. 4.2a) and significantly reduced aphid abundance in the Alb ($F_{1,49} = 5.50$, $P = 0.023$) but not in the Hainich ($F_{1,55} = 0.32$, $P = 0.578$). The negative effect increased with time and was significant from the third observation onward ($F_{1,110} = 7.98$, $P = 0.006$; Appendix 4.2)—i.e. 5 weeks after aphid exposure on the grass pots. In contrast, the interaction between endophyte infection and the management intensity of the surrounding grasslands was not significant, indicating that management did not significantly influence the effects of endophyte on aphid abundance. The interaction of endophyte infection with the predation treatment was also not significant for aphid abundance, indicating that endophyte infection did not induce a significant predator preference for aphids. The presence of the endophyte did not significantly affect predator abundance (Table 4.1b).

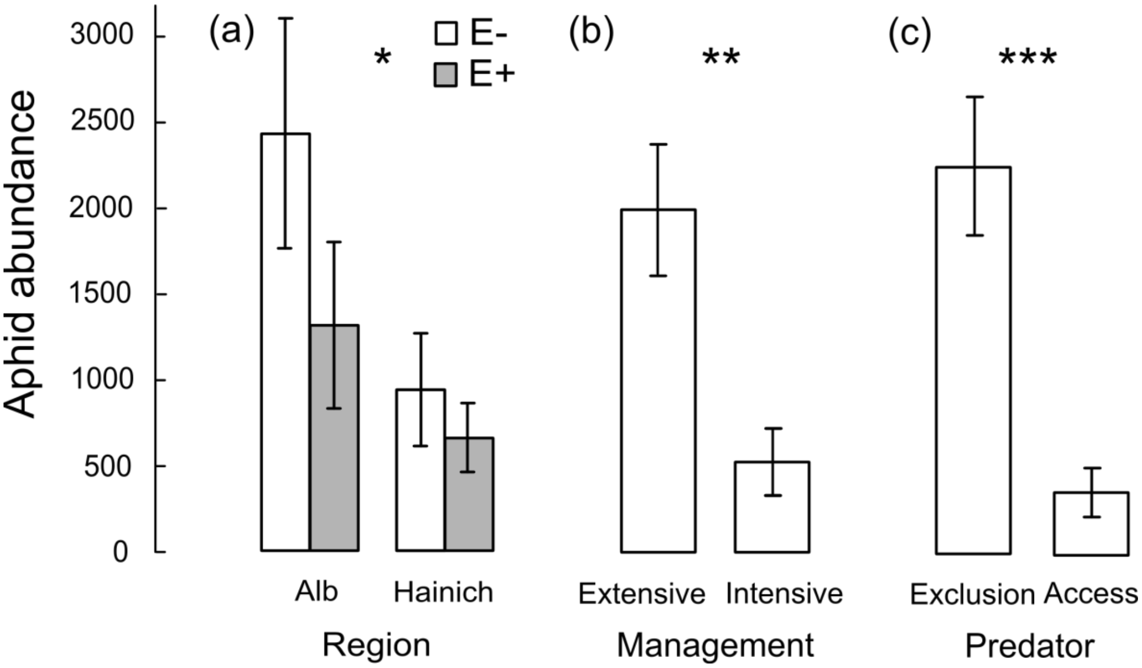

Figure 4.2 Effects on aphid abundance. **(a)** Significant interaction effect of region and endophyte infection ($F_{1,111}$ = 4.53, P = 0.036) on aphid abundance (*Rhopalosiphum padi*) (number of individuals per pot over all 6 counts). Aphid abundance was significantly lower on grass pots with an infection of *Neotyphodium lolii* (E+) than without an infection (E–) in the study region Alb but not in the study region Hainich, **(b)** higher on extensively compared to intensively managed grasslands, and **(c)** higher on pots with predator exclusion than on pots with free access for predator. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; mean ± SE is shown.

As intended by the study design, we recorded more aphidophagous predators when predators had free access to the grass pots (see Table 4.1b, Fig. 4.3c). Aphid abundance was six times lower on the predator-accessed pots (see Table 4.1a, Fig. 4.2c) compared with predator-exclusion pots. Predator abundance was not significantly correlated with aphid abundance (r = –0.087, P = 0.289). For detailed analyses of predator groups see Appendix 4.3. Contrary to expectation, aphid abundance was lower (see Table 4.1a, Fig. 4.2b) and predator abundance was higher (see Table 4.1b, Fig. 4.3b) on intensively managed grasslands than on extensively grasslands, independent of study region. Only 0.02% of all counted aphids (0.22 ± 0.06 individuals per pot) were winged, indicating that the role of potential aphid dispersal during aphid population growth was of little importance.

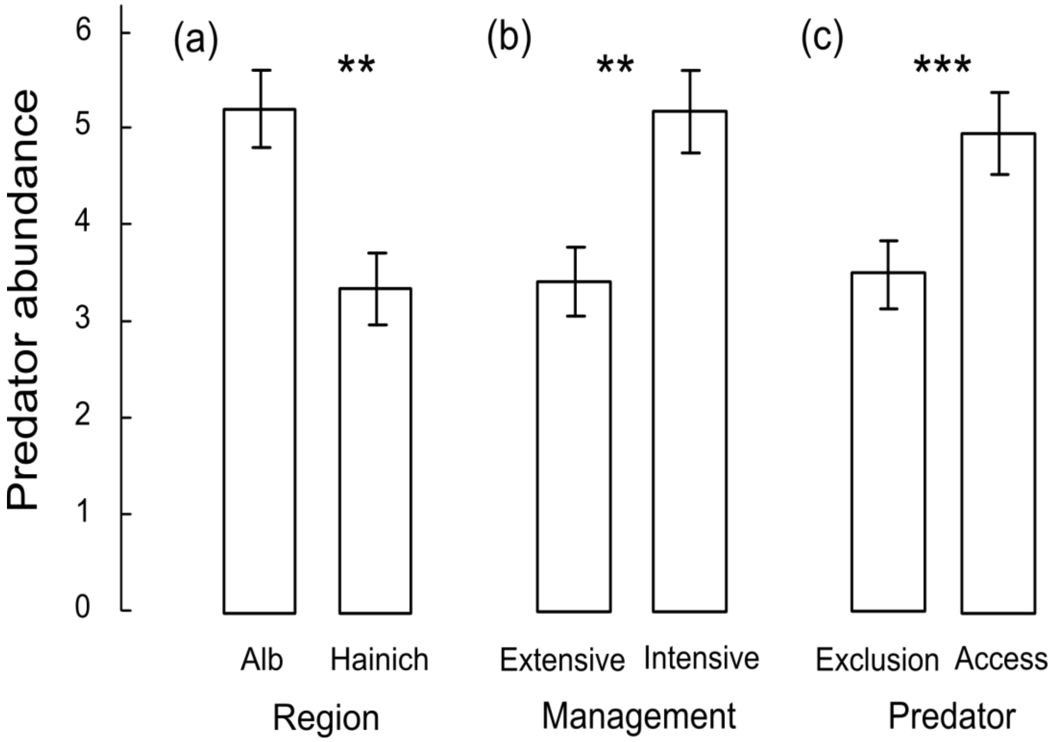

Figure 4.3 Effects on predator abundance. Predator abundance (number of individuals per pot over all 6 counts) in the experimental pots was **(a)** higher in the region Alb than in the region Hainich, **(b)** lower in extensively managed compared with intensively managed grasslands, and **(c)** lower in pots with predator-exclusion treatment. $^{**}P < 0.01$, $^{***}P < 0.001$; mean ± SE is shown.

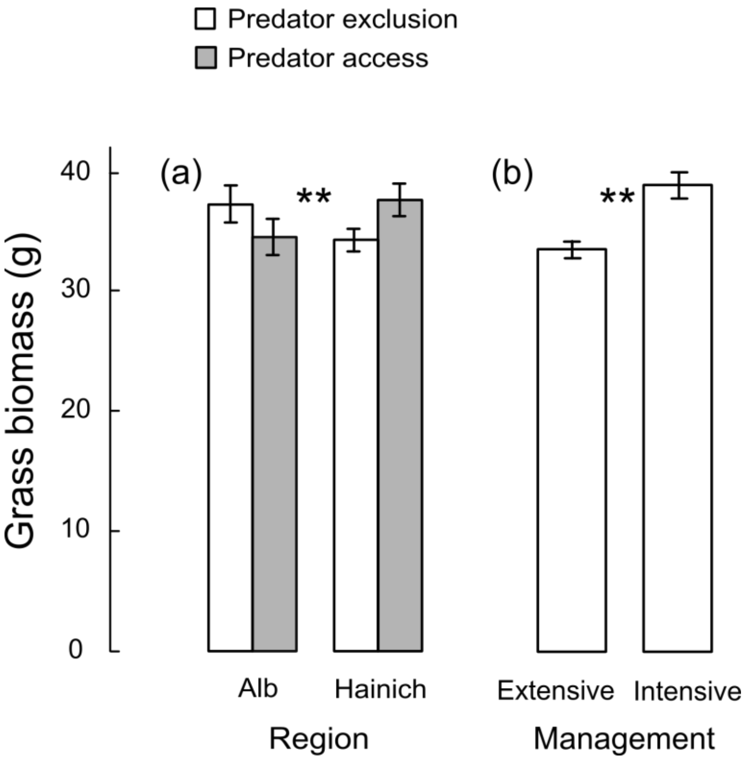

Figure 4.4 Effects on grass biomass. Grass biomass in the experimental pots was **(a)** higher in the region Alb with predator-exclusion treatments but higher in the region Hainich with predator-access treatments and **(b)** higher on intensively managed than on extensively managed grasslands. $^{**}P < 0.01$; mean ± SE is shown.

Grass biomass of the experimental pots was not significantly affected by endophyte infection (Table 4.1c). Pots on intensively managed grasslands exhibited a significantly higher plant biomass compared with those on extensively managed grasslands (see Table 4.1c, Fig. 4.4b). In the Alb, biomass was higher in the closed cages, whereas in the Hainich, biomass was higher in the cages with cut-outs (see Table 4.1c, Fig. 4.4a). Grass biomass was negatively correlated with aphid abundance ($r = –0.197$, $P = 0.015$).

Analyses including predator abundance ($F_{1,106} = 1.26$, $P = 0.265$) or biomass ($F_{1,106} = 4.75$, $P = 0.032$) as additional cofactors in the full linear mixed-effects models with the response variable aphid abundance did not substantially change the effects of the explanatory variables: region, management intensity, endophyte infection, and predator treatment.

Table 4.1 Results of mixed effects models. **(a)** Aphid abundance, **(b)** predator abundance, and **(c)** biomass as response variables.*

		(a) Aphid abundance		(b) Predator abundance		(c) Biomass	
	df	F	P	F	P	F	P
Region	1,34	1.93	0.174	**8.96**	**0.005**	0.002	0.965
Management	1,34	**11.75**	**0.002**	**6.96**	**0.012**	**11.51**	**0.002**
Infection	1,107	1.64	0.202	2.48	0.117	0.53	0.470
Predation	1,107	**69.94**	**<0.001**	**13.15**	**0.0004**	0.15	0.696
Region: Management	1,34	0.02	0.891	0.05	0.823	2.86	0.100
Region: Endophyte	1,107	**4.53**	**0.036**	1.23	0.271	0.37	0.543
Region: Predation	1,107	1.16	0.284	2.47	0.119	**7.04**	**0.009**
Management: Endophyte	1,107	1.33	0.252	0.10	0.749	0.02	0.880
Management: Predation	1,107	1.04	0.311	1.16	0.285	0.20	0.656
Endophyte: Predation	1,107	0.51	0.478	1.34	0.250	1.07	0.303

*Significant effects are bold and depicted in Figures 2, 3, and 4.between endophyte infection

DISCUSSION

In general, our results indicate that endophyte presence in the agronomic grass species *L. perenne* is less important in herbivore suppression than top-down control by predators. Comparing two study regions, we found that endophyte infection with *N. lolii* significantly reduced aphid abundance of *R. padi* in one but not in the other region.

Previous field studies have shown that endophytes of the genus *Neotyphodium* affect the performance of herbivores inconsistently, raising questions about whether endophyte-grass symbiosis is always defensive mutualism or parasitism (Cheplick & Faeth 2009; Saikkonen et al. 2010). In addition to considering the effects of native versus agronomic grass species and the species identity of the interacting partners, it is speculated that environmental context might explain the contradicting results in field studies (Müller et al. 2005; Hartley & Gange 2009; Faeth & Shochat 2010). Our study shows evidence environmental contexts can affect the significance and strength of endophyte effects on herbivores. Several studies have shown that endophyte infection can influence plant fitness under low nutrient or low water availability (Malinowski & Belesky 2000; Hesse et al. 2003; Saona et al. 2010). This influence can also have consequences at higher trophic levels. For instance, fertilisation of uninfected plants can lead to a higher reproduction rate of aphids, because plants offer better food quality, whereas fertilisation of infected plants can be a disadvantage for aphids probably owing to a higher production of mycotoxins, which require nitrogen (Lehtonen et al. 2005; but see Rasmussen et al. 2007). Saona et al. (2010) have demonstrated the interaction of environmental context with the endophyte *Epichloë festucae* in a field study. Endophyte presence increases with increasing availability of nutrients and grazing pressure on dry but not on moist sites whereas the fitness of infected plants is not influenced on dry sites but increases with nutrient availability and grazing pressure on moist sites (Saona et al. 2010). We assume that the understanding of how plants, endophytes, and herbivores interact in the field under different environmental conditions is incomplete. Our experimental design allows us to exclude the most frequently studied variable, nutrient availability, from interacting with endophyte infection, because the soil conditions were constant in all of our experimental pots. We also used interacting species with the same genetic background exclusively; however, different regions might differ in a large number of environmental conditions—e.g. abiotic conditions such as precipitation, temperature, and ultraviolet radiation, and biotic conditions such as species communities of plants, herbivores, and predators. Therefore, we conclude that inconsistent results can occur owing to the environmental contexts in the locations of field studies.

Different management intensity of the grasslands had no significant interacting effects with endophyte presence on the abundance of aphids. A Swedish study has shown, however, that management intensity (grazing pressure and nutrient availability) can affect the influence of endophyte infection on plant fitness (Saona et al. 2010), which can cascade up the food chain and influence herbivore abundance (Lehtonen et al. 2005).

Predator abundance was not significantly influenced by the presence of the fungus *N. lolii*. Further, the interaction of endophyte presence with predation was also not a significant influence on aphid abundance. Therefore, we can assume that predators do not avoid aphids feeding on infected plants. Similarly, previous field experiments have shown no effects from *N. lolii* on predator abundance (Härri et al. 2008b; Jones et al. 2011). Experiments showing that predators are negatively affected have been conducted only in laboratory studies (de Sassi et al. 2006). In contrast to these experiments, in our study, predators had a wide choice of prey and presumably did not feed exclusively on aphids from endophyte-infected grass pots. We assume that the toxic effect of alkaloids is too low to affect predators, and they are not forced to choose between prey from infected and uninfected grass individuals. Further, some predators might be unable to distinguish between herbivores from endophyte-infected hosts and uninfected hosts, whereas other predators might be able to distinguish between preys from both host plants after a learning period (Härri et al. 2008a).

In contrast to relatively weak endophyte effects, our results showed strong top-down control of aphids in all grass pots. Such strong impact on herbivore populations has been recorded in natural (Müller & Godfray 1999) and agricultural ecosystems (Schmidt et al. 2003). Our result is also in line with another study on the same endophyte–grass association, in which natural enemies are more important and faster acting than endophytes for aphid abundance control (Härri et al. 2008b).

Independent of study region and opposite to our expectations, experimental pots on intensively managed grasslands had consistently lower aphid abundance and higher predator abundance compared to those in pots on extensively managed grasslands. We expected better aphid control on extensively managed grasslands because extensive land-use systems have been shown to attract more aphid predators (Roschewitz et al. 2005). In our study, extensively managed grasslands were mostly calcareous grasslands with few pasture grasses, which are considered the main host of *R. padi*. Grass species on calcareous grasslands could be less attractive for aphids because they are often dry during the summer months. On intensively managed grasslands, aphids in our pots were surrounded by sowed pasture grasses and aphids, which allow higher aphidophagous predator abundance. Intensively managed grasslands also produce larger plant biomass owing to higher nutrient availability in the soil or fertilisation than those on extensively managed grasslands (Lee et al. 2010). Higher plant biomass should, therefore, enhance herbivore and predator abundance in the surrounding grassland, leading to the conclusion that intensively managed grasslands provide high predator abundance (Siemann 1998). Weiner et al. (2011) have conducted a study on sites in the Alb that overlapped with ours, and demonstrate a higher abundance of syrphid flies, one of the main aphid predators, on intensively managed grasslands compared with extensively managed grasslands.

The abundance of herbivores can be resource-driven by the biomass of the host plant (Price 2002). It is unclear, however, whether our experimental system is mainly top-down or bottom-up controlled. We found neither a consistent effect of endophyte on biomass—in contrast to laboratory studies (Hesse et al. 2003; Schardl et al. 2004)—nor a positive biomass–aphid correlation. In fact, grass biomass was even negatively correlated with aphid abundance, which does not support the hypothesis that the biomass and quality of plants restrict the number of aphids feeding on them (Price 2002). Rather, it implies that high aphid abundance can significantly reduce host plant biomass—a pattern that might occur in our caged pots, because we placed additional aphids at the beginning of the season, but which is less plausible for other experimental designs (e.g. Jones et al. 2011) or natural grasslands.

CONCLUSIONS

We have shown that the direct impact of the endophytic fungus *N. lolii* on the abundance of aphid *R. padi* is less important than the impact of predators and management intensity. Effects of endophytes on aphid abundance can depend on study region and, therefore, on environmental context, which cannot easily be controlled at the field or regional scale. Conclusions drawn from studies conducted in laboratories, therefore, do not necessarily hold true for field experiments or even for studies conducted in natural ecosystems. Field studies might not be repeatable in other study regions if the impact of an explanatory variable is weak. When the impact of a variable is as strong as our experiment demonstrates predator exclusion or management to be, however, the results should have a higher probability of holding for many regions. To address disagreement on the nature of endophytes in grasses—that is, whether they are defensive mutualists or parasites (Faeth 2010; Rudgers et al. 2010)—we suggest more experimental studies to identify abiotic and biotic factors that can modify the effects of endophytes on the host grass and alkaloid production as well as on herbivore and predator performance. Further, these factors and their interactions must be tested in field studies in different regions to ensure their general validity. As long as environmental conditions that influence the impact of endophytes on aphid abundance are unknown, the outcomes of such experiments remain difficult to predict.

ACKNOWLEDGEMENTS

We thank Elisabeth K. V. Kalko, Eduard K. Linsenmair, François Buscot, Dominik Hessenmöller, Jens Nieschulze, Ingo Schöning, Ernst-Detlef Schulze, and Wolfgang W. Weisser for setting up the Biodiversity Exploratories project, and Swen Renner, Sonja Gockel, Andreas Hemp, Martin Gorke, and Simone Pfeiffer for maintaining plot and project infrastructure. We also thank farmers who permitted us to conduct our experiment on their fields. Further, we thank Jonathan Newman, Simone Härri, Teja Tscharntke, and an anonymous reviewer for helpful comments on the manuscript. Our thanks also go to David Hume for the provision of *L. perenne* seeds. Stefanie Socher and colleagues from University of Bern kindly provided unpublished data about the plant diversity of the sites. We are grateful to Jochen Fründ and Christoph Scherber for statistical support. This work has been funded by the Deutsche Forschungsgemeinschaft Priority Program 1374 'Infrastructure-Biodiversity-Exploratories' (KL 1849/3-1). Fieldwork permits were given by the responsible state environmental offices of Baden-Württemberg and Thüringen (according to § 72 BbgNatSchG).

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APPENDIX 4.1

Table A4.1 Location and management of the study sites in the regions Alb and Hainich (2009).

Region	EP Plotid	Plotid	Easting	Northing	Management type	Livestock	Mowing frequency	Fertilisation
Alb	AEG2	A39275	3535100	5360000	meadow	no	3	yes
Alb	AEG3	A48112	3539490	5363590	meadow	no	2	yes
Alb	AEG4	A31160	3531105	5360425	mown meadow	cattle	1	yes
Alb	AEG7	A24854	3527985	5361580	meadow	sheep	0	no
Alb	AEG9	A43687	3537310	5361995	pasture	sheep	0	no
Alb	AEG11	A20470	3525780	5372020	meadow	no	2	yes
Alb	AEG14	A46088	3538500	5359900	meadow	no	2	yes
Alb	AEG15	A35767	3533240	5372265	meadow	no	3	yes
Alb	AEG19	A35463	3533200	5362300	mown meadow	cattle and horse	1	yes
Alb	AEG21	A21850	3526505	5367220	pasture	cattle and horse	0	yes
Alb	AEG22	A45310	3538090	5363080	meadow	no	1	no
Alb	AEG25	A7116	3519300	5361995	pasture	sheep	0	no
Alb	AEG26	A28932	3529985	5362000	pasture	sheep	0	no
Alb	AEG30	A36956	3533900	5368820	mown meadow	sheep	0	no
Alb	AEG32	A41172	3536000	5369900	pasture	sheep	0	no
Alb	AEG35	A11495	3521400	5371500	meadow	no	2	yes
Alb	AEG42	A24657	3527920	5362310	mown meadow	cattle	1	yes
Alb	AEG47	A35283	3533100	5364700	pasture	sheep	0	no
Alb	AEG49	A43953	3537400	5368900	pasture	sheep	0	no
Alb	AEG50	A38525	3534700	5363200	meadow	no	2	no
Hainich	HEG1	H4580	4388100	5649700	meadow	no	2	yes
Hainich	HEG3	H8709	4390100	5652600	meadow	no	3	yes
Hainich	HEG4	H20510	4390599	5665418	mown meadow	cattle	1	yes
Hainich	HEG9	H30212	4386991	5677799	pasture	cattle	0	no
Hainich	HEG13	H4651	4387020	5681780	mown meadow	cattle	3	no
Hainich	HEG16	H12643	4392000	5656500	pasture	sheep	0	no
Hainich	HEG18	H16581	4390200	5684380	pasture	sheep	0	no
Hainich	HEG23	H20426	4383790	5667898	mown meadow	cattle	1	no
Hainich	HEG24	H20438	4384619	5664180	mown meadow	cattle	1	no
Hainich	HEG25	H37029	4382400	5655585	pasture	sheep	0	no
Hainich	HEG28	H20535	4395199	5682299	mown meadow	cattle	1	yes
Hainich	HEG32	H20106	4399720	5661020	mown meadow	cattle	1	yes
Hainich	HEG34	H30402	4387397	5676720	mown meadow	cattle	1	yes
Hainich	HEG35	H30417	4389100	5677299	mown meadow	cattle	1	yes
Hainich	HEG37	H20245	4395901	5656297	mown meadow	sheep	1	yes
Hainich	HEG42	H13708	4392500	5660490	pasture	sheep	0	no
Hainich	HEG43	H19577	4391020	5686400	pasture	sheep	0	no
Hainich	HEG44	H20215	4393508	5658884	pasture	sheep	0	no
Hainich	HEG46	H30915	4412998	5675399	pasture	sheep	0	no

APPENDIX 4.2

Figure A4.2 Aphid population growth over the six surveys in the two study regions Hainich and Alb. Mean and standard error are shown.

Figure legend

E-: endophyte free grass

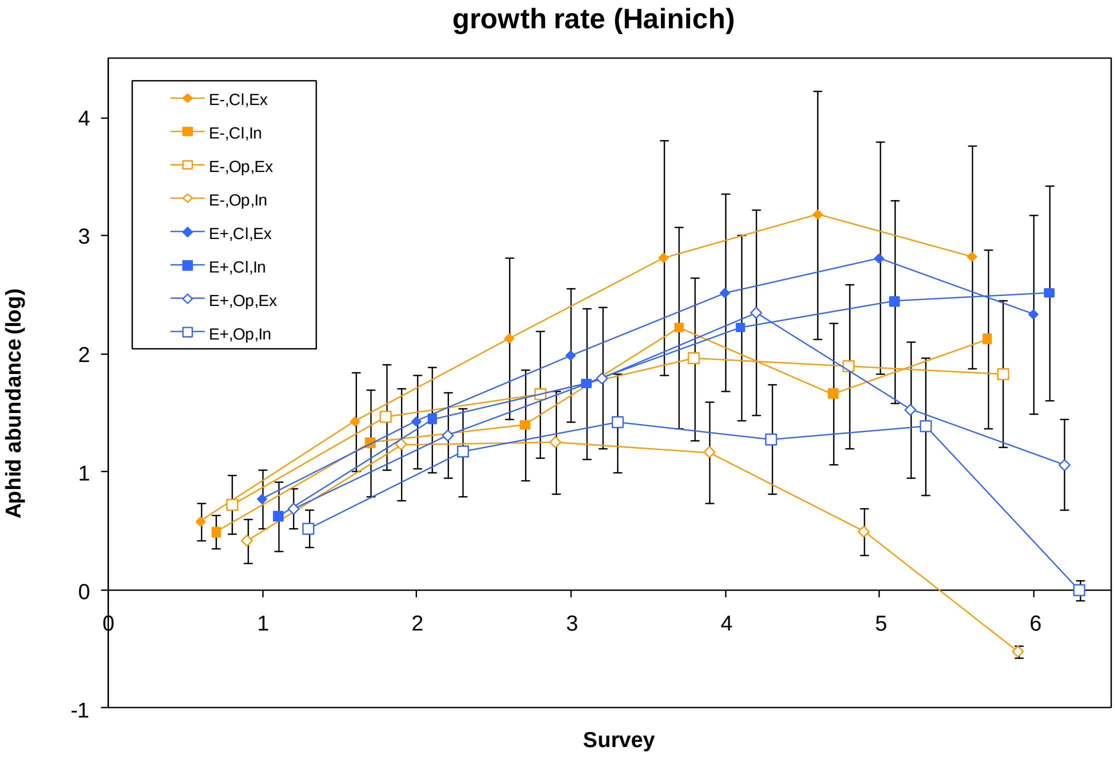
E+: endophyte infected grass

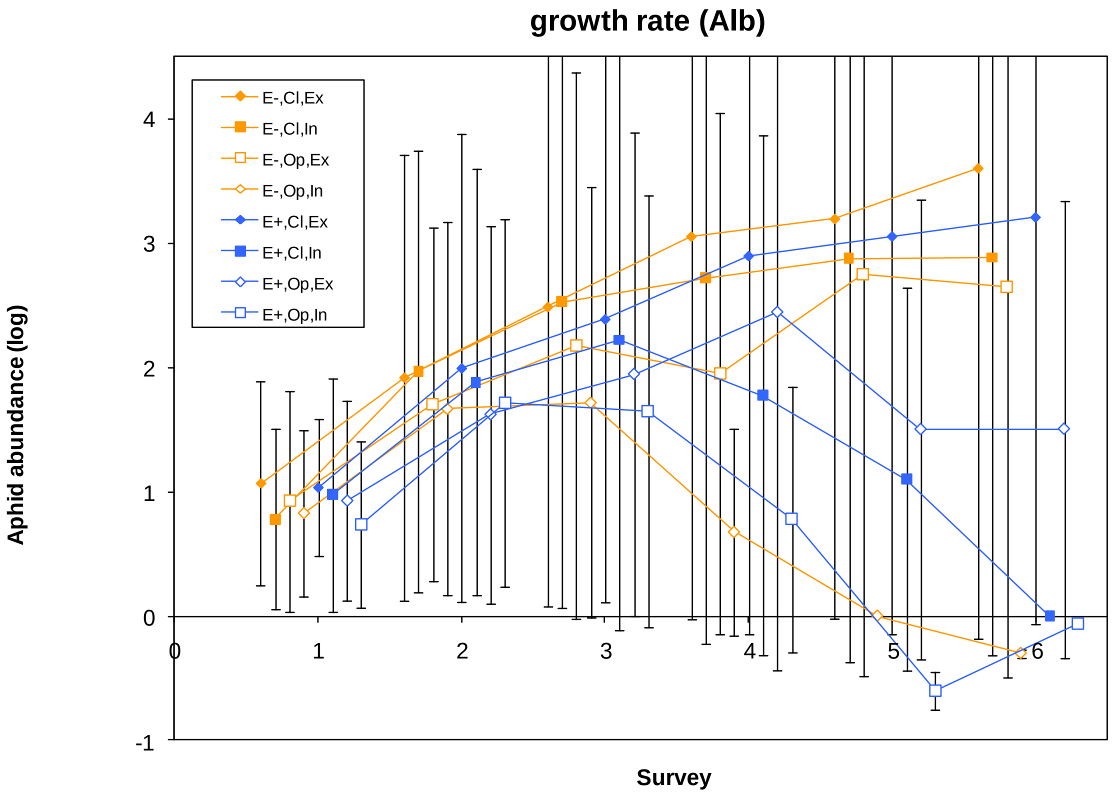
Cl: closed cage, predator exclusion

Op: open cage, predator access

Ex: extensive grassland management

In: intensive grassland management




growth rate (Alb)
Aphid abundance (log)
Survey
E-,Cl,Ex
E-,Cl,In
E-,Op,Ex
E-,Op,In
E+,Cl,Ex
E+,Cl,In
E+,Op,Ex
E+,Op,In
4
3
2
1
0
-1
0
1
2
3
4
5
6

APPENDIX 4.3

Additional analyses of predators

Materials and methods

Statistical analyses

In the manuscript we analysed correlation for predator abundance and aphid abundance. Here we additionally analysed correlations for predator without earwigs and earwigs separately because approximately half of the predators were earwigs.

Results

In the manuscript we said that predator abundance and aphid abundance were not correlated. But predator abundance without earwigs (r = 0.257, $P = 0.001$) was positively correlated with aphids, while earwigs separately analysed were negatively correlated with aphid abundance (r = -0.338, $P < 0.0001$). Adequate linear mixed effects models could not be conducted for abundance of earwigs and other predators separately due to the low numbers of predator individuals observed in our surveys. Approximately 50% of all predator individuals were earwigs, which were more abundant on intensively managed grasslands in the study year and which occasionally entered the exclusion cages at the beginning of the experiment, while later in the season most predators were larvae of ladybirds and lacewings.

Discussion

In our study only earwigs were negative correlated with aphid abundance and represent 50% of all recorded predators. Earwigs were shown to be highly effective of controlling aphid abundance in other study systems like orchards (e. g. Piñol et al. 2009). In our study earwigs were more abundant on intensively compared to extensively managed grasslands and occasionally entered the exclusion cages. Nonetheless, we wish to be cautious with the interpretation of the recorded predator abundance as our experiment was designed to quantify aphid abundances rather than predator abundances. Some aphid predators are highly mobile and some are active at night, shy, barely visible and might have visited the grass pots only for short time periods (Vickerman & Sunderland 1975).

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CHAPTER V

Synthesis

SYNTHESIS

I studied in three regions the effects of land-use intensity on species richness and abundance and the occurrence of endangered Lepidoptera species (chapter II), changes of ecological and life-history trait characteristics in butterfly communities (chapter III) and multitrophic interactions with the focus on an endophyte, its host grass, plant sucking aphids and their natural enemies. I was also interested, whether effects depended on the studied region or were consistent over the three regions.

In chapter II I showed that the land-use intensity effect on Lepidoptera species richness and abundance differed between regions. In one region Lepidoptera did not respond on land-use intensity, while in the other two regions Lepidoptera species richness and abundance decreased with increasing land-use intensity. Similarly, the occurrence of endangered species decreased with increasing land-use intensity. Region dependent responses to land-use intensity might be driven by the regional species pool and the number of endangered species. In a region with a large species pool and many endangered species, the effect of land-use intensity was stronger than in regions with a small species pool and few endangered species. Endangered species—most are specialists—disappear first when land-use intensity increases (Kleijn et al. 2011). A species poor region without endangered species holds mostly common species, which are not or less sensitive to increasing land-use intensity. Thus increasing land-use intensity has more severe effects in a species rich region with many endangered species.

The absence of calcareous grasslands in the species poor region plays an important role for the overall low diversity, because calcareous grasslands are one of the most important habitat for Lepidoptera (van Swaay et al. 2006).

The amount of fertiliser did not affect Lepidoptera species richness and abundance, rather it was more important whether a grassland site was fertilised or not. This is in line with a study about effects of nitrogen application on plant species richness (Kleijn et al. 2009), showing the same pattern as Lepidoptera in our study. Increasing mowing frequency had against our expectation no effect, while increasing grazing intensity reduced species richness and abundance of Lepidoptera.

I conclude that extensively managed grasslands are of major importance to maintain high Lepidoptera species richness and abundance. Extensive land-use intensity has a stronger positive effect on species richness and abundance in regions with a large species pool and many endangered species than in regions with a small species pool and few endangered species. Consequently incentives will be more affective in regions where still a considerable regional species pool with endangered species is present. For extensive grassland management fertiliser should abandoned and grazing intensity should be low.

In chapter III I showed that ecological and life-history trait characteristics in butterfly communities changed with changing land-use intensity. Eight of ten tested traits shifted with increasing land-use intensity from specialist to generalist characteristics. The trait characteristics changed to a broader feeding niche, higher dispersal ability, higher migration propensity, higher distribution range, lower population density, brood of more than one generation per year, hibernation in a more advanced larval stage and a longer flight period. The egg maturation time and the number of eggs did not respond to land-use intensity. This indicates that species with generalists' trait characteristics can compensate better for high disturbance frequencies by mowing or grazing and recurrent food resource removal than species with specialists' trait characteristics. A comparison of species trait characteristics and the species threat status in the Red List of Germany 2008 implicates that highly specialised species showing several identified trait characteristics specific to extensively managed grasslands are endangered.

An increase of generalist species coupled with a reduction of species richness with increasing land-use intensity lead to functional homogeneity in butterfly communities on grassland sites, which can have severe consequences for ecosystem functioning (Clavel et al. 2010). The knowledge of species trait characteristics can help to predict the species threat status due to land-use intensification and can help to protect species before they become rare (Kotiaho et al. 2005). Therefore, trait data are highly valuable for conservation issues and more efforts should be spend on gathering such data. To protect highly specialised species, grasslands of extensive land-use intensity are most important and should be protected by incentives.

In chapter IV I showed for a multitrophic system that the land-use intensity and top-down control by predators had a strong effect on aphid abundance in a field experiment. Contrasting, the bottom-up effect by endophytes on aphid abundance was weak and depended on the study region, reducing aphid abundance significantly only in one but not the other region. Land-use intensity and predators had no additional modifying effect on the endophyte effect on aphid abundance. This leads to the conclusion that the direct impact of endophytes on herbivores in field studies is less important than top-down control by predators and the environmental context offered by different land-use intensity. The weak endophyte effect on herbivores depended on the study region and therefore on the environmental context, which has to be considered in further studies.

In conclusion, I found a strong land-use intensity effect in all three studies on all response variables. Increasing land-use intensity reduced species richness and abundance and the occurrence of endangered species. High land-use intensity disadvantaged species with trait characteristics of specialists. Land-use intensity also affected aphid abundance in the multitrophic study. Consequently land-use intensity has a great impact on many aspects in ecology and is an important factor to consider in future studies.

Further, some effects differed between regions; this shows the importance conducting observations or experiments in more than one region to be able to draw more general conclusions or to point out regional dependent effects.

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Summary

Land-use intensification is identified as one of the major threats for biodiversity worldwide. The number of endangered species is increasing and only few species mostly generalists seem to benefit from land-use intensification. Consequently, land-use intensification affects species with specific life-history trait characteristics stronger than others. Different levels of land-use intensity provide different biotic and abiotic contexts for interacting species and thus can influence multitrophic interactions differently. Land-use intensity influences many ecological aspects, but regions with different environmental context can modify such effects.

Therefore, in my thesis I asked how land-use intensity affects ecological variables such as species richness, abundance and the occurrence of endangered species (chapter II), ecological and life-history trait characteristics (hereafter referred as traits) (chapter III) and multitrophic interactions (chapter IV). I was interested, if effects differ between regions.

For my study in chapter II I used butterflies and day-active moths (hereafter termed Lepidoptera) as a target group. I obtained the threat status from Red Lists of Germany to identify endangered species. I expected a decline of Lepidoptera species richness, abundance and the occurrence of endangered species with increasing land-use intensity dependent on the region. In chapter III I excluded day-active moths from the dataset used in chapter II, because only for butterflies sufficient data on ten selected ecological and life-history traits were available in the literature. I expected traits to change with increasing land-use intensity from characteristics of specialists to such of generalists due to frequent habitat disturbances. Further, I expected species with trait characteristics of specialists to be endangered. For these investigations I caught butterflies and day-active moths on 137 grassland sites using fixed transect walks in three different regions in Germany. The selected grasslands showed a gradient from very extensively to very intensively land-use intensity in each region. To assess land-use intensity I used a land-use intensity index comprising amount of fertiliser, mowing frequency and grazing intensity.

In chapter IV I used a multitrophic system of four levels with the asexual in grass living endophyte of the genus *Neotyphodium* (bottom-up control), the host grass, leaf-sucking aphids and natural enemies of aphids (top-down control). Former field studies led to contrasting conclusion on the nature of the endophyte-grass symbiosis; some studies

showing defensive endophytic effects on herbivores (mutualism) others supportive endophytic effects on herbivores (parasitic). I expected the endophyte effect on aphid abundance to be weak and to be modified by different environmental context of different regions or different land-use intensities and predators preferring aphids from uninfected plants. For this study, I selected 40 grassland sites in two regions, in each region ten intensively and ten extensively managed ones to account for effects of the environmental context. On each grassland site I placed four grass pots *(Lolium perenne)* with aphids (*Rhopalosiphum padi*). The aphid population size was regularly documented. The grass was a) either infected with an endophyte (*Neotyphodium lolii*) or not and b) predators were excluded or had free access to the pots.

In all three studies (chapter II-IV) a strong land-use intensity effect was shown on the tested response variables and some responses were modified by the region.

The Lepidoptera species richness and abundance studied in chapter II decreased with increasing land-use intensity only in two regions significantly, but not in the third; similarly was the occurrence of endangered Lepidoptera affected by land-use intensity. The region dependent responses were probably caused by different regional species pools and the related number of endangered species and the presence of calcareous grasslands in only two regions. In species rich regions with many endangered species effects of land-use intensity were strong contrasting to species poor regions. Species poor regions have mainly common species, which are less sensitive to increasing land-use intensity than endangered species. Calcareous grasslands, which are lacking in the species poor region, are an important habitat for high species diversity. I showed that fertilisation and increasing grazing intensity, but not increasing mowing frequency decreased Lepidoptera species richness and abundance significantly.

In chapter III I showed that eight of the ten tested traits of butterfly communities changed with increasing land-use intensity from specialist characteristics to generalist characteristics: broader feeding niche, higher dispersal ability, higher migration propensity, higher distribution range, lower population density, brood of more than one generation per year, hibernation in a more advanced larval stage and a longer flight period. The species threat status in the Red List 2008 compared with the trait characteristic combination for each species showed that species with several trait characteristics specific to extensively managed grasslands are endangered. This indicates that only species with generalist trait characteristics are able to cope with high a disturbance frequency and unreliable food resource availability caused by high land-use intensity.

I showed in chapter IV for the multitrophic system that aphid abundance was strongly affected by the region, land-use intensity and predators. In contrast, the effect of endophytes was weak and region dependent, reducing aphid abundance only in one, but not the other region. Land-use intensity and predators had no further modifying effects on the endophyte effect. This indicates that endophyte effects on herbivores might be less important in natural systems compared to other forcing factors on herbivores such as region, land-use intensity and predators and that endophyte effects depend on the environmental context.

In chapter II and III calcareous grasslands and other grasslands with extensive management intensity were shown to be of major importance especially in regions with a high species pool for high Lepidoptera species richness, abundance, endangered species and specialists. Ecological and life-history traits of species can be considered to evaluate the threat status of a species and are therefore highly valuable for conservation. In chapter IV I showed that the endophyte effect on herbivores was dependent on the environmental context, which has to be considered in future field studies. The importance of endophyte effects on herbivores has to be reconsidered in comparison with other variables.

In conclusion, changing land-use intensity affected the response variables in all three studies. Consequently, land-use intensity has a great influence on many aspects in ecology and is an important factor to consider in future studies. Some effects differed between regions; this shows the importance to repeat surveys and experiments in different regions to draw reliable general conclusions from the results or to point out possible regional differences.

Zusammenfassung

Zunehmende Landnutzungsintät ist eine der Hauptursachen für den weltweiten Biodiversitätsverlust. Die Zahl der gefährdeten Arten steigt stetig an und nur wenige Arten - meist Generalisten - profitieren von der Landnutzungsintensivierung. Folglich beeinträchtigt steigende Landnutzungsintensität Arten mit bestimmten „life-history traits“ stärker als andere. Durch unterschiedlich intensive Landnutzung unterscheiden sich die biotischen und abiotischen Gegebenheiten für interagierende Arten und können deshalb multitrophische Interaktionen beeinflussen. Landnutzungsintensität beeinflusst viele Bereiche in der Ökologie, aber diese Effekte können in Regionen mit unterschiedlichen Umweltbedingungen verschieden ausfallen.

In meiner Arbeit stellte ich die Frage, wie Landnutzungsintensität ökologische Aspekte wie Artenvielfalt und –abundanz und das Vorkommen gefährdeter Arten (Kapitel II), ökologische und „life-history traits“ (im Weiteren als „Traits“ abgekürzt) von Lebensgemeinschaften (Kapitel III) und multitrophische Interaktionen (Kapitel IV), beeinflusst. Mich interessierte, ob sich Effekte zwischen Regionen unterscheiden.

In meiner Studie in Kapitel II habe ich Tagfalter und tagaktive Nachtfalter (im Weiteren als Schmetterlinge bezeichnet) als Zielgruppe ausgewählt. Aus Roten Listen Deutschlands habe ich die Gefährdungssituation von Arten erhalten. Ich erwartete einen Rückgang der Schmetterlingsarten, ihrer Abundanz und des Vorkommens gefährdeter Arten mit zunehmender Landnutzungsintensität in Abhängigkeit von der Region. In Kapitel III habe ich die tagaktiven Nachtfalter aus dem Datensatz des Kapitels II herausgenommen, da nur für Tagfalter verlässliche Daten zu zehn ausgewählten „Traits“ in der Literatur verfügbar sind. Ich erwartete, dass sich „Traits“ mit zunehmender Landnutzungsintensität von für Spezialisten typischen hin zu für Generalisten typischen verschieben. Weiterhin erwartete ich, dass Arten, die überwiegend „Traits“ von Spezialisten aufweisen, gefährdet sind. Um dies zu untersuchen, habe ich Schmetterlinge auf Transekten auf 137 Graslandflächen in drei verschieden Regionen Deutschlands gefangen. Die ausgewählten Graslandflächen zeigten in jeder Region einen Gradienten von sehr extensiver bis sehr intensiver Landnutzungsintensität. Um die Landnutzungsintensität zu berechnen, habe ich einen Landnutzungsintensitätsindex verwendet, der sich aus der Menge Dünger, Mahdhäufigkeit und der Beweidungsintensität zusammensetzt.

In Kapitel IV behandle ich ein multitrophisches System, das aus vier Stufen besteht: Endophyten der Gattung *Neotyphodium*, die sich asexual in Gräsern vermehren („Bottom-up“-Kontrolle), der Wirtspflanze, an der Pflanze saugenden Blattläusen und deren natürlichen Feinden („Top-down“-Kontrolle). Frühere Feldstudien führten zu kontroversen Schlüssen bezüglich des Charakters der Symbiose zwischen Endophyten und ihren Wirtspflanzen, da einige Studien negative Wirkungen (Mutualismus), andere positive Wirkungen auf Herbivore (Parasitismus) zeigten. Ich nahm an, dass Effekte von Endophyten auf Blattlausabundanzen von Umweltbedingen in unterschiedlichen Regionen und unterschiedlich intensiv genutzten Graslandflächen und von Prädatoren, die eventuell Blattläuse von Endophyten-freien Pflanzen bevorzugen, beeinflusst werden. Für diese Studie habe ich in zwei Regionen 40 Graslandflächen ausgewählt, von denen in jeder Region zehn intensiv und zehn extensiv genutzt waren, um auf die damit einhergehenden unterschiedlichen Umweltbedingungen zu testen. Auf jede Graslandfläche habe ich vier Grastöpfe (*Lolium perenne*) mit Blattläusen (*Rhopalosiphum padi*) ausgebracht. Die Blattlauspopulationsgröße wurde regelmäßig aufgenommen. Das Gras war a) entweder mit Endophyten (*Neotyphodium lolii*) infiziert oder nicht und b) Prädatoren wurden ausgeschlossen oder hatten freien Zugang zu den Töpfen.

In allen drei Studien (Kapitel II-IV) konnte ich einen starken Effekt der Landnutzungsintensität auf die getesteten Variablen feststellen und einige Effekte unterschieden sich zwischen den Regionen.

Die Schmetterlingsartenvielfalt und -abundanz, die in Kapitel II untersucht wurden, nahmen nur in zwei Regionen signifikant ab, aber nicht der dritten. Ähnlich war das Vorkommen gefährdeter Schmetterlingsarten durch die Landnutzungsintensität beeinflusst. Der Regionenunterschied des Landnutzungseffekts ist wahrscheinlich durch die Größe der unterschiedlichen regionalen Artenpools und der korrelierenden Anzahl gefährdeter Arten und des Vorhandenseins von Kalkmagerrasen in nur zwei Regionen bedingt. In artenreichen Regionen mit vielen gefährdeten Arten sind Landnutzungsintensitätseffekte stark ausgeprägt im Gegensatz zu artenarmen Regionen, in denen es meist nur gewöhnliche Arten gibt, die nicht stark auf intensive Landnutzung reagieren. Zudem stellen Kalkmagerrasen, die in der artenarmen Region fehlen, ein wichtiges Habitat für Schmetterlinge dar. Ich konnte zeigen, dass Düngung und steigende Beweidungsintensität, aber nicht die Mahdfrequenz die Artenvielfalt signifikant verringerten.

In Kapitel III zeigte ich, dass sich acht der zehn getesteten „Traits“ von Tagfaltergemeinschaften mit steigender Landnutzungsintensität von Spezialisten-typischen hin zu Generalisten-typischen „Traits“ veränderten (breitere Nahrungsnische, höhere Ausbreitungsfähigkeit, stärkeres Wanderverhalten, größeres Verbreitungsgebiet, geringere Populationsdichte, Schlupf von mehr als einer Generation pro Jahr, Überwinterung in einem höher entwickelten Raupenstadium und eine längere Flugzeit). Ein Vergleich des Gefährdungsgrades der Arten in der Roten Liste 2008 und einer Kombination von „Traits“ für jede Art zeigte, dass Arten mit mehreren „Traits“, die typisch für extensiv genutzte Graslandflächen und damit für Spezialisten sind, gefährdet sind. Das weist darauf hin, dass nur Arten mit „Traits“ von Generalisten in der Lage sind, mit häufigen Störungen im Habitat und unbeständiger Futterpflanzenverfügbarkeit, welches beides durch hohe Landnutzung verursacht ist, umgehen können.

In dem multitrophischen System in Kapitel IV konnte ich starke Effekte durch Landnutzungsintensität und Prädatoren auf die Blattlausabundanz feststellen. Der Effekt der Endophyten dagegen war schwach und Regionen-abhängig. Endophyten verringerten die Blattlausabundanz nur in einer der beiden Regionen. Prädatoren und Landnutzungsintensität hatten keinen zusätzlichen Einfluss auf den Effekt der Endophyten. Insgesamt weist dies darauf hin, dass Effekte von Endophyten auf Herbivore eine geringere Bedeutung in natürlichen Systemen haben, wenn sie mit anderen Herbivoren-beeinflussenden Faktoren wie Region, Landnutzungsintensität oder Räubern verglichen werden, und dass die Effekte der Endophyten abhängig von den Umweltbedingungen sind.

In Kapitel II und III wurde die Bedeutung von Kalkmagerrasen und anderen Graslandflächen mit extensiver Landnutzungsintensität in Regionen mit einem hohen Artenpool für den Schutz hoher Schmetterlingsartenvielfalt, -abundanz, gefährdeter Arten und spezialisierter Arten gezeigt. „Trait“-Daten von Arten können herangezogen werden, um den Gefährdungsgrad einer Art zu bewerten, und sind daher höchst wertvoll für den Naturschutz. Im vierten Kapitel habe ich gezeigt, dass der Effekt von Endophyten auf Herbivore von Umweltbedingungen abhängig ist, was in zukünftigen Feldstudien berücksichtigt werden muss. Die Bedeutung von Endophyten in Gräsern im Vergleich zu anderen Herbivoren-beeinflussenden Faktoren muss für Feldstudien überdacht werden.

Zusammenfassend konnte ich zeigen, dass die Landnutzungsintensität in allen Studien die Untersuchungsobjekte beeinflusst hat. Daher gilt Landnutzung als ein wichtiger Faktor, der in zukünftigen ökologischen Studien berücksichtigt werden muss. Manche Effekte unterschieden sich zwischen den Regionen, weshalb Beobachtungen und Experimente in verschiedenen Regionen zu wiederholen sind, um generelle Aussagen über Ergebnisse machen zu können oder um regional bedingte Unterschiede aufzudecken.

Acknowledgements

My special thanks go to PD Dr. Jochen Krauss and Prof. Dr. Alexandra-Maria Klein for enabling me to work in this exciting project and for being such positive and supportive supervisors throughout the years. The spatial distance to my supervisors was not a drawback and they gave extremely helpful and constructive feedback on my work. I thank Jochen Krauss for being in my committee and putting an exceptional great effort into discussions about the manuscripts. I am very grateful to Prof. Dr. Teja Tscharntke for being in my committee, very helpful comments on my manuscripts, friendly encouraging words, and for being head of such a nice working group. I thank PD Dr. Dirk Gansert for organising the PhD program "Biological Diversity and Ecology" in which I took part. Laszlo Somay from Hungary kindly helped with the identification of some critical butterfly species.

Financial support was provided by the Deutsche Forschungsgemeinschaft within the framework of the DFG Priority Program 1374 "Infrastructure-Biodiversity.Exploratories". Field work permits were issued by the responsible state environmental offices of Baden -Württemberg, Thüringen, and Brandenburg (according to § 72 BbgNatSchG). I acknowledge Swen Renner, Sonja Gockel, Andreas Hemp and Martin Gorke and Simone Pfeiffer for their work in maintaining the plot and project infrastructure, and Markus Fischer, Elisabeth Kalko, Eduard Linsenmair, Dominik Hessenmöller, Jens Nieschulze, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze and Wolfgang W. Weisser for their role in setting up the Biodiversity Exploratories project. In the field Jörg Hailer, Ralf Lauterbach und Martin Fellendorf were very helpful and supportive in the Alb, Claudia Seilwinder, Matthias Gross and Norbert Leber in the Hainich and Ute Schumacher in the Schorfheide. This thesis could only be conducted because many farmers and land owners provided permanent permissions to accede the study sites.

I would also like to thank my dear colleagues and friends Juliane Steckel, Michaela Bellach, Christoph Rothenwöhrer, Astrid Bütof and Lars von Riedmatten for nice company during the field seasons in the summer months in the exploratories. I express my cordial thanks to all my dear colleagues of the Agroecology group for the extremely warm and supportive working atmosphere. We shared a long nice time not only in the University and

I will greatly miss you all. Vesna Gagic and Elli Vockenhuber are especially acknowledged for their true and reliable friendships as well as for fruitful scientific and non-scientific discussions at afternoon tea brake or other occasions in the evenings: I thank Elli Vockenhuber, Dr. Jochen Fründ and Dr. Christoph Scherber for their valuable help and discussions on statistical problems; Ines Vollhardt gave helpful comments on previous versions of some chapters; Jutta Gilles for help with administrative troubles; Jan Tönjes, Tom Österreich and Siggi Opolka for any help with computer troubles and Mareen Gollnow, who also became a close friend of mine, for her support in the field season 2009. I also want to acknowledge the welcoming atmosphere in the Würzburg (former Bayreuth) working group when I visited Jochen Krauss and the nice evenings with the working group. I finally thank all my rock climbing friends for the opportunity to deal not only with scientific challenges. Last and by means not least, I am extremely thankful to my family for their great support and for just being like they are.

Publication list

Journal articles

Börschig, C. Klein, A.-M., Somay, L., Krauss, J. (to be submitted to Biodiversity and Conservation or Insect Conservation and Diversity): Management intensity and region predict species richness and the occurrence of endangered day-active Lepidoptera in grasslands.

Börschig, C., Klein, A.-M., Krauss J. (under review in Ecography): Traits of butterfly communities change from specialist to generalist characteristics with increasing land-use intensity.

Börschig, C., Klein, A.-M., Krauss, J. (under review in Oecologia): Effects of endophytic fungi on aphid abundance depend on environmental context and are difficult to predict.

Talks

Börschig, C., Klein, A.-M., Krauss J. (2010): Endophytic fungi reduce aphid abundance depending on study region. 40th Anniversary Meeting of the Ecological Society of Germany, Austria and Switzerland (GFÖ), Gießen, 30th Aug. - 3rd Sept. 2010.

Posters

Börschig, C., Klein A.-M., Krauss J. (2011): Stochasticity: Effects of endophytic fungi on aphids unpredictable? Annual meeting of the biodiversity-exploratories, Bad Blankenburg, 14th – 18th Feb. 2011.

Thesis declaration

Declaration of the author's own contribution to manuscripts with multiple authors

The chapters II to IV are a series of manuscripts that have or will be submitted to peer-reviewed journals. I am the main author of all manuscripts presented in this thesis. I have personally collected and analysed the data for all manuscripts, developed the main ideas presented in the manuscripts, written all manuscripts, made the tables, figures, and appendices, and contributed significantly to the sampling design. My supervisors laid out the study design and helped with analysis, discussions and writing. The other co-authors contributed to the discussions of results.

Declaration plagiarism

I hereby confirm that I have written this doctoral thesis independently that I have not used other sources or facilities others than the ones mentioned, that I have not used unauthorised assistance and that I have not submitted this thesis previously in any form for another degree at any university or institution.

Göttingen, February 2012