Influence of land use on *Plantago lanceolata* L. and its higher trophic levels at different spatial scales and in three geographic regions

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Christine Hancock, geb. Herbst
geboren in Heilbronn, Deutschland

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Mitglieder der Promotionskommission:

Vorsitzender: Prof. Dr. Wolfgang Rössler

Gutachter : Prof. Dr. Elisabeth Obermaier

Gutachter: Prof. Dr. Johannes L. M. Steidle

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Ich widme diese Doktorarbeit meiner Familie.

Vielen Dank, dass ihr für mich da seid!!

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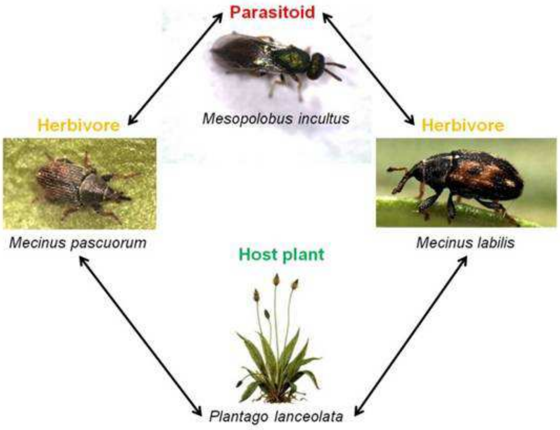

Summary

Nowadays, agriculturally used areas form a major part of the German landscape. The conversion from natural habitats to agriculturally used grasslands fundamentally influences the diversity of plants and animals. Intensive use of these areas increases indeed the productivity of crop or biomass on meadows as food source for cattle. How these influences affect biodiversity, ecosystems and trophic interactions over years is still not understood completely.

To understand biodiversity functions in an agriculturally used area my study focused on the influence of land use (fertilization, grazing and mowing) on a herbivore-parasitoid system of *Plantago lanceolata*. The ribwort plantain is a generalist herb of cosmopolitan distribution. It can grow in a very broad range of ground conditions (both in wet and dry habitats), which makes *P. lanceolata* an ideal model system for investigating tritrophic interactions in a gradient of land use intensity. The weevils *Mecinus labilis* and *M. pascuorum* feed and oviposit on *P. lanceolata*. *Mesopolobus incultus* is a generalist parasitoid that parasitizes different insect orders. However its only hosts on *P. lanceolata* are the two weevil species mentioned before.

The intention of my study was to investigate the influence of land use on a tritrophic system and its surrounding vegetation (structure, density and species richness) at different spatial scales like subplot, plot and landscape level in three different regions (north, middle and south of Germany). I studied the influence of land use intensity not only correlative but also experimentally. Additionally I aimed to reveal how vegetation composition changes host plant metabolites and whether these changes impact higher trophic levels in the field.

Interactions at the subplot level

In the investigated subplots (6m x 6m) experimental fertilization enhanced the growth of the vegetation but decreased the plant species richness and host plant abundance. Additional fertilizer application increased the proportion of grasses because they may handle the nutritional input better than forbs which decreased with fertilization. Due to the higher proportion of grasses *P. lanceolata* seems to grow more likely in less fertilized sites with a lower vegetation cover where the competition with other plants is less. Biomass and rosette diameter of the ribwort plantain however increased with fertilization. To be competitive against the rising vegetation height the host plant seems to invest in growth when fertilized. The nitrogen content of *P. lanceolata* increased with fertilization in the subplots whereas the carbon content was not influenced.

Increased size and heightened leaf nitrogen content did not influence the abundance of the weevils positively although a better plant quality is known to support the performance of herbivores. The

abundance of *M. labilis* and *M. pascuorum* decreased with the additional application of fertilization in the subplots. This reduced herbivore abundance due to fertilization, which was astonishingly the most important factor influencing the weevils in the subplot, can additionally be explained by indirect effects via the lower abundance of the host plant and by changed patterns of host localisation within higher vegetation. Fertilization indirectly affected the third trophic level *M. incultus* negatively by cascading up via the abundance of its prey. The host plant and changed vegetation structure did not influence the parasitoid at all.

Interactions at the plot level

In every region investigated the plots (50m x 50m) were treated by farmers in different ways generating three different land use types (meadows, mown pastures and pastures) and two land use intensities (fertilized or not fertilized plots).

Land use intensity (fertilization by farmers) and type (mowing vs. grazing) increased the vegetation structure (height and density) and affected the plant species richness negatively. The influence of land use on the surrounding vegetation was consistent for both levels investigated.

Furthermore plant species richness was positively correlated with secondary metabolite content in *P. lanceolata* leaves but these changes did not cascade up through higher trophic levels. However, the leaf nitrogen content was negatively correlated with the abundance of the weevils. This means the higher the nitrogen content (fertilized > unfertilized), the lower the abundance of the two herbivores (unfertilized > fertilized).

The abundance of *M. labilis* and *M. pascuorum* decreased in fertilized and mown plots although the host plant quality (nitrogen content) and size (plant growth and biomass) increased with intensive land use. On the other hand herbivores are mostly linked to their food and oviposition plant. Not only the size of the host plant is important, but also the density. The more host plants are available the better the chance of finding a mating partner and a place to feed and oviposit. Therefore the decreased abundance of *P. lanceolata* in fertilized and mown plots may explain the decreased weevil abundance although the plants are taller.

In the field, *M. pascuorum* is positively associated with plant diversity, which was enhanced in unfertilized or grazed plots. The laboratory bioassays showed that both insect groups in this tritrophic system can perceive and respond to different odors tested and that larger plant diversity does not prevent host location. Odors from a plant-rich community enhanced the “searching” activity of *M. pascuorum* in the olfactometer.

The third trophic level the parasitoid was, like in the investigated subplots, not influenced by host plant quality, quantity and vegetation composition. However *M. incultus* occurred more often in unfertilized or grazed plots where the abundance of his prey was higher. Maybe the parasitoid is like in the subplots indirectly influenced by land use via the herbivores. Interestingly the parasitization rate was not influenced by fertilization.

The results shown were consistent in two of the three regions. In Hainich-Dün and Schwäbische Alb where similar land use was implemented the results were steadily the same. In the Schorfheide-Chorin due to a higher proportion of organic farming and less livestock on the plots results were less pronounced.

Different indices were used to identify one “ideal” diversity index which describes the “real world” in the field. Indices aim to describe general properties of communities that allow us to compare different regions, taxa, and trophic levels. The performance of different indices (species richness (S), Shannon’s diversity (H’), Simpson’s diversity (D_1), Simpson’s dominance (D_2), Simpson’s evenness (E), and Berger Parker dominance (BP)) was assessed largely but if effects like of the land use or relationships between organism groups are not strong, indices could not detect effects. This demonstrates that while common diversity indices may appear interchangeable in simple analyses, when considering complex interactions the choice of index can profoundly alter the interpretation of results. Compared to the results measured on the plot the calculated diversity of organisms was astonishingly little influenced by land use changes like increased fertilization, grazing and mowing. In this analysis no effect of land use on aboveground arthropods, belowground insect larvae, *P. lanceolata* chemical (compound) diversity was found. It is clear that relationships between diversity indices do not always follow mathematically predicated patterns, and it is therefore important to perform analyses such as these on real data to ensure that conclusions will be valid in the field.

Interactions at the landscape scale

Land use did not only influence trophic interactions on the two mentioned local scales (subplot and plot), but also on larger scales like landscape. Therefore the influence of land use (here: extensive vs. intensive habitat) at different spatial scales around the investigated plots (100m - 2000m) on the tritrophic system was investigated. The three insect species investigated were affected by extensively and intensively managed grassland, however in opposite ways. Rising proportion of extensively managed (semi-natural) grassland had a positive effect on insect abundances (radii of 100m – 2000 m). Intensively managed grassland on the other hand had a negative effect on the two herbivores and their natural enemy (radii of 100m – 2000 m). Like seen for the subplots and the plots the species abundance was most strongly affected by increased semi-natural habitat (e.g. extensively used habitats like nutrient poor grassland and meadows with scattered fruit trees). Semi-natural habitats are important environments where a high number of species occurs and a high biodiversity is supported compared to intensively used grasslands where biodiversity is reduced and species richness is less.

The parasitoid, *M. incultus*, was directly positively affected only by the density of its two host species and only indirectly, via its hosts, by land use intensity like demonstrated for the subplot and plot level, too.

The three insect species however differed in the spatial scale at which they responded to the landscape diversity. While the abundance of the herbivore *M. labilis* was best explained by larger spatial scales (r = 1500 – 2000 m), *M. pascuorum* and the parasitoid *M. incultus* responded most strongly to landscape diversity at smaller scales (r = 100 – 500 m). *Mecinus pascuorum* feeds monophagous on *P. lanceolata*, while *M. labilis* feeds oligophagous on *P. lanceolata* and *P. sempervirens* and may need a larger range for host plant food search. *Mecinus pascuorum* may prefer smaller ranges due to host plant density. In the subplots or plots *P. lanceolata* may be more abundant than in larger scales where suitable growing habitats are less abundant due to a higher proportion of forest and paved areas. At smaller scales *M. pascuorum* may search only a short time to find his host plant.

The parasitoid responded best on smaller spatial scales, too. This was contrary to other studies where higher trophic levels like parasitoids depend in most cases on larger spatial scales. *Mesopolobus incultus* abundance is, just as seen in the subplots and plots influenced by the abundance of his main host *M. pascuorum* which preferred to occur on smaller scales. Likewise, in other studies less specialized parasitoids were more likely to be found on patches with high host density, while patch size and isolation seem to be less important.

Intensive land use like high fertilizer application, frequent mowing and high livestock densities on grasslands influenced the abundances of both herbivores and their parasitoid negatively.

The herbivores decreased in intensive used grasslands and increased in extensive used grasslands. Both weevils were directly influenced by land use on different spatial scales. The parasitoid on the other hand depended on his prey and was more abundant in extensive used grasslands. *Mesopolobus incultus* was indirectly influenced by the land use via the herbivores. This indicates that extensive land use with less fertilizer application, a low frequency of mowing and a low livestock are major causes to preserve multitrophic interactions in such habitats.

Chapter II - Zusammenfassung

Zusammenfassung

Heutzutage prägen landwirtschaftlich genutzte Flächen einen großen Teil der deutschen Landschaft. Die Umwandlung von natürlichen Lebensräumen zu bewirtschaftetem Grünland beeinflusst grundlegend die Vielfalt von Pflanzen und Tieren. Zwar erhöht die intensive Nutzung dieser Flächen die Produktivität der Pflanzen oder die Biomasse als Viehfutter auf den Wiesen. Wie diese Einflüsse auf die Artenvielfalt, Ökosysteme und trophische Interaktionen, im Laufe der Jahre wirken ist jedoch immer noch nicht vollständig verstanden.

Um die Funktionen der Biodiversität in einer landwirtschaftlich genutzten Fläche zu verstehen konzentrierte sich meine Arbeit auf den Einfluss der Landnutzung (Düngung, Beweidung und Mahd) auf ein Herbivor-Parasitoid-System von *Plantago lanceolata*. Der Spitzwegerich ist ein generalistisches Kraut mit kosmopolitischem Vorkommen. Er kann in einem sehr breiten Spektrum von Bodenverhältnissen (sowohl in nassen und auch in trockenen Lebensräumen) vorkommen und ist daher ein ideales Modellsystem zur Untersuchung tritrophischer Systeme in einem Landnutzungs-intensitätsgradienten. Die Rüsselkäfer *Mecinus labilis* und *M. pascuorum* ernähren sich von *P. lanceolata* und legen dort ihre Eier ab. *Mesopolobus incultus* ist ein generalistisch lebender Parasitoid, der verschiedenen Insektenordnungen parasitiert. Die einzigen Wirte auf *P. lanceolata* sind jedoch die beiden erwähnten Rüsselkäferarten. Das Ziel meiner Studie war es, den Einfluss der Landnutzung auf ein tritrophisches System und seiner umgebenden Vegetation (Struktur, Dichte und Artenreichtum) auf unterschiedlichen räumlichen Skalen wie Subplot, Plot und Landschaftebene in drei verschiedenen Regionen (Nord-, Mittel- und Süddeutschland) zu untersuchen. Ich untersuchte den Einfluss der Nutzungsintensität nicht nur korrelativ, sondern auch experimentell. Zusätzlich zielte ich darauf ab, aufzuzeigen wie die Vegetationszusammensetzung die Metabolite der Wirtspflanze verändert und ob diese Veränderungen Auswirkungen auf höhere trophische Ebenen im Feld haben.

Interaktionen auf der Subplotebene

In den untersuchten Subplots (6m x 6m) verbesserte die experimentelle Düngung das Wachstum der Vegetation, verminderte aber die Pflanzenartenanzahl und die Wirtspflanzendichte. Die zusätzliche Düngung erhöhte den Anteil der Gräser, da diese, besser als krautige Pflanzen, die durch die Düngung verringert wurden, die Nährstoffe verarbeiten können. Aufgrund des höheren Anteils an Gräsern scheint *P. lanceolata* eher auf weniger gedüngt Flächen mit einer niedrigeren Vegetation, auf denen die Konkurrenz mit anderen Pflanzen geringer ist, zu wachsen. Die Biomasse und der Rosettendurchmesser des Spitzwegerichs wurden allerdings mit der Düngung vergrößert. Um bei Düngung gegen die ansteigende Vegetationshöhe wettbewerbsfähig zu sein scheint die Wirtspflanze in ihr Wachstum zu investieren. Der Stickstoffgehalt von *P. lanceolata* erhöhte sich durch die Düngung in den Subplots während der Kohlenstoffgehalt nicht beeinflusst wurde.

Erhöhte Wirtspflanzengröße und Blattstickstoffgehalt hatten keinen positiven Einfluss auf die Käferabundanz obwohl eine verbesserte Pflanzenqualität sich positiv auf pflanzenfressende Insekten auswirken sollte. Die Abundanz von *M. labilis* und *M. pascuorum* nahm mit der zusätzlichen Dünung auf den Subplots ab. Die aufgrund der Düngung, welche erstaunlicherweise der wichtigste Einflussfaktor auf die Käfer darstellte, reduzierte Anzahl der Herbivoren kann zusätzlich durch indirekte Effekte einer geringeren Wirtspflanzendichte und durch veränderte Muster der Wirtsfindung aufgrund der höheren Vegetation erklärt werden. Düngung beeinflusste indirekt die dritte trophische Ebene (*M. incultus*) durch die Herbivoren negativ. Die Wirtspflanze sowie die veränderte Vegetationsstruktur beeinflussten den Parasitoiden hingegen nicht.

Interaktionen auf der Plotebene

In jeder Region wurden die Plots (50m x 50m) unterschiedlich von den Landwirten bewirtschaftet was zur Bildung von drei verschiedene Landnutzungstypen (Wiesen, Mähweiden und Weiden) sowie zu zwei Landnutzungsintensitäten (gedüngt oder ungedüngt) führte.

Die Landnutzungsintensität (Düngung durch die Landwirte) und der Landnutzungstyp (Mahd gegen Beweidung) erhöhten die Vegetationsstruktur (Höhe und Dichte) und beeinflussten den Pflanzenartenreichtum negativ. Der Einfluss der Landnutzung auf die umgebende Vegetation erwies sich auf beiden Ebenen als sehr beständig.

Darüber hinaus korrelierte der Artenreichtum positiv mit dem Sekundärmetabolitgehalt in *P. lanceolata* Blättern. Jedoch wirkte sich dies nicht auf die höheren trophischen Ebenen aus. Der Blattstickstoffgehalt allerdings korrelierte negativ mit den Rüsselkäfern. Dies bedeutet, je höher der Gehalt an Stickstoff (gedüngte > ungedüngte Flächen), desto geringer ist die Abundanz der beiden Herbivoren (ungedüngte > gedüngte Flächen).

Obwohl die Wirtspflanzenqualität (Stickstoffgehalt) und Größe (Pflanzenwuchs und Biomasse) mit intensiver Landnutzung zunahmen kamen *M. labilis und M. pascuorum* weniger häufig auf gedüngten und gemähten Flächen vor. Allerdings sind pflanzenfressende Insekten meist von ihrer Wirtspflanze, die auch der Ort der Eiablage ist, abhängig. Nicht nur die Größe der Wirtspflanze, sondern auch deren Häufigkeit auf einer Fläche ist entscheidend. Je mehr Wirtspflanzen vorkommen, desto höher ist die Chance, einen passenden Partner zur Paarung, einen Eiablageplatz und eine Futterquelle zu finden. Deshalb könnte die geringere Anzahl von *P. lanceolata* auf den gedüngten und gemähten Plots die geringe Anzahl von Rüsselkäfern, trotz der dort größeren Wirtspflanzen, erklären.

Im Freiland ist *M. pascuorum* positiv mit der Pflanzenartenvielfalt, welche auf ungedüngten oder beweidet Flächen größer war, verbunden. Laborstudien zeigten, dass beide Insektengruppen des tritrophischen Systems verschiedene Gerüche wahrnehmen und darauf reagieren können und dass eine erhöhte Pflanzenartenvielfalt die Wirtsfindung nicht verhindert. Gerüche einer pflanzenreichen Zusammenstellung steigerten das Suchverhalten von *M. pascuorum* im Olfaktometer.

Die dritte trophische Ebene, der Parasitoid, war, wie auch in den Subplots nachgewiesen wurde, nicht von der Wirtspflanzenqualität, ihrer Quantität und der Zusammensetzung der Vegetation beeinflusst. Jedoch kam *M. incultus* häufiger auf ungedüngten oder beweidet Flächen, auf denen seine Wirte zahlreicher anzutreffen waren, vor. Möglicherweise ist der Parasitoid, wie für die Subplots gezeigt werden konnte, indirekt über die Herbivoren durch die Landnutzung beeinflusst. Interessanterweise wurde die Parasitierungsrate nicht durch Düngung beeinflusst.

Die gezeigten Ergebnisse waren in zwei der drei Regionen konsistent. Im Hainich-Dün und auf der Schwäbischen Alb, in denen ähnliche Landnutzung betrieben wird, waren die Effekte dauerhaft dieselben. In der Schorfheide-Chorin, in der biologischer Landbau betrieben wird und geringe Viehbestände auf den Flächen gehalten werden, waren die Ergebnisse etwas abgeschwächt.

Unterschiedliche Indizes wurden verwendet um einen „idealen“ Diversitätsindex zu finden, welcher die "reale Welt" im Freiland beschreibt. Indizes zielen darauf ab allgemeine Eigenschaften von Gemeinschaften zu beschreiben welche uns ermöglichen sollen verschiedenen Regionen, Taxa und trophische Ebenen zu vergleichen. Die Effizienz der verschiedenen Indizes wurde weitgehend bewertet (species richness (S), Shannon’s diversity (H’), Simpson’s diversity (D_1), Simpson’s dominance (D_2), Simpson’s evenness (E), and Berger Parker dominance (BP)), jedoch wenn Effekte wie Landnutzung oder Beziehungen zwischen Organismengruppen nicht stark sind, konnten die Indizes diese Effekte nicht erkennen. Dies zeigt, dass während gewöhnliche Diversitätsindizes in einfachen Analysen nicht austauschbar erscheinen, wenn man komplexe Wechselwirkungen beschreibt, dann kann die Wahl des Index tiefgreifend die Interpretation der Ergebnisse verändern. Verglichen mit den Ergebnissen, die auf dem Plot gemessen wurden, war die berechnete Organismenvielfalt erstaunlich wenig von den Änderungen durch die Landnutzung wie eine erhöhte Düngung, Beweidung und Mahd beeinflusst. In dieser Analyse wurden keine Auswirkungen der Landnutzung auf oberirdische Arthropoden, unterirdisch lebende Insektenlarven und die Vielfalt der chemischen Inhaltsstoffe von *P. lanceolata* gefunden. Es ist klar, dass die Beziehungen zwischen Diversitätsindizes nicht immer mathematisch vorhergesagten Mustern folgen und daher ist es wichtig, Analysen wie diese mit realen Daten durchzuführen, um sicherzustellen, dass die Schlussfolgerungen für das Freiland valide sind.

Interaktionen auf der Landschaftsebene

Landnutzung beeinflusste trophische Interaktionen nicht nur auf den beiden erwähnten lokalen Skalen (Subplot und Plot), sondern auch auf größeren wie der Landschaft. Daher wurde der Einfluss der Landnutzung (hier: extensiv gegen intensiv bewirtschaftetes Habitat) um die untersuchten Flächen auf unterschiedlichen räumlichen Skalen (Radius: 100m - 2000m) auf das tritrophische System untersucht. Die drei untersuchten Insektenarten wurden von extensiv und intensiv bewirtschaftetem Grünland beeinflusst. Dies geschah allerdings in gegensätzlicher Weise. Steigender Anteil von extensiv bewirtschaftetem (semi-natürlichen) Grünland hatte einen positiven Effekt auf die Insektenabundanzen (100m - 2000 m). Auf der anderen Seite hatte intensiv bewirtschaftetes Grünland einen negativen Effekt auf die zwei Herbivoren und ihre natürlichen Feind (100m - 2000 m). Wie für die Subplot- und Plotebene gezeigt werden konnte wurde die Abundanz der Arten am stärksten durch den ansteigenden Anteil von semi-natürlichem Habitat (extensiv genutzte Lebensräume wie Magerrasen und Streuobstwiesen) beeinflusst. Semi-natürliche Habitate sind wichtige Lebensräume, in denen eine große Anzahl von Arten auftritt und eine hohe Biodiversität unterstützt wird. Im Gegensatz dazu wird die biologische Vielfalt auf intensiv bewirtschaftetem Grünland reduziert und der Artenreichtum reduziert.

Der Parasitoid, *M. incultus*, wurde ausschließlich von seinen beiden Wirtsarten beeinflusst und nur indirekt durch die Landnutzungsintensität wie auch schon für die untersuchten Subplots und Plots gezeigt wurde.

Allerdings unterschieden sich die drei Insektenarten in der räumlichen Skala, in welcher sie auf die Landschaftsdiversität reagierten. Während die Abundanz von *M. labilis* am besten durch größere räumliche Skalen (1500 - 2000 m) erklärt wurde, reagierten *M. pascuorum* und der Parasitoid *M. incultus* am stärksten auf die Landschaftsdiversität in kleineren Skalen (100 - 500 m). *Mecinus pascuorum* frisst monophag an *P. lanceolata*, während *M. labilis* sich oligophag von *P. lanceolata* und *P. sempervirens* ernährt und könnte daher einen größeren Radius für die Suche nach der Wirtspflanze benötigen. *Mecinus pascuorum* hingegen könnte aufgrund der Wirtspflanzendichte einen kleineren Radius bevorzugen. Auf den Subplot- und Plotflächen könnte *P. lanceolata* häufiger zu finden sein als auf größeren Radien, wo aufgrund eines höheren Anteils von Wald und gepflasterten Flächen für die Pflanze seltener geeignete Habitate vorkommen. Auf kleineren Skalen dürfte *M. pascuorum* daher eine kürzere Zeit benötigen, um seine Wirtspflanze zu finden.

Der Parasitoid reagierte ebenfalls am besten auf kleinere räumliche Skalen. Dies steht im Widerspruch zu anderen Studien, in denen höhere trophische Ebenen wie Parasitoide in den meisten Fällen auf größere räumliche Skalen angewiesen sind. *Mesopolobus incultus* ist, ebenso wie in den Subplots und Plots gezeigt, durch die Abundanz seines Hauptwirtes *M. pascuorum*, der auf kleineren Skalen bevorzugt vorkommt, beeinflusst. Ebenso wurden in anderen Studien weniger spezialisierte

Parasitoide eher auf Flächen mit einer hohen Wirtsdichte gefunden, während die Flächengröße und ihre Isolation hier weniger wichtig zu sein scheinen.

Eine intensive Landnutzung wie beispielsweise durch hohen Düngungemitteleinsatz, häufige Mahd und intensive Tierhaltung auf Grünlandflächen beeinflussen das Vorkommen und die Häufigkeit der beiden Herbivoren und ihres Parasitoiden negativ. Die Rüsselkäferanzahl sank auf intensiv bewirtschaftetem Grünland und stieg auf extensiv bewirtschaftetem Grünland an. Beide Käferarten wurden auf unterschiedlichen räumlichen Skalen direkt durch die Landnutzung beeinflusst. Der Parasitoid hingegen ist von seinem Beutetier abhängig und war häufiger auf extensiv bewirtschaftetem Grünland zu finden. *Mesopolobus incultus* wurde daher nur indirekt durch die Herbivoren von der Landnutzung beeinflusst. Dies zeigt, dass extensive Landnutzung mit geringem Düngemitteleinsatz, einer weniger häufigen Mahd und einem niedrigen Viehbesatz eine Hauptursache darstellt um multitrophische Interaktionen in solchen Lebensräumen zu bewahren.

Chapter III - General Introduction

1. Introduction

1.1 Effects of land use type and intensity on vegetation and upper trophic levels

Anthropogenic interference from natural habitats to agriculture fundamentally influences the diversity of plants and animals (Foley et al. 2005). In cultural land, intensive land use represents one major negative impact not only for plant species richness, but also for higher trophic levels. However, still little is known about how intensification in land use influences species of higher trophic levels apart from simply reducing species numbers (van der Putten et al. 2004).

The land use type like grazing and mowing lead to changes in vegetation complexity and plant growth (Kruess & Tscharntke 2002). Even the frequency and time period of mowing can cause changes in the vegetation composition that further can influence the diversity of herbivorous arthropods (Köhler et al. 2004; Unsicker et al. 2006). Changes in the diversity of organisms of one trophic level may influence the diversity of the next level (Hartley & Jones 2003). Insects can be negatively as well as positively affected by intensive management of grasslands. Direct effects can be seen for example in an increased mortality due to high mowing frequencies (Tscharntke & Greiler 1995). Indirect effects of land use intensification may become apparent as changes in developmental parameters, host finding capacity, or movement patterns due to changes in host plant quality or vegetation complexity.

Fertilization results not only in an increase in plant quality of the individual (host) plant, but also in a changed structural complexity of the whole vegetation (Hartley, Gardner & Mitchell 2003; Gratton & Denno 2003). Unfertilized dry and wet grasslands belong to habitats showing the highest plant species richness (Ellenberg 1996). With the development of mineral fertilizer many of these habitats were transformed into intensively used grassland with high livestock density and/or several harvests per year. The number of plant species decreases with increasing soil fertility (Ellenberg 1996). This negative correlation between intensive land use and plant species richness is already well documented for many ecosystems (Baessler & Klotz 2006; Smart et al. 2006). Therefore the amount of fertilizer applied within a habitat often represents the degree of land use intensity at this site. The effects of fertilization on higher trophic level organisms mostly remain unknown. Not only plant species diversity is influenced by fertilizer application. Looking at plant performance, fertilization usually results in an increase in the biomass and in leaf nitrogen content of the focal plant, often in combination with a change in the vertical distribution of the biomass of this plant or of the whole plot treated. Fertilizer application therefore results not only in a change in plant quality of the individual plant, but also in a changed structural complexity of the whole vegetation (Bobbink et al. 1988; Hartley et al. 2003; Gratton & Denno 2003) with possibly dramatic consequences for phytophagous insects and their natural enemies.

Fertilization is known to increase food plant quality for herbivores and as a result herbivore abundance (Price et al. 1980; Gratton & Denno 2003; Stiling & Moon 2005) and in some cases also herbivore species richness (Hartley et al. 2003). Increased plant nitrogen (N) should enhance herbivore fitness by relieving protein limitation, thus increasing herbivorous insect populations (Cease et al. 2012). In other studies nutrient availability was shown to alter patterns of parasitoid related mortality (Moon et al. 2000; Yarnes & Boecklen 2006). However, also populations of natural enemies seem to increase after fertilizer application especially with regard to changes in vegetation structure with variable consequences for their prey. Nevertheless increased plant nitrogen seems to be not always beneficial to herbivorous insects (Fischer & Fiedler 2000; Cease et al. 2012).

1.2 Effects of the host plant and the surrounding vegetation on herbivores, parasitoids and host-parasitoid interactions

Vegetation structure may interact with the behavior of insects in many different ways. Vegetation can influence the motility of insects. For example the complexity of habitats and host plant architecture influence the ability of herbivores and parasitoids to move, as well as their host finding process (Goodwin & Fahrig 2002; Obermaier et al. 2008). Furthermore tall and dense vegetation in the neighborhood of host plants may either visually mask or decrease the access to the target plant (Perrin 1977; Åsman, Ekbom & Rämert 2001). Thus, different parameters of the vegetation may affect the access to resources like the host plant, and with it the population dynamics of phytophagous arthropods. Vegetation structure and host plant parameters are important environmental factors that are able to mediate host-parasitoid interactions (Heisswolf, Obermaier & Poethke 2005; Obermaier et al. 2008). Furthermore, the nutritional supply and availability of the host plant plays a fundamental role for both the occurrence and abundance of herbivores (Mohd Norowi et al. 1999; Heisswolf, Obermaier & Poethke 2005).

1.3 Factors influencing the occurrence of organisms within landscapes

Within a landscape, potential habitat patches can differ in quality, size, and isolation, which in turn can influence the occurrence of an organism on a patch (Thomas et al. 2001; Tscharntke et al. 2002; Hanski & Gaggiotti 2004). The metapopulation theory states that sub-populations of a species are interdependent within a landscape and that long-term persistence depends on a balance across the landscape between local extinctions of individual populations and new colonizations of vacant habitat patches (Hanski 1998; Moilanen & Hanski 1998). Based on this theory, the occurrence of a species within a patch is supposed to increase with increasing patch size and decreasing isolation. However, several recent studies emphasized that, in addition to the size, also the quality of a habitat patch determines its carrying capacity and may thus increase the survival chance of a population (Thomas et al. 1998; Wiegand et al. 1999; Binzenhöfer et al. 2007; Heisswolf et al. 2009). Habitat quality in grassland habitats of agricultural landscapes is most dominantly affected by land use intensity and type of management influencing host plant availability and quality (for insect herbivores) or host abundance (for parasitoids) as well as vegetation composition, chemical diversity and structural complexity.

1.4 Chemical composition of the vegetation

Multitrophic interactions are important drivers for ecosystem functioning and stability, especially because they can influence nutrient cycling (reviewed by Weisser & Siemann 2004; Schmitz 2010). To figure out the effects of plant species diversity on multitrophic interactions (Hooper et al. 2005; Unsicker et al. 2006; Shennan 2008) until now only a small number of studies have been performed in a natural context (Fischer et al. 2010). Plant species diversity can influence insects for example by disruption of olfactory or visual host location processes (Finch & Collier 2000; Randlkofer et al. 2007). An essential event in insects' life histories is host location. Herbivores as well as parasitoids use volatile cues of the host plant, the host or the microhabitat for locating their hosts at longer distances (Godfray 1994; Vet et al. 1995; Bruce et al. 2005; Steidle & van Loon 2002). However, multitrophic interactions take place in a heterogeneous and complex environment which is formed mainly by non-host plants (Casas & Djemai 2002). Non-host plants and high plant species diversity can generate a complex odor bouquet (Randlkofer et al. 2010) which insects have to cope with while searching for their host.

Vegetation composition (plant diversity and host plant density) can also impact trophic interactions indirectly by influencing host plant metabolites (Broz et al. 2010; Scherling et al. 2010). For the performance of a focal plant, plant neighbor identity can be very important (Barbosa et al. 2009) and thereby shape multitrophic interactions.

The composition of the vegetation surrounding a single plant can affect nutrient and secondary metabolite allocation in a focal plant (Cipollini & Bergelson 2001; Broz et al. 2010; Scherling et al. 2010). These changes in metabolite levels might influence plant performance, for example via the alteration of herbivory levels (Agrawal 2004), and by interactions with surrounding plant species or changing competition levels (Barbosa et al. 2009). Higher plant species diversity can enhance (Mraja et al. 2011) as well as lower (Whitehouse & Bayley 2005) plant nitrogen content. Plants grown together with conspecific neighbors exhibit increased concentrations of secondary metabolites (Barton & Bowers 2006; Broz et al. 2010).

In general primary metabolites and the nutritional value of a plant correlate positively with herbivore abundance (Mattson 1980). Otherwise, plants with high nitrogen content caused a higher larval and pupal mortality of a caterpillar (Fischer & Fiedler 2000). Secondary metabolites in plants function as defense compounds against herbivores, but insects have evolved different ways to handle them (reviewed by Opitz & Müller 2009). Many differences in the ability to cope with phytochemicals can be explained by the specialist or generalist life-style (Schoonhoven et al. 2005). Besides the influence of nutrient composition of a host plant on herbivores it may also affect the next trophic level. The nutritional value of the host plant might affect parasitoids by an altered parasitization rate (Thompson 1999; Sarfraz et al. 2009a). Furthermore secondary metabolite content of a host plant may as well affect parasitoids (Poelman et al. 2008; Gols et al. 2009). For example, parasitoid´s performance was negatively influenced by caterpillar hosts feeding on plants containing the alkaloid nicotine (Barbosa et al. 1986).

1.5 Choosing and using diversity indices

Land use, surrounding vegetation with its chemical composition and spatial scales not only influence mutitrophic interactions in detail, but can also influence the whole biodiversity in a habitat. Biodiversity displays the variety and heterogeneity of organisms at all trophic levels. Normally we focus on species diversity, but other forms like genetic and chemical diversity, are also important to have a look on. To quantify biodiversity remains problematic because there is no single index that adequately summarizes the concept (Hurlbert 1971, Purvis & Hector 2000). The number of species present (richness), is the simplest metric used to represent diversity (Whittaker 1972). Species abundance is also important for diversity, and the proportional abundance of species can also be incorporated into indices representing diversity. Berger and Parker proposed the simplest of these indices. It has an analytical relationship with the geometric series of the species abundance model (May 1975, Caruso et al. 2007), and reports the proportional abundance of only the most abundant species in the population (Berger & Parker 1970).

An important basis for biodiversity studies is to understand how changes in biodiversity of one trophic level affect biodiversity of other trophic levels. By including trait based diversity measures in analyses additional insights into community dynamics can be obtained. For example, when modeling changes in species diversity throughout a community, knowledge of the genetic and chemical diversity of the primary producer in this system would provide mechanistic insights into any changes in herbivorous insect diversity that could be related to the complexity of herbivore defenses or attractants displayed by the plant (Barto et al. submitted). It is still unclear which diversity index is most effective at this type of complex community level analysis.

2. The Biodiversity Exploratories

Biodiversity research explores drivers and consequences of biodiversity changes. Modifications in land use are major factors for changes in biodiversity and biological ecosystem processes and services. However, the influences of land use on species diversity are well documented only for a few taxa and trophic networks. We still do not know how different components of biodiversity and their responses to land use changes are interrelated. Furthermore we hardly know about the interacting effects of land use on ecosystems. Thus, overall goals are to understand the effects of land use on biodiversity and to understand the modifying role of biodiversity change for land-use effects on ecosystem processes (Fischer et al. 2010). To address these important questions, a new large-scale and long-term project for functional biodiversity was established (Fig. 1), the Biodiversity Exploratories (www.biodiversity-exploratories.de). They comprise a hierarchical set of standardized field plots in three different regions of Germany covering manifold management types and intensities in grasslands and forests (Fischer et al. 2010). They serve as a joint research platform for 40 projects involving over 300 people studying various aspects of the relationships between land use, biodiversity and ecosystem processes through experiments, monitoring and observations (Fischer et al. 2010).

Fig. 1: Schema on the location of the three different exploratories in Germany.

The term “Exploratories” is used to emphasize that the project is not only based on observation and between-plot comparison, which could have been described by the term “observatories”, but that the project very importantly also involves replicated field experimentation in order to gain causal insights (Fischer et al. 2010).

Design of the Biodiversity Exploratories

A key aspect of the Biodiversity Exploratories is the system of standardized study plots, which are used by all involved research groups. Such a study design is important for statistical comparisons across land use types, taxa, or geographical regions (Fischer et al. 2010). To allow for the test of consistency and generality of land-use effects across geographic regions, the Biodiversity Exploratories were established in three different regions of Germany (Fig. 1, 2).Within each region a pattern of experimental plots in grasslands and forests of different land use types and intensities was established. All plots within one region are characterized as “exploratory” (Fischer et al. 2010). Within each exploratory, there are three levels of study plots, on which research of different intensity and detail is conducted. In my study we used the 50 experimental plots per exploratory in grasslands, which are a selected subset of grid plots serving as a platform for more thorough biodiversity assessment and environmental monitoring, as well as for several manipulative experiments.

As a first step for establishing the Biodiversity Exploratories three study regions were selected (Fischer et al. 2010): (1) the UNESCO Biosphere Reserve Schorfheide-Chorin, which is situated in the lowlands of North-eastern Germany, a young glacial region with many wetlands, (2) the National Park Hainich-Dün and its surrounding areas, situated in the hilly lands of Central Germany, and (3) the UNESCO Biosphere Reserve Schwäbische Alb (Swabian Jura), which is situated in the low mountain ranges of South-western Germany (Fig. 2). Each of these areas represents a clear variation in typical land use types for grasslands and forests in Germany, from hardly managed grasslands and unmanaged beech forests to highly fertilized and intensively used meadows and pastures, and intensively managed forests (Fischer et al. 2010).

Fig. 2: Overview of the three different exploratories. Located from north to south: Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb.

Selection of experimental plots

100 locations in each exploratory were used (50 in forests and 50 in grasslands) in which so-called experimental plots (EPs) were established. Experimental plots have a size of 50 m × 50 m in grasslands. The selection was based on a stratified random sampling, with strata representing land use and several other criteria (Fischer et al. 2010). Two particularly important criteria were that the EPs should cover the variation in land use intensities and in soil depth found in each exploratory. Moreover, land owners had to allow long-term studies and experiments on the plots. Additional criteria were consistency and constancy of soil type, homogeneity of land use and vegetation composition within plots, and that they should have a slope of less than 20% (Fischer et al. 2010). A particularly challenging aspect of experimental plot selection was soil type. Up to ten different soil types occurred in each exploratory, and soil type variation was partly confounded with land use type. To reduce the variation caused by soil type, experimental plots were restricted to the two most dominant and characteristic soil types in forests and grasslands, respectively, in each exploratory (Fischer et al. 2010).

3 Study system

Plantago lanceolata (Plantaginacea), the ribwort plantain, is a generalist herb of cosmopolitan distribution (Van der Aart & Vulto 1992) that can grow up to a size between 5 and 50 cm and forms a rosette (Fig. 3). It blossoms from May to September (Schmeil & Fitschen 2003). Rosettes facilitate vegetative propagation (Wu & Antonovics 1975). According to Ellenberg (1996) and Opitz v. Boberfeld (1994) *P. lanceolata* grows both in wet and dry habitats. It grows in a very broad range of ground conditions (Dierschke & Briemle 2002), which makes *P. lanceolata* an ideal model system for investigating tritrophic systems in a gradient of land use intensity.

Mecinus labilis and *M. pascuorum* (Curculionidae) live monophagously on *P. lanceolata* (Fig. 3). Additionally *M. labilis* can live oligophagous when *P. sempervirens* is available. Both weevil species hibernate as adults and become active in May (Mohd Norowi 1999). Females oviposit on newly-formed seedheads in June and July (Dickason 1968). The hatched larvae bore into a seedhead where they feed and develop in individual seeds (Mohd Norowi 1999). The larvae are fully developed by July and pupate in the seedheads (Mohd Norowi 1999). Adults emerge from late August to September and move to the hibernation sites. *M. labilis* can mainly be found on mown and dry pastures. Adults have a size of 1.7 to 2.2 mm (Lohse 1983). The seed-feeder *M. pascuorum* lives on dry grasslands and has a size of 1.5 to 2.1 mm.

Mesopolobus incultus Walker (Hymenoptera: Pteromalidae) is a generalist parasitoid that parasitizes different insect orders (http://www.nhm.ac.uk/ research-curation/ research/ projects/ chalcidoids/ [December 2008]) (Fig. 3). This pteromalid wasp is a solitary species. Its only hosts on *P. lanceolata* are the two weevil species described above. No details of ovipositional behavior are available and most parts of its life cycle are unknown. *M. incultus* parasitizes the larvae of the weevils during summer (Mohd Norowi 2000), and hatches from the seedheads at the same time as the adult beetles (C. Herbst, pers. observ.).

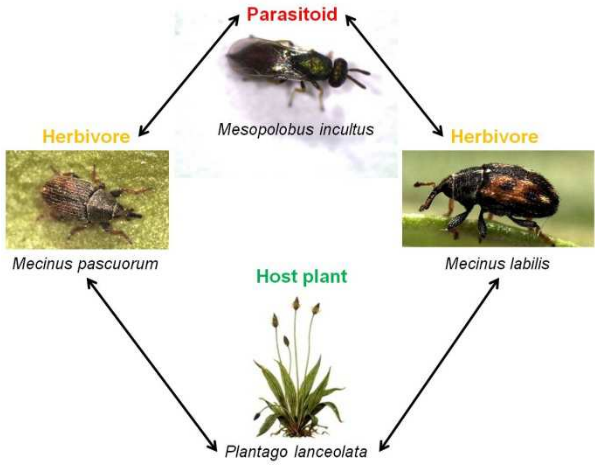

Fig. 3: Tritrophic system consisting of the host plant *Plantago lanceolata*, the herbivorous weevils *Mecinus labilis* and *M. pascuorum* as well as their parasitoid *Mesopolobus incultus*.

4. Study design

Between May and June in the years 2008, 2009 and 2010 different host plant and vegetation parameters were measured on the experimental grassland plots of the three exploratories (Fig. 4). In 2008 we investigated 10 randomly selected *P. lanceolata* plants per plot and collected mycorrhizas from five individuals. In 2009 and 2010 we investigated three plants on each plot. Host plant abundance was determined in a radius of 1 m around the selected *P. lanceolata* plant. Host plant parameters like stalk number was counted and maximum stalk height (cm), rosette diameter (cm) and rosette height (cm) were measured in a radius of 15 cm around the investigated host plant.

Vegetation structure was determined by height (maximum height of forbs or grass) and density (weighted mean vegetation height) of the investigated vegetation. Plant species diversity was calculated as plant species richness. We recorded plant species richness by counting the number of different forb species in a radius of 15 cm around the investigated host plant. The vegetation structure was investigated by estimating the vertical cover of forbs, grasses, open ground and the focus host plant (%) by looking at the top of the vegetation in the 15 cm radius. The horizontal cover of the vegetation (%) on the other hand was achieved by looking at the vegetation from the lateral side. The horizontal cover was assessed in 0.10 m steps from 0 m up to 1.70 m using a white board as background (Sundermeier 1999). We also calculated the weighted mean and measured the maximum vegetation height (cm) around the randomly chosen host plants (r = 0.15 m) per plot.

Invertebrate sampling

Insects were collected by sampling 100 inflorescences of *P. lanceolata* plants per plot in the year 2008 and 60 inflorescences for the fertilizer experiment in the following two years. The collection was conducted between August and September 2008, 2009 and 2010 in all three regions. The inflorescences were stored per subplot in plastic boxes (17.0 x 12.5 x 5.6 cm) with gauze covering (0.12 mm) under constant conditions (11:13 LD, temperature: 22°C, 50% rH). After hatching the insects were identified and counted.

Fertilization experiment

The number of plots investigated for the experimental fertilization were 76 in 2009 (34 plots Schwäbische Alb, 21 plots Hainich-Dün and 21 plots Schorfheide-Chorin) and 81 in the year 2010 (33 plots Schwäbische Alb, 25 plots Hainich-Dün and 23 plots Schorfheide-Chorin). Two subplots (6 m x

6 m) in one corner of every experimental plot were chosen for the experiment (Fig. 4). We selected one subplot as control and the other for experimental fertilization. The latter was fertilized in April and October 2009 and in April 2010 with 200 g per m² (N = 6,67%, P_2O_2 = 0,85%, K_2O = 0,86%) of an organic fertilizer (Agrobiosol®, SW-Düngesysteme, Wolfenbüttel). The control subplot was not experimentally fertilized.

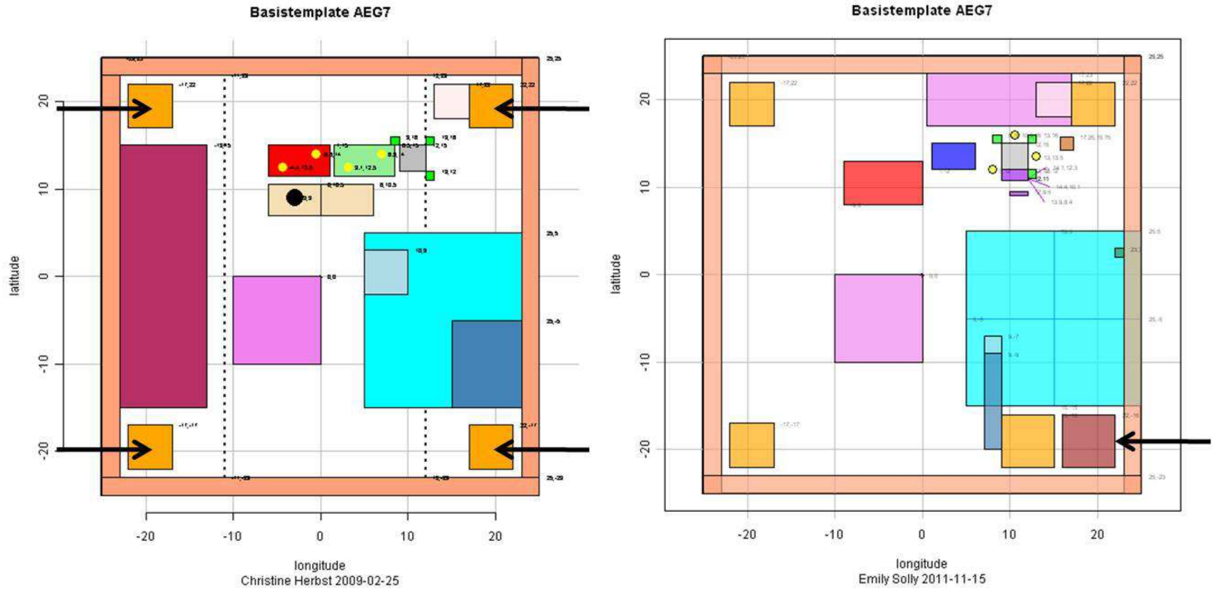

Fig. 4: Experimental plot (50 m x 50 m) on which the investigations took place. Left: Arrows show the four investigated subplots in the year 2008. Right: Arrow show one control and fertilized subplot for the fertilizer experiment in the years 2009 and 2010 (www.biodiversity-exploratories.de).

5. Thesis Outline

My PhD-Thesis aimed to investigate the influences of land use, surrounding vegetation structure and diversity as well as its chemical composition on a tritrophic system of P. lanceolata in three different regions and on different spatial scales. I had a closer look on how land use (grazing and mowing) and fertilization affected vegetation (structure and diversity), the host plant and the upper levels of the investigated system. Additionally the effects of chemical composition on the herbivores and their natural enemy were analyzed. I also had a closer look on different spatial scales from the single plot over a habitat to larger scales like landscapes. All these parameters not only influenced a single system, but also the whole biodiversity in the three regions investigated. Therefore at last I tried to give an overview by trying to measure biodiversity via different indices.

My thesis is based on the following manuscripts:

Chapter IV:

Land use intensification in grasslands: higher trophic levels are more negatively affected than lower trophic levels

Intensification in land use is known to influence biodiversity and eventually multitrophic interactions, but its effect on the performance of species of higher trophic levels is still little understood. To clarify the influence of land use on the different trophic levels of the tritrophic system of a common grassland herb, I asked whether land use intensity and type affect (1a) the host plant *P. lanceolata* and (1b) the vegetation complexity surrounding the host plants, (2a) the specialized herbivores and (2b) their parasitoid in the inflorescences of the host plant, and (3) the tritrophic system of *P. lanceolata* in three different geographic regions and years.

Chapter V:

Fertilizer application decreases insect abundance on *Plantago lanceolata* – a large scale experiment in three geographic regions

In this study a closer look on the effects of experimental fertilization on the investigated tritrophic system and its surrounding vegetation was taken. I addressed the following questions: How does experimental fertilization affect (1) the vegetation structure and plant species richness, (2) the host plant abundance and quality as well as (3) the two weevil species and their common parasitoid?

Chapter VI:

Landscape wide land use intensity determines local abundance of higher tropic levels in grassland habitats

Here the weevil-parasitoid community of *P. lanceolata* was analyzed on two different habitat types (intensively used and extensively used grassland) and also on different landscape scales to answer the following questions: 1) Does the land use intensity at landscape scale has a negative influence on the abundance of the three insect species, 2) are there differences in the response of the second and the third trophic level, 3) is the abundance of the two herbivores more strongly influenced by the surrounding landscape at smaller spatial scales than at larger spatial scales to which the parasitoid should react more?

Chapter VII:

Defensive plant compounds relate to plant species richness but plant nitrogen content mediates multitrophic interactions in cultural landscapes

Here, the question how vegetation composition impacts plant metabolites of the host plant and thus higher trophic levels associated to this plant in three different geographic regions was investigated. It was hypothesized that (a) vegetation composition (plant species richness and host plant density) affect nitrogen and IG (aucubin and catalpol) leaf content of *P. lanceolata* and that (b) host plant metabolite concentrations are correlated with the abundances of the herbivores *M. pascuorum* and *M. labilis,* their parasitoid *M. incultus* as well as the interaction between herbivores and parasitoid (parasitization success).

Chapter VIII:

Habitats as complex odorous environment: How does plant diversity affect herbivore and parasitoid orientation?

In this study field investigations were combined with a laboratory approach to reveal the impact of habitat (odor) diversity in tritrophic interactions. In the field the impact of plant diversity and host plant density on the abundance of (1) the herbivore *M. pascuorum* and (2) the parasitoid *M. incultus* by surveying vegetation and insects in different diverse grassland plots was studied. In the lab a new olfactometer assay to rebuild natural odorous conditions and tested (3) whether *M. pascuorum* is attracted by its host plant, and if so (4) whether this attraction is affected by enhanced plant diversity was established. Further it was checked whether the parasitoid *M. incultus* (5) is attracted to the host complex (host plant + host), and (6) hampered by enhanced plant diversity.

Chapter IX:

Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories

All the influences on a single tritrophic system which were investigated in the upper studies are transferable to the biodiversity in habitats. Therefore three hypotheses related to performance of diversity indices were set out to test. (1) Correlations between diversity indices, and which indices discriminate sites. (2) Dependence of effects of land use on diversity indices. (3) Dependence of community dynamics on diversity index chosen. This is the first analysis to compare performance of diversity indices when quantifying complex community dynamics. The results should provide guidelines for appropriate use and interpretation of diversity indices in future studies exploring biodiversity and community dynamics.

Chapter IV

Land use intensification in grasslands: higher trophic levels are more negatively affected than lower trophic levels

Chapter IV

Land use intensification in grasslands: higher trophic levels are more negatively affected than lower trophic levels

Christine Herbst*[1], Nicole Wäschke[2], E Kathryn Barto[3], Sabrina Arnold[1], Daniel Geuß[2], Ivonne Halboth[1], Sabina Reschke[2], Jakob Sänger[1], Torsten Meiners[2], Elisabeth Obermaier[1]

[1] Department of Animal Ecology and Tropical Biology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

[2] Institute of Biology, Applied Zoology / Animal Ecology, Freie Universität Berlin, Haderslebener Strasse 9, 12163 Berlin, Germany

[3] Institute of Biology, Dahlem Center of Plant Sciences, Freie Universität Berlin, Altensteinstrasse 6, 14195 Berlin, Germany

*Corresponding author. Present address:
Department of Animal Ecology and Tropical Biology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
Tel.: + 49 (0) 171-76 49 916
E-mail address: christine-herbst@t-online.de

Keywords: Land use; mutitrophic interactions; plant species richness; ribwort plantain; vegetation structure

Abstract

Increasing land use intensity and human influence are leading to a reduction in plant and animal species diversity. However, little is known about how these changes may affect higher trophic levels, apart from simply reducing species numbers. Here we investigated, over three years, the influence of different land practices on a tritrophic system (the host plant *Plantago lanceolata* L., two monophagous weevils – *Mecinus labilis* Herbst and *Mecinus pascuorum* Gyllenhal – and their parasitoid *Mesopolobus incultus* Walker). We measured, at over 70 sites, across three geographic regions in Germany, plant species diversity and vegetation structure, as well as abundance of *P. lanceolata*, the two weevils, and the parasitoid. Land use intensity (fertilization) and type (mowing vs. grazing) negatively affected not only plant species richness, but also the occurrence of the two specialized herbivores and their parasitoid. In contrast, land use had a mostly positive effect on host plant size, vegetation structure and parasitization rate. Our study reveals that intensification of land use influences higher trophic organisms even without affecting the availability of the host plant. The observed relationships between land use, vegetation complexity, and the tritrophic system are not locally restricted; rather they are measureable along a broad range of environmental conditions and years throughout Germany. Our findings may have important implications for the conservation of insect species of nutrient poor grasslands.

Introduction

The recent worldwide anthropogenic conversion of natural habitats to agriculture profoundly affects the diversity of plants and animals (Foley et al., 2005). A negative correlation between plant species richness and land use intensity in terms of fertilization is well documented for many ecosystems (Baessler & Klotz, 2006; Smart et al., 2006). At the same time field studies and experiments have revealed a positive relationship between plant species richness and herbivore diversity (Schläpfer & Schmid, 1999; Pearson & Dyer, 2006; Crutsinger et al., 2006; Schaffers et al., 2008). In grasslands, intensive mowing, heavy grazing, and fertilization represent major negative impacts for plant species richness. However, little is known about how intensification in land use influences the performance of species at higher trophic levels, apart from simply reducing species numbers (van der Putten et al., 2004).

Insects are key players in grassland ecosystems, and management practices can affect them in numerous ways. For example, heavy grazing and high mowing frequencies negatively affect survival (Tscharntke & Greiler, 1995) because grazing and mowing reduce available food resources, and lead to changes in plant growth and vegetation complexity (Kruess & Tscharntke, 2002). The frequency of mowing is also important because it can cause changes in the vegetation composition that in turn can influence the occurrence and diversity of herbivorous arthropods (Köhler et al., 2004; Unsicker et al., 2006). Species at higher trophic levels may also be affected because the abundance and diversity of organisms at lower trophic levels are reduced (Hartley & Jones, 2003).

However, intensive land management may sometimes positively affect insects. For instance, fertilization can increase host plant quantity and quality, which can affect the occurrence and abundance of herbivores (Mohd Norowi et al., 1999; Heisswolf et al., 2005). Furthermore host plant quality is an important factor for food web structure even for very high trophic levels (Bukovinszky et al. 2008). Fertilization can also increase host plant patch size and density, which can in turn affect insect herbivore densities (Kareiva, 1985; Heisswolf et al., 2005). These could have positive effects at the next trophic level. On the other hand, and in contrast to the nitrogen limitation hypothesis (White, 1993), Fischer and Fiedler (2000) showed that high nitrogen contents of host plants, caused by fertilization, can negatively affect insect herbivores. In the butterfly species *Lycaena tityrus* Poda, fertilization caused high pupal and larval mortality and a reduction in adult size in species inhabiting nutrient poor grasslands. Nutrient availability also was shown to alter patterns of parasitoid related mortality (Moon et al., 2000; Yarnes & Boecklen, 2006). However, populations of natural enemies seem to increase after fertilizer application, especially with regard to changes in vegetation structure with variable consequences for their prey.

Little is currently known about how land use practices affect the abundance and occurrence of organisms of higher trophic levels. In this study we investigate the impact of land use on all levels of a tritrophic system, and on the surrounding vegetation, in multiple regions in Germany, and across multiple years. The targeted plant, *Plantago lanceolata* L. (Plantaginaceae), was chosen because it is widespread throughout Germany, and occurs under different land use intensities. Two herbivorous weevils, *Mecinus labilis* Herbst and *Mecinus pascuorum* Gyllenhal (Coleoptera: Curculionidae), are commonly found on this plant. Both weevils oviposit on newly formed seedheads, and this is where larvae feed and develop. These two weevils also share a common parasitoid, the pteromalid wasp *Mesopolobus incultus* Walker (Hymenoptera: Pteromalidae). We studied this tritrophic system across three different regions in Germany, which differed considerably in their soil and vegetation types, and the amount of precipitation they received (Fischer et al., 2010). This enabled us to investigate the influence of land use on the same tritrophic system under different abiotic and biotic conditions. We hypothesized that land use intensity (fertilization) and type (mowing vs. grazing): (1) increase the size and abundance of our target plant, decrease the plant species richness and change the surrounding vegetation, (2) and therefore increase the occurrence of the specialized herbivores, their parasitoid and its parasitization success. At last (3) we wanted to find out in which way these parameters differ across the three different geographic regions and years.

Material and methods

Study sites and plot design

In the framework of the large-scale German Biodiversity Exploratories project (http://www.biodiversity-exploratories.de), we investigated experimental plots on grasslands with different land use types and intensities in three different regions – Schorfheide-Chorin (Brandenburg), Hainich-Dün (Thuringia), and Schwäbische Alb (Baden Württemberg) (Fischer et al. 2010). In each region we investigated plots from three different land use types (meadows, mown pastures and pastures, Table A1) and two different land use intensities (fertilized and unfertilized, Table A2); both as defined by the farmers (Fischer et al., 2010). Meadows were mown one to three times a year. Mown pastures were mown one time a year and have different livestock densities. Pastures range from high livestock densities to fairly used plots (see Fischer et al., 2010).

The three regions differ considerably in their soil, vegetation, precipitation and agricultural use, and therefore enable the investigation of the influence of land use on the same tritrophic system under very different abiotic and biotic conditions on a larger geographical scale. In every geographical region, 50 experimental grassland plots were provided (Fischer et al., 2010). According to the occurrence of our host plant (*P. lanceolata*), the number of investigated plots was 76 plots in the year 2008, 78 in 2009 and 80 plots in the year 2010 (Table A1, 2). Every plot had a size of 50 m x 50 m and had a north-south alignment.

Study system

Our study system consisted of the ribwort plantain *Plantago lanceolata L.*, two associated weevil species *Mecinus labilis* Herbst *and Mecinus pascuorum* Gyllenhal and the parasitoid *Mesopolobus incultus Walker*.

Plantago lanceolata L. is an ubiquitous perennial herb that can grow up to a size between 5 and 50 cm and forms a rosette. It blossoms from May to September in Germany (Schmeil & Fitschen, 2003).

According to Ellenberg (1996), *P. lanceolata* grows both on wet and dry habitats. It grows in a very broad range of ground conditions (Dierschke & Briemle, 2002), which makes *P. lanceolata* an ideal model system for investigating tritrophic systems in a gradient of land use intensity.

Mecinus labilis Herbst and *Mecinus pascuorum* Gyllenhal live monophagously on *P. lanceolata*. Both weevil species hibernate as adults and become active in May (Mohd Norowi et al., 1999). Females oviposit on newly-formed seedheads in June and July (Dickason, 1968). The hatched larvae bore into a seedhead where they feed and develop in individual seeds (Mohd Norowi et al., 1999). The larvae are fully developed by July and pupate in the seedheads (Mohd Norowi et al., 1999). Adults emerge from late August to September and move to the hibernation sites. *Mecinus labilis* can mainly be found on mown and dry pastures; adults range in size from 1.7 to 2.2 mm (Lohse, 1983). The seed-feeder, *M. pascuorum,* lives on dry grasslands and ranges in sizes of 1.5 to 2.1 mm.

Mesopolobus incultus Walker is a generalist parasitoid that parasitizes different insect orders (http://www.nhm.ac.uk/research-curation/research /projects /chalcidoids/ [December 2008]). This pteromalid wasp is a solitary species. Females range in sizes of 1.5 to 2.4 mm and males in sizes of 1.4 to 1.8 mm (Baur et al., 2007).Its only hosts on *P. lanceolata* are the two weevil species described above. No details of ovipositional behavior are available and most parts of its life cycle are unknown. *Mesopolobus incultus* parasitizes the larvae of the weevils during summer (Mohd Norowi et al., 2000), and hatches from the seedheads at the same time as the adult beetles (C. Herbst, pers. observ.).

Sampling design

Between May and June in the years 2008, 2009 and 2010 we measured different host plant and vegetation parameters on the experimental grassland plots of the three regions.

In 2008 we investigated 10 *P. lanceolata* plants per plot. Because we averaged plant data for each plot and because of the large plot number, we only investigated three *P. lanceolata* plants in 2009 and 2010. We determined host plant abundance in a radius of 1 m around the selected *P. lanceolata* plant. Additionally we recorded host plant parameters like stalk number, maximum stalk height (cm), rosette diameter (cm) and rosette height (cm).

In our study, vegetation structure was defined by height (maximum height of forbs or grass) and density (weighted mean vegetation height) of the investigated vegetation. Plant species diversity was calculated as plant species richness. We recorded plant species richness by counting the number of different forb species in a radius of 15 cm around the investigated host plant. The vegetation structure was investigated by estimating the horizontal cover of the vegetation (%) in the same radius. It was assessed in 0.10 m steps from 0 m up to 1.70 m using a white board as background (Sundermaier, 1999). We also calculated the weighted mean and measured the maximum vegetation height (cm) around the randomly chosen host plants (r = 0.15 m) per plot.

Invertebrate sampling

We collected insects by sampling 100 inflorescences of *P. lanceolata* plants per plot. The collection was conducted between August and September 2008, 2009 and 2010 in all three regions. Afterwards we stored the inflorescences per subplot in plastic boxes (17.0 x 12.5 x 5.6 cm) with gauze covers (0.12 mm) under constant conditions (11:13 LD, temperature: 22°C, 50% rH). After hatching we identified and counted the insects.

Statistical Analyses

The influence of land use on vegetation structure, host plant parameters, plant species richness, as well as on weevil and parasitoid occurrence, was analyzed using multiple logistic regression and one-way ANOVA (Jongman et al., 1995; Hosmer & Lemeshow, 2000), followed by correction for multiple comparisons, the false discovery rate by Benjamini & Hochberg (1995). Parasitization rate was analyzed by using generalized linear mixed models. Prior to analysis, we excluded collinearity of vegetation structure and host plant parameters by principal component analysis. The resulting factor "host plant size" consists of the rosette height and number of leaves. The weighted mean vegetation height (GMV, vegetation density) was calculated from data on horizontal vegetation cover

(Sundermaier, 1999). The factor “vegetation” consists of the vegetation density and the maximum vegetation height.

We used binary logistic regression to test the influence of land use intensity (fertilized or unfertilized) and type (meadow, mowed pasture, pasture) on the occurrence of the three insect species. To evaluate the goodness-of-fit of the logistic regression models the coefficient of determination R^2 from Nagelkerke (1991) was used. We used the one-way ANOVA to compare the categorical variables land use intensity and type with host plant and vegetation parameters. Residuals were tested for normal distributions. These analyses were conducted with the software package SPSS Statistics Version 18.0 (SPSS Inc., 2010). We analyzed the influence of land use intensity and type on the parasitization rate of *M. incultus* with generalized linear mixed models. Exploratory and plot number were used as random effects. We used land use intensity and type as fixed factors. Models were calculated with the lmer function with Laplace approximation (package lme4 Version 0.999375-37) in R version 2.14.0 (R Development Core Team, 2011). For parasitization rate we applied the binomial error distribution (link = logit) and bound the number of successes (number of hatched parasitoids) and number of failures (number of hatched herbivorous hosts not parasitized by the parasitoid) as the response variable (Crawley, 2007).

Furthermore, we used structural equation models (SEM) to model direct and indirect effects of land use (LUI) on vegetation structure and diversity, and the host plant in all three years of the study. Vegetation structure was represented as a latent variable indicated by the manifest variables maximum vegetation height and weighted mean vegetation height. We represented host plant size also as a latent variable indicated by the manifest variables maximum stalk height, rosette height, and diameter. Including latent variables in the model allows us to include more than one relevant measure for each metric without arbitrarily deciding which is most important, as well as improving parameter estimates by assigning measurement error to the manifest variables (Grace, 2006). Site replication was too low for reliable SEMs, so we focused on effects of land use intensity on different vegetation parameters. We defined land use intensity (here as Land use index, LUI), according to Blüthgen et al. (2012), as one continuous factor combining information on the amount of fertilizer (kg N ha^{-1}), the frequency of mowing (y^{-1}), and the livestock density (livestock units × days ha^{-1}) for every plot. We analyzed data from each exploratory separately, and data were transformed as necessary to improve normality. As tests of model fit, we report χ^2 *P*-values, where values less than 0.05 suggest a poor model fit. Excellent model fits are indicated by Root Mean Square Error of Approximation (RMSEA) values less than 0.05, and Tucker Lewis Non-Normed Fit Index (TLNNFI) values greater than 0.9. These analyses were done with correlation matrices in the 'sem' package version 0.9-16 (Fox 2006) in R version 2.9.0 (R Development Core Team 2008).

Results

Influence of land use intensity (fertilization)

Land use intensity positively influenced host plant size in Schwäbische Alb in all three years (Figure 3; Table 1). Rosette height and number of leaves of *Plantago* plants were larger on fertilized than on unfertilized plots (Table A3). However, host plant abundance was not affected. For the other two regions we found neither a positive nor a negative effect of fertilization on the host plant parameters.

Fertilization influenced plant species richness either negatively or not at all (Figure 3, 2). In Schwäbische Alb we found this negative effect in all three years. In Hainich-Dün and Schorfheide-Chorin we found it in some, but not in all years of the study (Table 1, A3).

In contrast, fertilization mostly affected vegetation structure either positively or not at all (Figure 3, 4). In Schwäbische Alb vegetation structure was denser and higher on fertilized plots than on unfertilized plots in all three years (Table 1, A3). In Schorfheide-Chorin fertilization did not influence the vegetation structure and in Hainich-Dün vegetation structure was influenced only in 2008. In this year the vegetation grew denser and higher on unfertilized compared to fertilized plots.

Fertilization affected the occurrence of the three investigated insect species either negatively or not at all (Figure 1, 3, 4). In Schwäbische Alb and Hainich-Dün insects occurred more often on unfertilized than on fertilized plots in all three years (Figure 1, 3; Table 2, A4). In Schorfheide-Chorin there was no effect of fertilizer on either the herbivores or the parasitoid (Figure 1, Table A4). Although the occurrence probability of the parasitoid was lower on fertilized plots compared to unfertilized, the parasitization rate of *M. incultus* was higher on fertilized plots in 2008 (Table 3). There was no significant effect of fertilization on parasitization rate in the other two years examined (Table 3).

In summary there was an overall negative influence of fertilizer on plant species richness, and on the occurrence of the investigated insect species (Figure 4). In contrast, there was a positive effect on vegetation structure and parasitization rate in one year.

Influence of land use type

Land use type (mowing vs. grazing) had either a positive effect on host plant size or none at all. Rosette height and number of leaves were smaller on pasture plots compared to mown pastures and meadows in Hainich-Dün and Schwäbische Alb in most years of the study (Figure 3, 4, Table 1). In Schorfheide-Chorin there was no effect of mowing and grazing on host plant size (Table 1, A3). Additionally, land use type had no effect in Hainich-Dün and Schorfheide-Chorin or an indifferent effect in Schwäbische Alb on host plant abundance.

Land use type (mowing vs. grazing) influenced vegetation structure positively in all three regions and almost all years (Figure 3, 4). In Schorfheide-Chorin the highest and most dense vegetation grew on meadows followed by mown pastures and pastures (Table 1, A3). In Hainich-Dün and Schwäbische Alb we observed the same results, except in the year 2008 in Hainich-Dün. Here the highest and most dense vegetation grew on pastures followed by mown pastures and meadows (Table 1, A3).

Mowing and grazing affected plant species richness as well. More plant species were found on pastures than on mown pastures and meadows in Hainich-Dün and Schwäbische Alb in all three years (Figure 3, 4, Table 1, A3). Only in Schorfheide-Chorin in the year 2008 we found higher plant species richness on meadows compared to pastures (Table 1, A3). In the following two years no further influence could be shown (Table 1).

Mowing affected the occurrence of the herbivores and the parasitoid in all three regions either negatively or not at all (Figure 2, 3, 4, Table A4). In Schwäbische Alb, except for the year 2009, and in Hainich-Dün we found a higher weevil and/or parasitoid occurrence on pastures than on the other two land use types (Figure 2, 3; Table A4). In Schorfheide-Chorin, except for the year 2010, there was no effect of land use type on the occurrence of any of the insect species (Figure 2; Table 2, A4). In contrast, the parasitization rate of *M. incultus* was higher on meadows compared to the other land use types in the years 2008 and 2010 (Table 3B).

In general mowing had a more positive effect on vegetation structure, the host plant and the parasitization rate than grazing, but a more negative effect on plant species richness and insect species occurrence.

Interaction of all parameters

In addition to an analysis of all possible interactions with land use by multiple logistic regression and ANOVA we used structural equation models (SEMs) for a combined analysis of vegetation, host plant and land use variables within one model. For every region, and year, a separate SEM was calculated (Table 4). Despite the occurrence of significant paths in each model, none of the models calculated provided a significant model fit, except Schorfheide-Chorin 2009. Attempts to improve model fit by adding paths based on modification indices generated poorer model fits, based on BIC scores (data not shown).

Discussion

Influence of land use on host plant and vegetation parameters

Although a negative correlation between land use intensity and plant species richness is well known and generally recognized (Di Tommaso & Aarssen, 1989; Gratton & Denno, 2003; Baessler & Klotz, 2006; Smart et al., 2006), few studies exist on the effects of land use on the performance of specific host plants and their associated insects. Since herbivores and their natural enemies may be affected not only directly by land use, but also indirectly by changes in their host plant quality and quantity, as well as vegetation diversity and structure, we additionally investigated the impact of increasing land use on the surrounding vegetation and its interaction with the tritrophic system.

In our study fertilization supported vegetation structure and increased host plant size, especially in Schwäbische Alb, and decreased plant species richness in all three regions investigated, and in most years. This result is supported by numerous studies showing that fertilization reduces plant species number (Gratton & Denno, 2003; Baessler & Klotz, 2006). Regarding individual plant performance and vegetation structure, fertilization usually results in an increase in leaf nitrogen content and in the biomass of the focal plant (McNeill & Southwood, 1978), often in combination with a change in the vertical distribution of the biomass of this plant or of the whole plot treated. Fertilization is responsible therefore not only for an increase in plant growth and quality of the individual (host) plant, but also for a changed structural complexity of the vegetation (Hartley et al., 2003; Gratton & Denno, 2003; Chen et al., 2010). In Schorfheide-Chorin there was no impact of fertilization on parameters of vegetation structure. In Hainich-Dün, we found a higher vegetation density and height on unfertilized compared to fertilized plots in the year 2008. The increased vegetation structure on unfertilized plots in 2008 is probably the result of mowing events on the fertilized plots. In Schwäbische Alb vegetation structure was highest and most dense on fertilized plots in all three years. Variability in vegetation structure could be traced to vegetation being affected by land use management, as well as fertilization. Unfortunately, mowing by farmers took place at different times in the season and could not always be taken into account when plots were investigated (although no plot was investigated earlier than two weeks after mowing). Interestingly, fertilization had no influence on host plant size in Schorfheide-Chorin and the Hainich-Dün, although fertilizer is in general known to support plant growth (Ellenberg, 1977). However, in Schwäbische Alb, fertilization increased host plant size in all three years. Host plant abundance, in contrast, was affected in none of the three regions, whereas in another study with experimental fertilization we showed a decrease of *P. lanceolata* abundance (Herbst et al. submitted).

Land use type significantly influenced plant species richness, vegetation structure and host plant size in all three regions. Plant species richness was highest on pastures compared to mown pastures and meadows in Hainich-Dün and Schwäbische Alb. Joern (2005) also describes a positive correlation between extensive grazing and plant species richness. At the same time agricultural influences in the form of intensive mowing represent one of the major negative impacts on plant species richness (Köhler et al., 2004). The opposite result, higher species richness on meadows, in Schorfheide-Chorin in the year 2008 might be explained by the reduced land use gradient, due to a high proportion of organic farming in this region. Land use type also affected the vegetation structure in all three regions. We measured the highest and most dense vegetation on meadows compared to the other two land use types, except again for Hainich-Dün in the year 2008. In Schwäbische Alb we found a significant positive effect of mowing vs. grazing on host plant size in all three years. In Hainich-Dün host plant size was positively influenced by mowing in the year 2009 and as a trend in 2010. The tallest plants occurred on meadows compared to mown pastures and pastures. In summary, consistent effects in all three regions were reduced plant species richness and an increased vegetation structure. Host plant size was supported by land use in two of three regions, whereas there was no influence on host plant abundance.

In summary, consistent effects in all three regions were reduced plant species richness and an increased vegetation structure. Host plant size was supported by land use in two of three regions, whereas there was no influence on host plant abundance.

Influence of land use on herbivore and parasitoid occurrence

Increasing land use intensity and human influence are known to lead to a serious reduction in plant and animal species diversity in different habitats (Unsicker et al., 2006). Gratton & Denno (2003) propose that fertilization may not only affect herbivore abundance and diversity directly, but suggest that interacting effects of features of the vegetation with host-parasitoid systems can provoke more complex interactions in a multitrophic context of bottom up and top down effects. In two of the three regions (Hainich-Dün and Schwäbische Alb) land use intensity (fertilization) consistently negatively affected the two weevil species. Likewise the third trophic level, the occurrence of the parasitoid, was partly negatively affected by fertilization in these regions. The missing effects of fertilization on insect occurrence in Schorfheide-Chorin might be due on the one hand to the mostly extensive and organic farming in this region. This might promote species numbers and species richness in contrast to conventional agriculture. On the other hand the differences might be caused by completely different soil types in this region. In Schorfheide-Chorin Histosols, Luvisols, and Gleysols are common soil types which limit the types of land use and change plant communities found there.

Parasitization rate was not influenced by fertilization in two of three years. Only in 2008 parasitization rate of *M. incultus* was higher on fertilized than on unfertilized plots. This might be due to higher plant species richness and therefore higher odor diversity on unfertilized plots. Host plant odors have been found to be masked by diverse environments and might reduce the host finding success of the parasitoid. Non-host plants and high plant diversity may generate a complex odor bouquet (Randlkofer et al., 2010) which insects have to cope with while searching for their host. Negative effects may occur because of disruption of olfactory host location by non-host plant volatiles (Perfecto & Vet 2003; Randlkofer et al. 2007).

Likewise, land use type can strongly affect insect communities because grazing and mowing may modify plant growth and quality and the complexity of the vegetation, as well as possibly interrupt the phenology of insects (Kruess & Tscharntke, 2002; Woodcock et al., 2005). Weevil occurrence was negatively affected by mowing in Schorfheide-Chorin (*M. pascuorum*), Hainich–Dün (*M. labilis, M. pascuorum*) and Schwäbische Alb (*M. labilis, M. pascuorum*). The same result we found for the parasitoid. *Mesopolobus incultus* occurred more often on unmown plots. Several studies illustrate that foraging success of parasitoids is linked to their motility and that vegetation properties change their movement patterns (Randlkofer et al., 2009). In most cases, complex vegetation structures (Coll & Bottrell, 1996; Tschanz et al., 2005) like on meadows exert a negative influence on the foraging efficiency of natural enemies. In contrast the parasitization rate on meadows was higher than on the other land use types. We hypothesized that his result may be due to odor masking by higher plant species richness on pastures which we had discussed before for land use intensity.

In summary, we found that the *Plantago*-weevils and their parasitoid occurred with a higher probability on unfertilized plots and pastures than on fertilized sites and meadows.

Interaction of all parameters

In order to explain the observed patterns of a consistent negative impact of land use on the higher trophic levels investigated, independently of the abundance and size of the host plant, we discuss three hypotheses: 1) A high and dense vegetation structure, as observed on fertilized and meadow plots, might render host searching by herbivores and parasitoids more difficult and reduce the host finding capacity of the species. This might reduce the probability of their occurrence on plots with high human impact. 2) Changes of patterns of nutrient availability of the host plant due to changes in land use might cause potential negative effects on the performance of the flowerhead insects. 3) The different management regimes in combination might affect the tritrophic system with a preference of the insects for unfertilized pastures due to an interrupted phenology on mown plots.

With respect to our first hypothesis, fertilization and mowing as part of the agricultural management positively influenced vegetation height and density in all three regions investigated. Experimental fertilization, performed on the same plots, likewise increased vegetation height, density, and the aboveground biomass of *P. lanceolata* and decreased the abundance of the three insect species (Herbst et al. unpublished data). Plant structures interfere with insect behavior in different ways. For example, the structural complexity of habitats and the architectural traits of single plants influence the movement ability of herbivores as well as their host finding process (Goodwin & Fahrig, 2002; Randlkofer et al., 2009). Tall and dense vegetation in the neighborhood of host plants may either visually mask the target plant or may impede access to the target plant (Perrin, 1977; Åsman et al., 2001). Several examples illustrate that foraging success is linked to the movement ability of insects, and that vegetation properties change the movement pattern. In most cases complex vegetation structure exerts a negative influence on the foraging efficiency of herbivores and natural enemies (Tschanz et al., 2005; Obermaier et al., 2008; Randlkofer et al., 2010). Thus, structural features of the vegetation may affect access to resources and with it the population dynamics of phytophagous and entomophagous arthropods. However, since parasitization rate was increased by fertilization in 2008 and by mowing vs. grazing in two of three years this hypothesis might not be appropriate for the system studied.

In terms of our second hypothesis, the nutritional quality of the host plant might change according to land use intensity and management, with implications for higher trophic level organisms. We found a consistently negative effect of fertilization on the flowerhead insects in all three regions. An analysis revealed a negative impact of total leaf nitrogen on the abundance of both weevil species investigated (Wäschke et al. unpublished data). Changes in climate, land use and biodiversity can alter an animal´s nutritional environment (Warbrick-Smith et al., 2006). Although many publications report on positive correlations between insect performance and host plant N concentrations (McNeill & Southwood, 1978; White, 1993; Obermaier & Zwölfer, 1999), Fischer & Fiedler (2000) found that high leaf nitrogen contents were not generally beneficial to a butterfly species investigated. In a study by Joern & Behmer (1998) three grasshopper species responded in different ways to increased levels of nitrogen, and very recently a grass-feeding grasshopper was shown to perform best on low nitrogen diets, and do so where there was heavy grazing (Cease et al., 2012)

Finally, on mown plots the phenology of the insects may be interrupted (several times), and new flowerheads may no longer be infested by the weevils after a certain date (Tscharntke & Greiler, 1995). On pastures, in comparison, some flowerheads might remain and guarantee survival of the populations. The plot design might also explain the patterns in the three regions. Since the plots are not equally distributed between treatment groups with respect to land use intensity and type, management types might correlate with fertilization and lead to a general preference of unfertilized pastures by the herbivores.

This study suggests that increasing land use does not only lead to reduced plant species diversity and changed structural complexity of the vegetation, but has pronounced consistent effects on the higher trophic levels of a specialized tritrophic plant-herbivore-parasitoid system. Increasing human impacts increased parasitization rate, but reduced the probability of occurrence of the two weevil species and their parasitoid in the inflorescences of *P. lanceolata*. If land use intensification negatively influences specialized species of higher trophic levels even when the host plant is neutrally or positively affected, this will have important consequences for the preservation of multitrophic systems on nutrient poor grasslands.

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WEBSITE

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Figure legends

Figure 1 Proportion of fertilized and unfertilized plots (%) with occurrence of the three insect species *Mecinus labilis*, *Mecinus pascuorum* and *Mesopolobus incultus* for the three regions (Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin) in the year 2010. Significant differences at $P \leq 0.05$ are indicated by with different letters.

Figure 2 Proportion of meadows, mown pastures and pastures (%) with occurrence of the three insect species *Mecinus labilis*, *Mecinus pascuorum* and *Mesopolobus incultus* for the three regions (Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin) in the year 2010. Significant differences at $P \leq 0.05$ are indicated by different letters.

Figure 3 Influences of land use intensity and type on the tritrophic system, vegetation structure and plant species richness in the three regions and years. Possible interactions are marked with gray arrows and actual interactions with black arrows. Black arrows indicate significant results ($P < 0.05$) after correction with the false discovery rate (FDR) correction. Dashed arrows show possible trends. Land use intensity: plus signs indicate a positive influence while minus signs indicate a negative influence. Land use type: plus sign indicates a positive effect on meadows and a negative effect on pastures, while a minus sign indicates a negative effect on meadows and a positive effect on pastures.

Figure 4 Schematic interactions between land use, vegetation and the tritrophic system. Arrows show possible influences of the different levels.

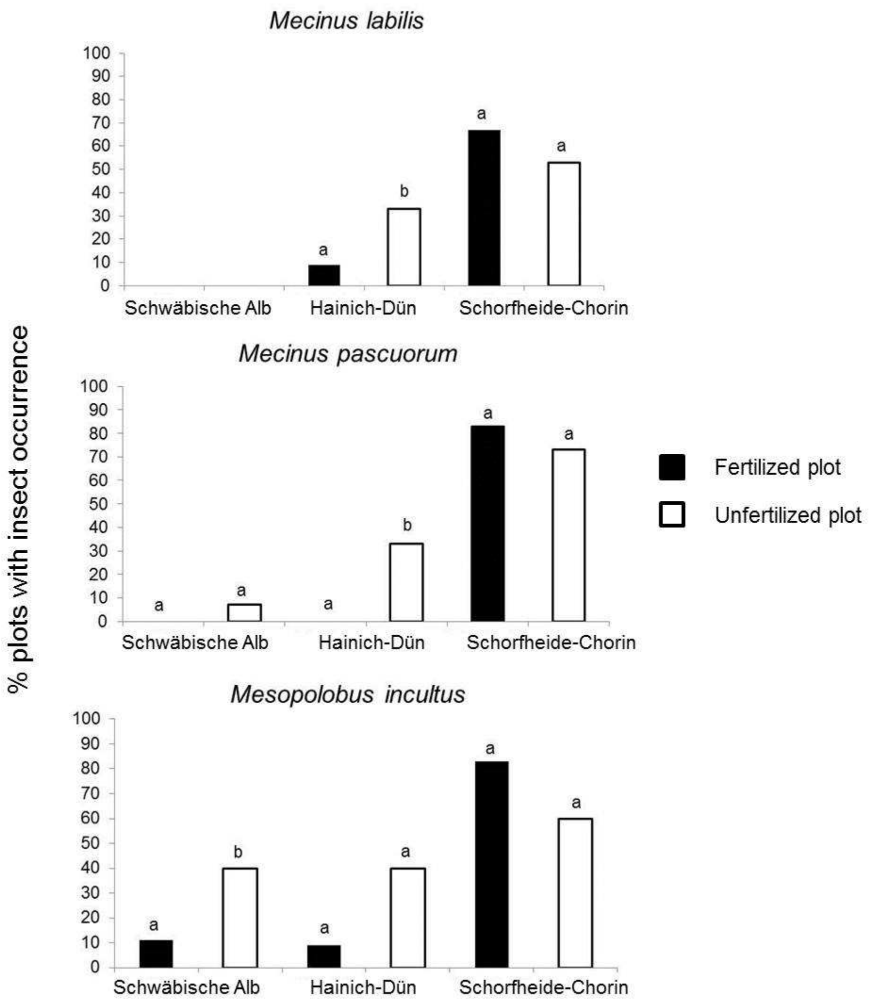
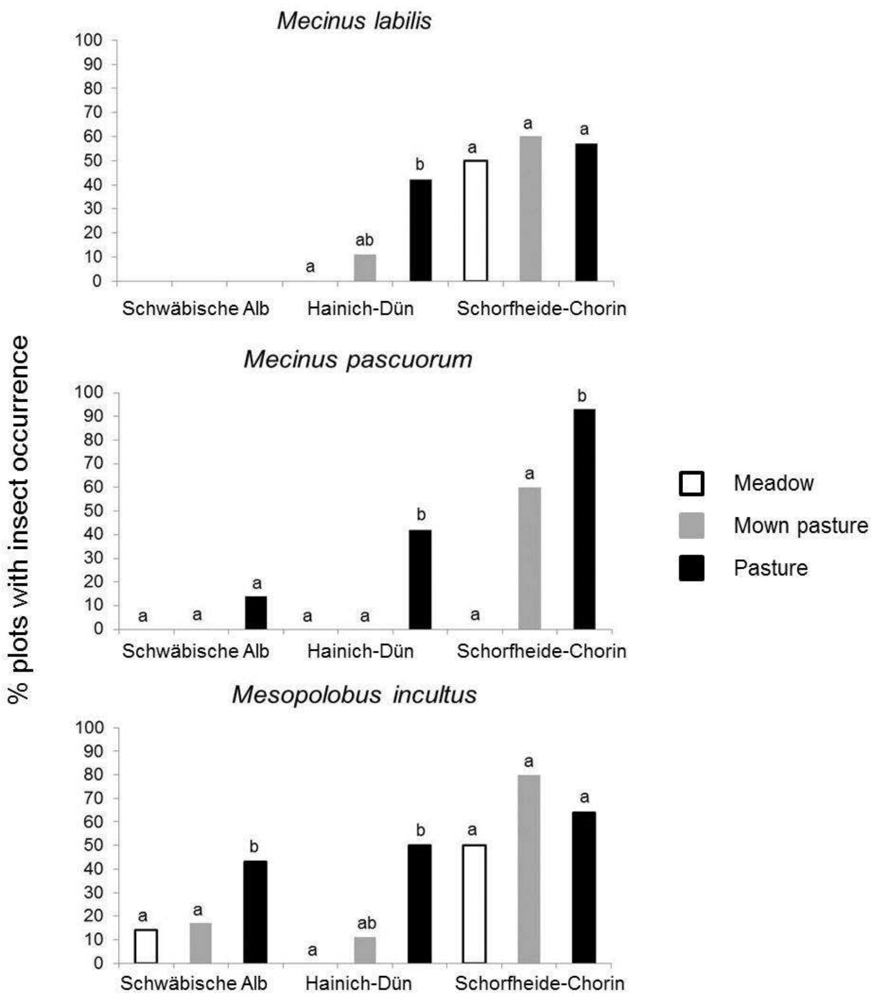

Mecinus labilis
Mecinus pascuorum
Mesopolobus incultus
% plots with insect occurrence
0
10
20
30
40
50
60
70
80
90
100
Schwäbische Alb
Hainich-Dün
Schorfheide-Chorin
a
b
Fertilized plot
Unfertilized plot

Figure 1



Mecinus labilis
Mecinus pascuorum
Mesopolobus incultus
% plots with insect occurrence
0
10
20
30
40
50
60
70
80
90
100
Schwäbische Alb
Hainich-Dün
Schorfheide-Chorin
a
ab
b
Meadow
Mown pasture
Pasture

Figure 2

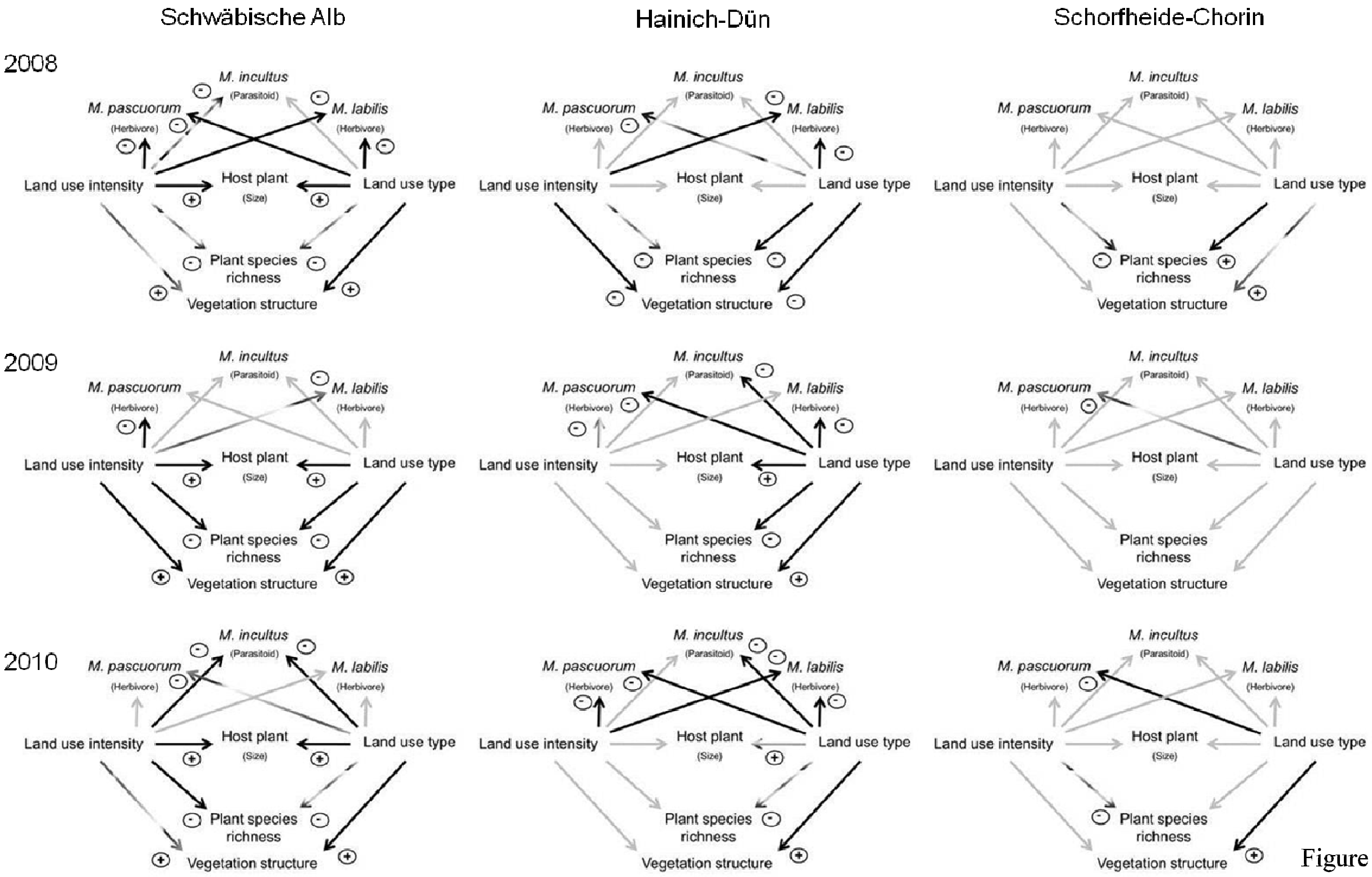

Schwäbische Alb
Hainich-Dün
Schorfheide-Chorin
2008
2009
2010
M. incultus
(Parasitoid)
M. pascuorum
(Herbivore)
M. labilis
(Herbivore)
Land use intensity
Host plant
(Size)
Land use type
Plant species
richness
Vegetation structure

Figure 3

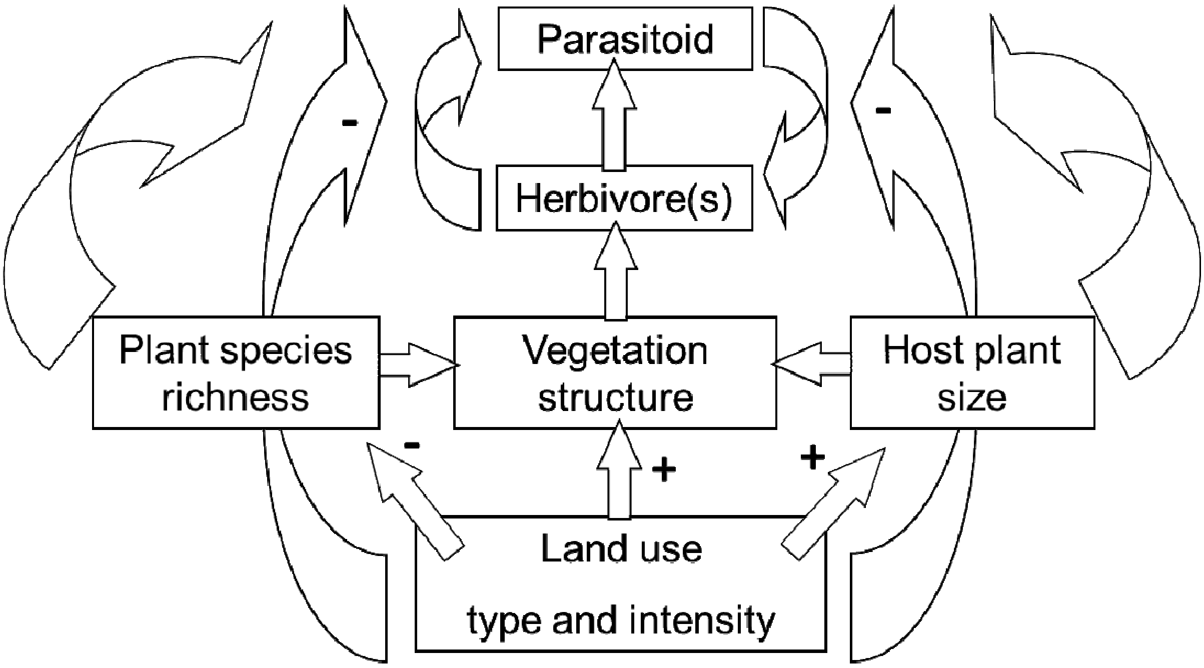
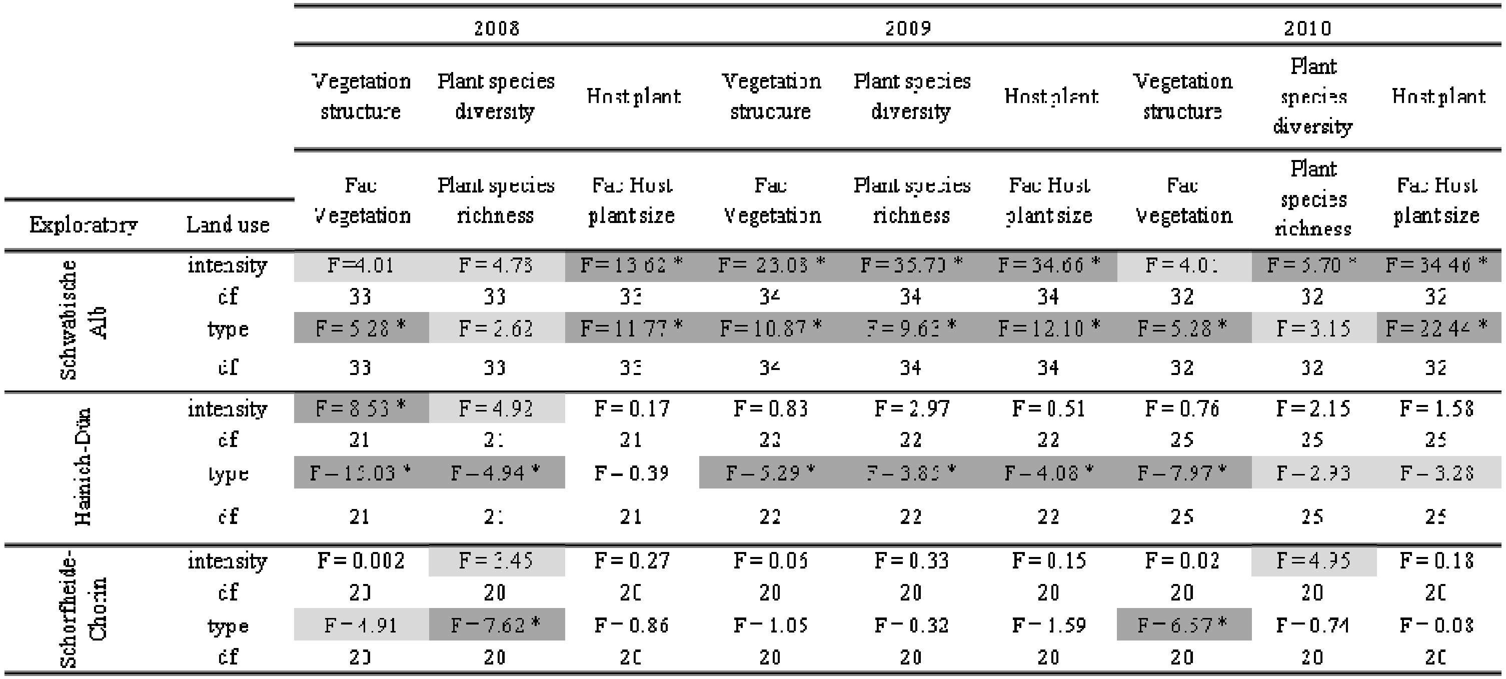

Parasitoid
Herbivore(s)
Plant species richness
Vegetation structure
Host plant size
Land use type and intensity
-
-
-
+
+

Figure 4

Table 1: One-way ANOVA models corrected by the false discovery rate (FDR) for the different vegetation and host plant parameters calculated for each land use intensity and type for the three regions and years. F-values in dark gray: significant difference after FDR at $P < 0.05$ (*).

F-values in light gray: effects significant by trend after FDR at $0.1 > P > 0.05$. Fac vegetation means calculated factor combining vegetation density (GMV) and maximum vegetation height. Fac host plant size means calculated factor combining rosette height and number of leaves.



		2008			2009			2010		
		Vegetation structure	Plant species diversity	Host plant	Vegetation structure	Plant species diversity	Host plant	Vegetation structure	Plant species diversity	Host plant
Exploratory	Land use	Fac Vegetation	Plant species richness	Fac Host plant size	Fac Vegetation	Plant species richness	Fac Host plant size	Fac Vegetation	Plant species richness	Fac Host plant size
Schwabische Alb	intensity	F=4.01	F=4.73	F=13.62 *	F=23.08 *	F=35.70 *	F=34.66 *	F=4.01	F=5.70 *	F=34.46 *
	df	33	33	33	34	34	34	32	32	32
	type	F=5.28 *	F=2.62	F=11.77 *	F=10.87 *	F=9.63 *	F=12.10 *	F=5.28 *	F=3.15	F=22.44 *
	df	33	33	33	34	34	34	32	32	32
Hainich-Dün	intensity	F=8.53 *	F=4.92	F=0.17	F=0.83	F=2.97	F=0.51	F=0.76	F=2.15	F=1.58
	df	21	21	21	22	22	22	25	25	25
	type	F=15.03 *	F=4.94 *	F=0.39	F=5.29 *	F=3.85 *	F=4.08 *	F=7.97 *	F=2.93	F=3.28
	df	21	21	21	22	22	22	25	25	25
Schorfheide-Chorin	intensity	F=0.002	F=3.45	F=0.27	F=0.05	F=0.33	F=0.15	F=0.02	F=4.95	F=0.18
	df	20	20	20	20	20	20	20	20	20
	type	F=4.91	F=7.62 *	F=0.86	F=1.05	F=0.32	F=1.59	F=6.57 *	F=0.74	F=0.08
	df	20	20	20	20	20	20	20	20	20

Table 2: Binary logistic regression models for the probability of occurrence of *Mecinus labilis, Mecinus pascuorum* and *Mesopolobus incultus* as affected by land use intensity and land use type for the three regions and years. Models are corrected by the false discovery rate (FDR). Given are the R^2 $_{\mathrm{Nagelkerke}}$- values. R^2 -values in dark gray: significant difference after FDR at $P < 0.05$ (*). R^2-values in light gray: effects significant by trend after FDR at $0.1 > P > 0.05$.

		2008			2009			2010		
		Mecinus labilis	*Mecinus pascuorum*	*Mesopolobus incultus*	*Mecinus labilis*	*Mecinus pascuorum*	*Mesopolobus incultus*	*Mecinus labilis*	*Mecinus pascuorum*	*Mesopolobus incultus*
Exploratory	Land use	Herbivores		Parasitoid	Herbivores		Parasitoid	Herbivores		Parasitoid
Schwäbische Alb	intensity	$R^2_{\mathrm{Nk.}} = 0.42$ *	$R^2_{\mathrm{Nk.}} = 0.41$ *	$R^2_{\mathrm{Nk.}} = 0.12$	$R^2_{\mathrm{Nk.}} = 0.15$	$R^2_{\mathrm{Nk.}} = 0.22$ *	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.10$	$R^2_{\mathrm{Nk.}} = 0.09$	$R^2_{\mathrm{Nk.}} = 0.26$ *
	type	$R^2_{\mathrm{Nk.}} = 0.44$ *	$R^2_{\mathrm{Nk.}} = 0.41$ *	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.11$	$R^2_{\mathrm{Nk.}} = 0.12$	$R^2_{\mathrm{Nk.}} = 0.11$	$R^2_{\mathrm{Nk.}} = 0.16$	$R^2_{\mathrm{Nk.}} = 0.14$	$R^2_{\mathrm{Nk.}} = 0.32$ *
Hainich-Dür.	intensity	$R^2_{\mathrm{Nk.}} = 0.33$ *	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.07$	$R^2_{\mathrm{Nk.}} = 0.27$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.36$ *	$R^2_{\mathrm{Nk.}} = 0.35$ *	$R^2_{\mathrm{Nk.}} = 0.04$
	type	$R^2_{\mathrm{Nk.}} = 0.46$ *	$R^2_{\mathrm{Nk.}} = 0.26$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.58$ *	$R^2_{\mathrm{Nk.}} = 0.39$ *	$R^2_{\mathrm{Nk.}} = 0.38$ *	$R^2_{\mathrm{Nk.}} = 0.43$ *	$R^2_{\mathrm{Nk.}} = 0.36$ *	$R^2_{\mathrm{Nk.}} = 0.36$ *
Schorfheide-Chorin	intensity	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.16$	$R^2_{\mathrm{Nk.}} = 0.02$	$R^2_{\mathrm{Nk.}} = 0.02$	$R^2_{\mathrm{Nk.}} = 0.07$
	type	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.09$	$R^2_{\mathrm{Nk.}} = 0.41$	$R^2_{\mathrm{Nk.}} = 0.11$	$R^2_{\mathrm{Nk.}} = 0.01$	$R^2_{\mathrm{Nk.}} = 0.53$ *	$R^2_{\mathrm{Nk.}} = 0.05$

Table 3 Generalized linear mixed models for the parasitization rate of *Mesopolobus incultus* on two weevils (*Mecinus labilis*, *Mecinus pascuorum)* calculated with land use intensity (A) and type (B) and nested for the three regions. Estimates (β) with standard errors (SE) are given for the minimal adequate model (evaluated by Akaike information criterion (AIC)). z-values in dark gray: significant difference at P ≤ 0.05.

A	Parasitization rate											
	2008				2009				2010			
Explanatory variables	β	SE	z value	P	β	SE	z value	P	β	SE	z value	P
Intercept	0.07	0.52	0.14	>0.05	-1.19	1.88	-0.53	>0.05	1.40	1.19	1.17	>0.05
Fertilized plots	1.98	0.25	7.94	<0.001	0.62	1.38	0.45	>0.05	0.10	0.63	0.13	>0.05
AIC	275.9				79.57				92.87			

B	Parasitization rate											
	2008				2009				2010			
Explanatory variables	β	SE	z value	P	β	SE	z value	P	β	SE	z value	P
Intercept	1.06	0.36	2.97	<0.01	-2.51	2.13	-1.18	>0.05	3.77	1.87	2.01	<0.05
Mown pasture	-0.42	0.37	-1.15	>0.05	3.20	2.70	1.18	>0.05	-1.61	1.58	-1.02	>0.05
Pasture	-0.58	0.33	-1.74	>0.05	1.50	1.36	1.12	>0.05	-2.62	1.50	-1.77	>0.05
AIC	337.6				80.17				90.30			

Table 4: Fit statistics for the structural equation models for the three regions. Schwäbische Alb (AL), Hainich-Dün (HA), and Schorfheide-Chorin (SC); RMSEA = root mean square error of approximation, and TLNNFI = Tucker Lewis non-normed fix index.

	N	χ^2 (P)	RMSEA	TLNNFI
2008				
AL	340	114.4 (<0.0001)	0.140	0.839
HA	207	55.5 (<0.0001)	0.115	0.877
SC	201	63.0 (<0.0001)	0.126	0.884
2009				
AL	100	57.9 (<0.0001)	0.170	0.852
HA	68	38.8 (0.0007)	0.154	0.891
SC	63	22.3 (0.1002)	0.089	0.948
2010				
AL	93	24.1 (0.0041)	0.135	0.887
HA	77	30.4 (0.0004)	0.177	0.741
SC	66	22.4 (0.0078)	0.151	0.828

Table A 1: Number of plots used to investigate land use type in the three different regions and over three years.

	2008			2009			2010		
	Meadow	Mown pasture	Pasture	Meadow	Mown pasture	Pasture	Meadow	Mown pasture	Pasture
Exploratory	Plot number								
Schwäbische Alb	14	4	16	15	6	13	13	7	13
Hainich-Dün	5	6	11	4	7	12	5	9	12
Schorfheide-Chorin	2	7	12	2	5	14	2	5	14

Table A 2: Number of plots used to investigate land use intensities in the three different regions and over three years.

	2008		2009		2010	
	Fertilized	Unfertilized	Fertilized	Unfertilized	Fertilized	Unfertilized
Exploratory	Plot number					
Schwäbische Alb	17	17	19	15	18	15
Hainich-Dün	11	11	8	15	11	15
Schorfheide-Chorin	11	10	7	14	6	15

Table A3: Means and standard deviations for land use intensity and type and the parameters of vegetation structure, plant species richness, and host plant in all three regions and years.

			2008					2009					2010				
			Vegetation structure		Plant species diversity	Host plant		Vegetation structure		Plant species diversity	Host plant		Vegetation structure		Plant species diversity	Host plant	
Exploratory	Land use		Vegetation height	GMV	Plant species richness	Rosette height	Leave number	Vegetation height	GMV	Plant species richness	Rosette height	Leave number	Vegetation height	GMV	Plant species richness	Rosette height	Leave number
Schwäbische Alb	intensity (mean ± sd)	unfertilized	61.84 ± 20.35	0.12 ± 0.03	22.24 ± 7.12	15.03 ± 7.99	7.48 ± 3.52	39.65 ± 9.89	0.08 ± 0.02	27.40 ± 6.07	10.40 ± 5.11	7.40 ± 2.17	53.11 ± 16.24	0.11 ± 0.03	23.53 ± 6.69	10.51 ± 4.27	5.17 ± 1.95
		fertilized	61.59 ± 30.71	0.13 ± 0.06	17.94 ± 3.86	22.96 ± 7.36	10.61 ± 3.18	58.00 ± 14.79	0.14 ± 0.04	17.05 ± 4.01	27.2 ± 8.72	9.05 ± 3.30	65.32 ± 24.29	0.15 ± 0.05	18.56 ± 5.29	19.32 ± 4.63	8.44 ± 2.21
	type (mean ± sd)	meadows	63.55 ± 31.53	0.13 ± 0.07	17.92 ± 3.92	24.65 ± 7.22	11.35 ± 3.12	58.76 ± 11.46	0.14 ± 0.03	17.27 ± 4.40	27.25 ± 7.86	9.60 ± 3.49	67.87 ± 23.18	0.15 ± 0.05	17.62 ± 3.60	19.28 ± 5.06	8.46 ± 2.45
		mown pastures	54.10 ± 32.68	0.11 ± 0.05	17.50 ± 5.07	17.93 ± 4.90	10.93 ± 4.48	52.50 ± 21.23	0.11 ± 0.04	21.00 ± 4.67	20.72 ± 8.67	7.89 ± 2.44	69.10 ± 22.64	0.15 ± 0.05	22.14 ± 7.03	17.81 ± 5.69	8.48 ± 1.85
		pastures	62.11 ± 19.85	0.13 ± 0.03	22.35 ± 6.94	14.92 ± 8.01	6.83 ± 2.50	38.49 ± 9.70	0.8 ± 0.02	26.92 ± 7.47	10.77 ± 8.97	7.05 ± 1.77	46.64 ± 11.94	0.10 ± 0.02	23.31 ± 7.23	10.00 ± 3.38	4.64 ± 1.13
Hainich-Dün	intensity (mean ± sd)	unfertilized	45.95 ± 16.63	0.11 ± 0.03	20.39 ± 7.51	11.45 ± 4.69	11.64 ± 6.38	61.71 ± 17.82	0.12 ± 0.04	23.73 ± 7.95	18.93 ± 6.66	8.11 ± 3.51	46.82 ± 16.79	0.11 ± 0.04	20.67 ± 9.46	15.29 ± 4.59	7.98 ± 3.24
		fertilized	33.79 ± 8.01	0.08 ± 0.01	14.67 ± 1.94	10.18 ± 2.33	14.17 ± 3.65	48.65 ± 28.33	0.11 ± 0.06	18.50 ± 4.21	17.54 ± 7.20	8.96 ± 5.61	49.57 ± 12.48	0.13 ± 0.03	16.27 ± 3.38	21.24 ± 12.81	8.82 ± 3.32
	type (mean ± sd)	meadows	29.26 ± 6.48	0.07 ± 0.01	14.60 ± 2.07	8.68 ± 1.81	14.30 ± 4.71	36.17 ± 16.79	0.08 ± 0.01	20.25 ± 2.87	12.00 ± 1.94	11.42 ± 7.57	47.07 ± 14.46	0.11 ± 0.02	17.60 ± 3.98	23.14 ± 17.92	8.00 ± 1.72
		mown pastures	36.03 ± 7.77	0.08 ± 0.01	14.71 ± 4.65	11.47 ± 1.74	12.40 ± 2.87	72.36 ± 28.51	0.15 ± 0.05	17.00 ± 4.24	24.62 ± 5.21	7.15 ± 1.68	58.89 ± 15.96	0.15 ± 0.04	14.78 ± 2.77	19.70 ± 7.11	9.70 ± 3.52
		pastures	50.29 ± 16.20	0.12 ± 0.03	22.10 ± 6.94	11.68 ± 5.29	12.05 ± 7.25	55.31 ± 12.83	0.11 ± 0.03	25.33 ± 7.97	17.00 ± 6.72	8.14 ± 7.24	14.20 ± 8.93	0.09 ± 0.02	22.33 ± 9.83	14.17 ± 3.55	7.45 ± 3.36
Schorfheide-Chorin	intensity (mean ± sd)	unfertilized	51.55 ± 18.18	0.14 ± 0.04	15.20 ± 3.52	13.80 ± 7.44	15.02 ± 3.71	32.29 ± 20.51	0.08 ± 0.04	9.29 ± 2.40	11.05 ± 6.98	6.10 ± 2.15	38.31 ± 20.85	0.09 ± 0.04	11.53 ± 2.97	15.22 ± 3.46	5.96 ± 2.09
		fertilized	55.30 ± 37.41	0.13 ± 0.07	12.00 ± 4.29	13.64 ± 8.86	16.07 ± 6.14	31.24 ± 10.86	0.07 ± 0.02	9.29 ± 2.56	12.10 ± 3.88	6.86 ± 1.73	35.50 ± 17.59	0.09 ± 0.04	8.50 ± 2.35	15.72 ± 4.70	5.95 ± 2.09
	type (mean ± sd)	meadows	110.20 ± 26.73	0.21 ± 0.06	22.00 ± 1.41	22.50 ± 2.97	9.80 ± 1.70	18.67 ± 4.72	0.06 ± 0.01	8.00 ± 4.24	10.50 ± 0.24	3.50 ± 0.71	74.00 ± 16.02	0.16 ± 0.04	11.50 ± 3.54	14.00 ± 3.30	5.84 ± 1.65
		mown pastures	50.67 ± 26.09	0.14 ± 0.06	12.29 ± 4.15	13.69 ± 8.53	17.60 ± 6.20	23.80 ± 10.59	0.06 ± 0.01	9.20 ± 2.60	8.87 ± 4.70	7.53 ± 1.22	36.20 ± 1949	0.09 ± 0.03	9.20 ± 2.28	15.20 ± 4.41	6.47 ± 0.38
		pastures	45.73 ± 21.39	0.12 ± 0.05	12.83 ± 2.76	12.67 ± 7.74	15.35 ± 4.01	36.74 ± 19.02	0.09 ± 0.04	9.50 ± 2.25	12.43 ± 6.75	6.33 ± 1.98	32.76 ± 14.94	0.08 ± 0.03	11.07 ± 3.32	15.62 ± 3.78	5.93 ± 2.26

Table A4: Number of plots with insect occurrence in the different land use categories. Given are total plot number and plot number with the occurrence of the three insect species *Mecinus labilis*, *Mecinus pascuorum* and *Mesopolobus incultus* for land use intensity and type in all three regions and years.

			2008				2009				2010			
				Herbivores		Parasitoid		Herbivores		Parasitoid		Herbivores		Parasitoid
Exploratory	Landuse		Total number of plots	*Mecinus labilis*	*Mecinus pascuorum*	*Mesopolobus incultus*	Total number of plots	*Mecinus labilis*	*Mecinus pascuorum*	*Mesopolobus incultus*	Total number of plots	*Mecinus labilis*	*Mecinus pascuorum*	*Mesopolobus incultus*
Schwäbische Alb	intensity	unfertilized	17	9	6	12	15	3	1	3	15	0	1	6
		fertilized	17	1	0	8	19	1	0	3	18	0	0	2
	type	meadows	14	1	0	6	15	1	1	2	13	0	0	1
		mown pastures	4	0	0	2	6	1	0	1	7	0	0	1
		pastures	16	9	6	12	13	2	0	3	13	0	1	6
Hainich-Dün	intensity	unfertilized	11	7	8	12	15	6	6	8	15	5	5	6
		fertilized	11	1	3	5	8	2	0	3	11	1	0	1
	type	meadows	5	0	2	2	4	2	0	2	5	0	0	0
		mown pastures	6	2	2	4	7	0	0	1	9	1	0	1
		pastures	11	6	7	9	12	6	6	8	12	5	5	6
Schorfheide Chorin	intensity	unfertilized	10	7	9	9	14	1	1	0	15	8	11	7
		fertilized	11	6	7	8	7	0	0	0	6	4	5	5
	type	meadows	2	2	2	2	2	0	0	0	2	1	0	1
		mown pastures	7	3	6	4	5	0	0	0	5	3	3	4
		pastures	12	8	8	11	14	1	1	0	14	8	13	7

Chapter V

Fertilizer application decreases insect abundance on *Plantago lanceolata:* a large-scale experiment in three geographic regions

Chapter V

Fertilizer application decreases insect abundance on *Plantago lanceolata:* a large-scale experiment in three geographic regions

Christine Hancock*[1], Nicole Wäschke[2], Uta Schumacher[3], Karl Eduard Linsenmair[1], Torsten Meiners[2], Elisabeth Obermaier[1, 4]

[1] Department of Animal Ecology and Tropical Biology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

[2] Institute of Biology, Applied Zoology / Animal Ecology, Freie Universität Berlin, Haderslebener Strasse 9, 12163 Berlin, Germany

[3] Institute of Biochemistry and Biology, University of Potsdam, Biodiversity Exploratory Schorfheide-Chorin, Hoher Steinweg 5-6, 16278 Angermünde Germany

[4] present address: Ecological Botanical Gardens, University of Bayreuth, 95440 Bayreuth, Germany

*Corresponding author. Present address:
Department of Animal Ecology and Tropical Biology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
Tel.: + 49 (0) 171-76 49 916
E-mail address: christine-herbst@t-online.de

Abstract

Humans have substantially altered the nitrogen cycle of ecosystems through application of agricultural fertilizer. Fertilization may not only affect plant species diversity, but also insect dynamics by altering plant nitrogen supplies. We investigated the effect of experimental fertilization on the vegetation, the ribwort plantain a focal plant, and higher trophic levels on differently managed grasslands throughout Germany.

Over a period of two years, we examined two specialist herbivores and their parasitoid on *Plantago lanceolata* L. and the composition and structure of the surrounding vegetation. Over 70 sites in three geographic regions within the large-scale project German Biodiversity Exploratories were included in the study. The model system consisted of the host plant *P. lanceolata* L., the monophagous weevils *Mecinus labilis* Herbst and *M. pascuorum* Gyllenhal, and their parasitoid *Mesopolobus incultus* Walker.

Fertilization decreased plant species richness and host plant abundance, whereas it enhanced total vegetation growth. The increased size and heightened leaf nitrogen content did not improve herbivore performance. On the contrary the abundance of the two herbivores was decreased by fertilization. The parasitoid depended on the abundance of one of its hosts, *M. pascuorum* (positively density-dependent). Reduced herbivore abundance due to fertilization might be explained by a lower abundance of the host plant, a lower stalk number and by changed patterns of host localisation within higher vegetation. Fertilization negatively affected the third trophic level by cascading up via host abundance. The relationships between fertilization, surrounding vegetation and the tritrophic system were measured throughout the three regions and over the two year period. Our findings present consequences of intensification for a plant-herbivore-parasitoid system and may have significant implications for the conservation of multitrophic systems in managed grasslands.

Keywords: Fertilization, nitrogen, tritrophic interaction, plant species richness, grassland management, vegetation structure

Introduction

Intensification of land use is one major factor explaining why many species of the German flora and fauna are endangered. Agricultural intensification in the form of fertilization represents one important threat to plant species richness (Gough et al. 2000; Rajaniemi 2002). Anthropogenic conversion of natural habitats to agricultural areas has increased dramatically, especially in the last century. Today agricultural land has become one of the largest terrestrial biomes (Asner et al. 2004). Increasing population growth and a growing demand in terms of living standards lead to an intensification of agriculture via the use of fertilizer, pesticides and expanded irrigation systems (Matson et al. 1997). It is becoming increasingly evident that these changes are not beneficial to biodiversity. To judge the impact of human activities on ecosystems and on biodiversity it is important to investigate the interactions which take place in these systems and to know how ecosystems function. In the past few years the number of studies of complex interactions in which three or more trophic levels are involved is increasing, because these reflect the natural conditions more adequately (Tscharntke and Hawkins 2002).

However, still little is known about how an increase in land use intensity influences biotic interactions (van der Putten et al. 2004). Unfertilized dry and wet grasslands belong to habitats showing the highest richness in plant species (Ellenberg 1996). However, with the development of mineral fertilizers many of these systems were converted into intensively used grassland with high livestock density or several harvests per year. Plant species richness decreases with increasing soil fertility and species composition is negatively affected (Ellenberg 1996). Therefore the amount of fertilizer applied within a habitat often represents the degree of land use intensity at this site. A negative correlation between land use intensity and plant species diversity is already well documented for many ecosystems (Baessler and Klotz 2006; Smart et al. 2006). The effect of fertilization on higher trophic level organisms and the underlying mechanisms, however, still remain mostly unknown.

Not only plant species richness, but also the single plant individual is affected by fertilizer application. Regarding individual plant performance, fertilization usually results in an increase in leaf nitrogen content (Davidson and Potter 1995) and in the biomass of the focal plant, often in combination with a change in the vertical distribution of the biomass of this plant or of the whole plot. Fertilization not only increases biomass and plant growth, but also the density of plants (Gratton and Denno 2003). Fertilization therefore results not only in a change in plant quality and quantity of the individual (host) plant, but also in a changed structural complexity of the entire vegetation (Bobbink et al. 1988; Hartley et al. 2003; Gratton and Denno 2003) with possibly profound consequences for phytophagous insects and their natural enemies.

Plant nitrogen content and secondary metabolites might be affected by fertilization as well. Iridoid glycosides (aucubin and catalpol) are important secondary metabolites in *P. lanceolata*. Jarzomski et al. (2000) documented that nutrient availability determined iridoid glycoside concentration to a greater extent than herbivory. However, fertilization is not the only factor influencing plant-defensive chemistry, but belowground herbivores can also change the aucubin content in shoots of *P. lanceolata* (Wurst et al. 2004).

Fertilization is known to increase food plant quality for herbivores and hence herbivore abundance (Price et al. 1980; Gratton and Denno 2003; Stiling and Moon 2005) and in some cases also herbivore species richness (Hartley et al. 2003). Increased plant nitrogen (N) should enhance herbivore fitness by relieving protein limitation, thus increasing herbivorous insect populations (Cease et al. 2012). Nevertheless higher plant nitrogen content may not always be beneficial for herbivorous insects (Fischer and Fiedler 2000; Cease et al. 2012).

Fertilization was also shown to affect the third trophic level. Populations of natural enemies together with their prey, as well as parasitization rates sometimes increase after fertilizer application (Bentz et al. 2006; Krauss et al. 2007). Nutrient availability was shown to alter patterns of parasitoid related mortality (Moon et al. 2000; Yarnes and Boecklen 2006) as well as fecundity (Kaneshiro and Johnson 1996).

In summary, fertilization may affect herbivore abundance and diversity directly via altered host plant quality and availability and indirectly via increased structural complexity and plant species diversity. Interacting effects of features of the vegetation with host-parasitoid systems could further alter more complex interactions in a multitrophic context of bottom up and top down effects (Gratton and Denno 2003).

Three different regions, in the northern, middle and southern part of Germany, were chosen to investigate the impact of experimental fertilization on the tritrophic system of *Plantago lanceolata* and its surrounding vegetation. The three regions selected differ in their soil and vegetation types and the amount of precipitation they receive. Thus they allow the investigation of fertilizer application on the same tritrophic system under different abiotic and biotic conditions (Fischer et al. 2010). The ribwort plantain *Plantago lanceolata* L. (Plantaginaceae) is an indicator species for a cultivated landscape (Ellenberg et al. 1992). It grows in a very broad range of ground conditions (Dierschke and Briemle 2002), which makes *P. lanceolata* a perfect model for investigating influences of fertilization on trophic interactions. It is widespread in the regions investigated and hosts two herbivorous weevils *Mecinus labilis* and *M. pascuorum* (both Coleoptera: Curculionidae) and their natural enemy *Mesopolobus incultus* (Hymenoptera: Pteromalidae) (Mohd Norowi et al. 2000).

In this study we address the following questions: How does experimental fertilization affect (1) the vegetation structure and plant species richness, (2) the host plant abundance and quality as well as (3) the two weevil species and their common parasitoid?

Material and Methods

Study site

In the context of the German Biodiversity Exploratories (http://www.biodiversity-exploratories.de) we investigated experimental grassland plots in three geographic regions (exploratories), Schorfheide-Chorin (Brandenburg), Hainich-Dün (Thuringia) and Schwäbische Alb (Baden Württemberg) (Fischer et al. 2010). In each region there are three different land use types (meadows, mown pastures and pastures) and two different land use intensities (fertilized and unfertilized) which both were cultivated by the farmers (Fischer et al. 2010). For a detailed description of the Biodiversity Exploratories see Fischer et al. (2010).

Experimental design

In every region, 50 experimental grassland plots have been determined by the large-scale project (Fischer et al. 2010). The number of plots we investigated were 76 in 2009 (34 plots Schwäbische Alb, 21 plots Hainich-Dün and Schorfheide-Chorin) and 81 in the year 2010 (33 plots Schwäbische Alb, 25 plots Hainich-Dün and 23 plots Schorfheide-Chorin). Every plot measured 50 m x 50 m with a north-south orientation. The two investigated subplots (6m x 6m) were situated in one corner of the experimental plots. We selected one control and one fertilized subplot. The latter was fertilized in April and October 2009 and in April 2010 with 200 g per m² (N = 6.67%, P_2O_2 = 0.85%, K_2O = 0.86%) of an organic fertilizer (Agrobiosol®, SW-Düngesysteme, Wolfenbüttel). The control subplot was not experimentally fertilized.

Study system

Plantago lanceolata L., the ribwort plantain, is a generalist herb of cosmopolitan distribution (Van der Aart and Vulto 1992) that can grow up to 50 cm and forms a rosette. It blossoms from May to September (Schmeil and Fitschen 2003). Rosettes facilitate vegetative propagation (Wu and Antonovics 1975). According to Ellenberg (1996) and Opitz v. Boberfeld (1994) *P. lanceolata* grows both in wet and dry habitats.

Mecinus labilis and *M. pascuorum* live monophagously on *P. lanceolata*. Both weevil species hibernate as adults and become active in May (Mohd Norowi et al. 1999). Females oviposit on newly-formed seedheads in June and July (Dickason 1968). The hatched larvae bore into a seedhead where they feed and develop in individual seeds (Mohd Norowi et al. 1999). The larvae are fully developed by July and pupate in the seedheads (Mohd Norowi et al. 1999). Adults emerge from late August to September and move to the hibernation sites. *Mecinus labilis* can mainly be found on mown and dry pastures. Adults have a size of 1.7 to 2.2 mm (Lohse 1983). The seed-feeder *M. pascuorum* lives on dry grasslands and has a size of 1.5 to 2.1 mm.

Mesopolobus incultus Walker is a generalist parasitoid that parasitizes different insect orders (http://www.nhm.ac.uk/ research-curation/ research/ projects/ chalcidoids/ [December 2008]). This pteromalid wasp is a solitary species. Its only hosts on *P. lanceolata* are the two weevil species described above. No details of ovipositional behavior are available and most parts of its life cycle are unknown. *Mesopolobus incultus* parasitizes the larvae of the weevils during summer (Mohd Norowi et al. 2000), and hatches from the seedheads at the same time as the adult beetles (C. Hancock, pers. observ.).

Sampling design

Host plant and vegetation parameters

Between May and June in the years 2009 and 2010 host plant abundance was determined in a radius of 1 m around three selected *P. lanceolata* plants in each subplot. Host plant parameters like rosette diameter (cm) and above ground biomass (Bowers and Stamp 1992) were recorded. Metabolite composition of leaves varies with factors like leaf age, genotype (Bowers and Stamp 1992), and herbivory (Stamp and Bowers 2000). Therefore, we sampled a mixture of leaves from 10 different individuals randomly, by walking a pattern along the control and treatment subplots in 2009. Carbon and nitrogen content of the dried and ground plant material was analysed by a C/N analyser (Euro EA 3000 Elementalanalyser, EuroVector, Milan, Italy).

Vegetation structure was defined by height (maximum height of forbs or grasses) and ground cover of grasses (%) of the investigated vegetation. Along with vegetation height we also calculated the generalized mean vegetation height (GMV), which is a measure for vegetation density (calculating the vertical vegetation cover for each height class). The parameter GMV was correlated with the vegetation height in this study like in many previous studies (e.g., Obermaier et al. 2008; Randlkofer et al. 2009). To avoid collinearity of variables we excluded the GMV and only used the vegetation height to represent the vegetation structure. Plant species diversity was calculated as plant species richness. We recorded plant species richness by counting the number of forbs in a radius of 15 cm around the investigated host plant.

Invertebrate sampling

Insects were collected by sampling 60 inflorescences in each subplot. The collection was conducted between August and September 2009 and 2010 in all three regions. The inflorescences were stored per subplot in plastic boxes (17.0 x 12.5 x 5.6 cm) with gauze cover (0.12 mm) under constant conditions (11:13 LD, temperature: 22°C, 50% rH). After hatching insects were identified and counted.

Statistical Analyses

The influence of fertilization on vegetation and host plant parameters, as well as on the abundance of the two herbivores and the parasitoid was analyzed using linear mixed-effects models. All calculations were performed using R (Version 2.15.1; R Development Core Team, 2012). We calculated mean values per subplot for vegetation and host plant parameters. For abundance data (2009 and 2010), nitrogen and carbon (only 2009) we received one subplot value each per plot. Region and plot number were nested and used as random effects for fertilized and unfertilized subplot separately. Treatment (fertilized and unfertilized) was used as fixed factor. Normal distribution of response variables was checked by looking at the residuals (Zuur et al. 2007; 2010). Response variables were transformed if necessary to fulfill model assumptions. Percentages were arcsin square root transformed. Models were calculated by the lmer function with Laplace approximation (package lme4 version 0.999375-42), the zeroinfl function (package pscl version 1.04.4) or by the lme function with the maximum likelihood method (package nlme version 3.1-102). For the response variables vegetation and host plant parameters, nitrogen and carbon we used the Gaussian error distribution (link=identity). For the zero-inflated response variables weevil and parasitoid abundance we used a zero-inflated regression model (Zeileis et al. 2007).

Results

Effect of fertilization on the vegetation

Fertilization increased vegetation height 3 months after the first application (2009: Intercept: value = 50.29, standard error = 0.01, df = 75, P < 0.001; treatment control: value -3.77, standard error = 1.14, df = 75, P < 0.01) (Fig. 1). This effect was even stronger in the second year (2010: Intercept: value = 57.05, standard error = 5.31, df = 80, P < 0.001; treatment control: value -8.88, standard error = 1.54, df = 80, P < 0.001). Fertilization at the same time reduced forbs in favor of grasses which showed a higher cover in the fertilized compared to the unfertilized subplots in the first year (Tab. 1). This effect is supported by looking at the number of plant species in the same radius. Plant species richness was higher in unfertilized than in fertilized subplots (Fig. 1). The relationship was significant, however, only in the second year of the fertilizer application (2009: Intercept: value = 13.97, standard error = 2.62, df = 75, P < 0.001; treatment control: value 0.18, standard error = 0.38, df = 75, P > 0.05; 2010: Intercept: value = 10.96, standard error = 1.72, df = 80, P < 0.001; treatment control: value 3.17, standard error = 0.58, df = 80, P < 0.001).

Effect of fertilization on the host plant

Fertilization reduced the abundance of the host plant *P. lanceolata* (2009: Intercept: value = 25.25, standard error = 4.86, df = 75, P < 0.001; treatment control: value 4.91, standard error = 3.99, df = 75, P > 0.05; 2010: Intercept: value = 2.16, standard error = 0.13, df = 80, P < 0.001; treatment control: value 0.46, standard error = 0.10, df = 80, P < 0.001). After the second year of fertilization the abundance of *P. lanceolata* in the control subplots was higher compared to the abundance in the fertilized subplots (Fig. 2). Rosette diameter on the other hand was larger in fertilized subplots than in the control. The effect was stronger in 2010 compared to 2009. Stalk number was nearly the same in both subplots in the first year, but lower in the fertilized subplot in 2010. Individual host plant biomass was lower in the control than in the fertilizer subplots in both years of the study (Tab. 1). Nitrogen content of leaves of fertilized *P. lanceolata* plants was higher compared to the leaves of the control subplots (Intercept: value = 2.30, standard error = 0.11, df = 72, P < 0.001; treatment control: value -0.12, standard error = 0.05, df = 72, P < 0.01). Carbon content on the other hand was not influenced by fertilization (Intercept: value = 43.16, standard error = 0.51, df = 72, P < 0.001; treatment control: value 0.06, standard error = 0.30, df = 72, P > 0.05).

Effects of fertilization on the herbivores

The abundances per subplot of the herbivores *M. labilis* (2009: Intercept ß = -3.696, SE = 0.489, z-value = -7.561, P < 0.001, df = 75; Treatment control (TC) ß = 0.760, SE = 0.233, z-value = 3.257, P < 0.01, AIC = 196.2; 2010: Intercept ß = -2.088, SE = 0.333, z-value = -6.275, P < 0.001, df = 80, herbivore incidence n = 17, no incidence n = 64; TC ß = 0.768, SE = 0.184, z-value = 4.169, P < 0.001, herbivore incidence n = 28, no incidence n = 53, AIC = 253.5; Tab. 2), and *M. pascuorum* (2009: Intercept ß = -8.542, SE = 1.896, z-value = -4.505, P < 0.001; TC ß = 1.344, SE = 0.188, z-value = 7.143, P < 0.001, AIC = 259.3; 2010: Intercept ß = -2.871, SE = 0.792, z-value = -3.627, P < 0.001; TC ß = 1.124, SE = 0.106, z-value = 10.588, P < 0.001, AIC = 488.9; Tab. 2) decreased with fertilization in both years (Fig. 3). We always found a higher abundance of the species in unfertilized than in fertilized subplots.

Additionally different host plant covariates together with the fertilizer treatment were included in the models. We tested these models for both weevil species separately. We present representative data from 2010 because they show stronger effects due to a longer period of fertilization. First we tested the effect of number of stalks of *P. lanceolata* on *M. labilis* (2010: Intercept ß = -1.925, SE = 0.343, z-value = -5.617, P < 0.001, df = 80; TC ß = 0.837, SE = 0.200, z-value = 4.192, P < 0.001, stalk number ß = -0.134, SE = 0.137, z-value = 0.983, P > 0.05, AIC = 254.6) and *M. pascuorum* (2010: Intercept ß = -2.575, SE = 0.717, z-value = -3.594, P < 0.001, df = 80; TC ß = 1.119, SE = 0.107, z-value = 10.494, P < 0.001, stalk number ß = -0.198, SE = 0.101, z-value = -1.970, P = 0.05, AIC = 487.2). The number of stalks did not influence the abundance of *M. labilis. M. pascuorum* was influenced by the treatment as well as the stalk number. Second we tested the effect of host plant abundance on *M. labilis* (2010: Intercept ß = -2.094, SE = 0.335, z-value = -6.256, P < 0.001, df = 80; TC ß = 0.785, SE = 0.215, z-value = 3.655, P < 0.001, host plant abundance ß = -0.001, SE = 0.004, z-value = -0.159, P > 0.05, AIC = 255.5) and *M. pascuorum* (2010: Intercept ß = -2.834, SE = 0.761, z-value = -3.724, P < 0.001, df = 80; TC ß = 1.010, SE = 0.113, z-value = 8.903, P < 0.001, host plant abundance ß = 0.010, SE = 0.003, z-value = 2.527, P = 0.01, AIC = 484.2). *Mecinus labilis* was only influenced by the treatment but not by the host plant abundance. *Mecinus pascuorum* was positively affected by the abundance of the host plant. Third we tested the effect of maximum vegetation height on *M. labilis* (2010: Intercept ß = -1.081, SE = 0.639, z-value = -1.691, P > 0.05, df = 80; TC ß = 0.610, SE = 0.203, z-value = 3.003, P < 0.01, maximum vegetation height ß = 0.019, SE = 0.010, z-value = -1.830, P > 0.05, AIC = 252) and *M. pascuorum* (2010: Intercept ß = -6.795, SE = 1.403, z-value = -4.844, P < 0.001, df = 80; TC ß = 1.670, SE = 0.156, z-value = 10.717, P < 0.001, maximum vegetation height ß = 0.057, SE = 0.010, z-value = 5.664, P < 0.001, AIC = 459.3). *Mecinus labilis* was neither influenced by the host plant nor by the surrounding vegetation. Both weevil species were affected by the fertilization in every model. The host plant and the surrounding vegetation additionally influenced *M. pascuorum*. Besides the effect of the fertilization vegetation height seems to have the strongest effect on *M. pascuorum* based on the AIC value of this model.

Effects of fertilization on the parasitoid

Abundance of the parasitoid *M. incultus* (2009: Intercept ß = -3.787, SE = 2.082, z-value = -1.819, P > 0.05; TC ß = 0.571, SE = 0.113, z-value = 5.041, P < 0.001, AIC = 489.1; 2010: Intercept ß = -1.432, SE = 0.380, z-value = -3.769, P < 0.001; TC ß = 0.225, SE = 0.079, z-value = 2.857, P < 0.01, AIC = 647.0; Tab. 2) decreased with fertilization in both years (Fig. 3). We always found a higher abundance of *M. incultus* in unfertilized compared to fertilized subplots.

When host abundance of the two herbivores together with the treatment were included in the model, the abundance of the parasitoid was positively influenced only by its prey *M. pascuorum* (2009: Intercept ß = -3.113, SE = 1.826, z-value = -1.705, P ≤ 0.05; *M. pascuorum* ß = 0.101, SE = 0.016, z-value = 6.218, P < 0.001, AIC = 329.2). In this model neither the treatment nor *M. labilis* had significant effects on the parasitoid (2009: Treatment control ß = 0.033, SE = 0.158, z-value = -2.139, P > 0.05; *M. labilis* ß = -0.037, SE = 0.053, z-value = -0.709, P > 0.05). Herbivore abundance seems to be more important for the parasitoid than the treatment because this model had a better AIC than the model with treatment effect (2009: model treatment: AIC = 489.1; model herbivores + treatment: AIC = 329.2). Therefore fertilization turned out to affect the parasitoid only indirectly via its host.

Discussion

Effects of fertilization on vegetation composition and the host plant

We found changes in the vegetation in the first year of the investigation and after only one fertilizer application. Fertilization supported the general growth of the vegetation in the experimental subplots. These findings are corroborated by the studies of Gratton and Denno (2003), and Willems and van Nieuwstadt (2009). Grass species can use additional nutrients more quickly than forbs and therefore dominate fertilized areas (De Deyn et al. 2004). In contrast, plant species richness decreased with fertilization only after the third application in the second year. Gratton and Denno (2003) likewise showed that fertilization decreased plant species richness but increased plant biomass and density. Plant species composition reacts slowly to fertilizer application. After repeated applications a few forb species capitalize on the nutritional input leading to a dominance of these species (Foster and Gross 1998, Billeter et al. 2008).

Fertilization decreased host plant abundance in the experimental subplots. This could be due to the fact that *P. lanceolata* prefers to grow in less dense areas with a lower vegetation cover (Cavers et al. 1980). Possibly *P. lanceolata* is not sufficiently competitive against the rising cover of grasses. The number of stalks per subplot and consequently the number of potential food sources and oviposition sites decreased with fertilization whereas rosette diameter and above ground biomass increased. The host plant therefore seems to invest in growth when fertilized. As expected, *Plantago* leaf nitrogen content as a proxy for primary metabolites was higher in the fertilized subplots, whereas carbon content was not influenced. With increasing nutrient supply *P. lanceolata* seems to invest in biomass but not in reproductive organs like inflorescences.

Effects of fertilization on the upper trophic levels of P. lanceolata

Current paradigms assume that fertilization increases insect abundance and enhances its performance by increased plant nitrogen (N) (Mattson 1980; White 1993). In our study, in contrast, fertilization negatively affected the abundance of the weevil species living on *P. lanceolata* and their parasitoid. Decreasing insect abundances may be caused by effects of fertilization via the surrounding vegetation or via changes in resource availability or quality (Gratton and Denno 2003). Different hypotheses concerning the effects of fertilization on the different upper trophic levels are discussed:

The small insect abundances per subplot result from the fact that the two weevils and their parasitoid did not occur on every plot investigated. Therefore the boxplots show only low medians, but due to consistent differences between treatments and high numbers of replications we received explicit significant results.

Effects on upper trophic levels mediated via vegetation

Fertilization in this study influenced the vegetation in terms of an increased vegetation height and cover of grasses. Vegetation may interfere with insect behavior in different ways. The structural complexity of habitats and the architectural traits of single plants influence for example, the movement ability of herbivores and their host finding process (Goodwin and Fahrig 2002; Hannunen 2002). Particularly, the height and the density of the vegetation within habitats play a role here. Tall and dense vegetation, which we found in our study, in the neighborhood of host plants may either visually mask or structurally impair the access to the target plant (Perrin 1977; Åsman et al. 2001). Aside from visual camouflage, physical impediment caused by neighboring plants may diminish the dispersal rate of herbivores (Perrin and Phillips 1978). Dense vegetation may slow down the speed of movement by exerting a spatial resistance on individuals walking on the ground (Jopp 2006), crawling through the vegetation or on individuals conducting flights at low altitude as proposed by Coll and Bottrell (1994). Thus, vegetation structure may affect the access to resources and consequently determine to some extent the population dynamics of many phytophagous arthropods. The structure of non-host plants can also indirectly affect herbivores *via* their natural enemies. Non-host plant physical structures may provide shelter for natural enemies of herbivores (Langellotto and Denno 2004) or, in contrast, may render host search by the enemies more difficult (Tschanz et al. 2005, Obermaier et al. 2008).

Additionally we could show that fertilization decreased the plant species richness in the investigated subplots. Several investigations in agricultural systems showed a decrease of specialist herbivores in more diverse habitats resulting in specialist herbivores being negatively affected by plant diversity (Root 1973, reviewed by Finch and Collier 2000). However, in a natural context, most studies revealed a positive impact of plant diversity on herbivores and found higher herbivore abundance (Unsicker et al. 2006) or a higher degree of herbivory (Scherber et al. 2006) in more diverse habitats. A study by Wäschke et al. (unpublished) showed that the specialist weevil *M. pascuorum* living in an agricultural landscape is more abundant when its host plant is surrounded by a diverse vegetation. The herbivorous weevil occurs more often with higher plant diversity in the field, and odors from a plant species rich community enhanced its searching activity in an olfactometer assay which was adjusted to the field situation.

Regarding the parasitoid, several studies illustrate that foraging success is linked to parasitoid movement ability and that vegetation properties change their movement pattern (Randlkofer et al. 2010). In most cases complex vegetation structures (Coll and Bottrell 1996; Tschanz et al. 2005), which we found in the fertilized subplots, exert a negative influence on the foraging efficiency of enemies.

Effects on upper trophic levels mediated via host plant availability

Host plant abundance can be essential for the performance of individuals and the density of herbivore populations (Stilling and Moon 2005). Fertilization decreased host plant abundance and may therefore indirectly affect the herbivore species negatively via resource availability. However, in our study, *M.*

labilis was not influenced by the host plant abundance or the stalk number when these covariates were tested besides the treatment. The most important factor which influenced the weevil species was the treatment effect. On the other hand both covariates had a significant influence on the abundance of *M. pascuorum*. The treatment effect, however, was still stronger than that of the host plant. Lower host plant abundance and lower number of stalks per plant and therefore decreasing resource availability in fertilized plots might additionally be an important reason for the lower abundance of the specialist *M. pascuorum* in the fertilized subplots.

Effects on upper trophic levels mediated via host plant quality

Many publications report positive correlations between insect performance and N concentrations of host plants (McNeill and Southwood 1978; White 1993; Obermaier and Zwölfer 1999). The nitrogen limitation hypothesis (White 1993), for example, predicts a better herbivore performance with enhanced leaf nitrogen, but studies are arising which show an oviposition preference of herbivores for plants with lower nitrogen content (Bernays and Graham 1988, Denno et al. 1990, Berdegué et al. 1998) or a poorer performance in terms of higher pupal mortality and lower adult size on high nitrogen plants (Fischer and Fiedler 2000).

In this study we found decreased abundances of the three insect species in spite of increased nitrogen content of the host plants in fertilized subplots. Fischer and Fiedler (2000) likewise found that high leaf nitrogen contents were not generally beneficial to a butterfly species investigated. In a study of Joern and Behmer (1998) three grasshopper species responded in different ways to increased levels of nitrogen. Cease et al. (2012) showed that N fertilization and protein rich artificial diets can have consistent negative effects on the fitness of a locust. An explanation for a negative performance or avoidance of N-rich plants by herbivores could be that nutrients in the plant might be unbalanced due to nitrogen fertilization. Additional fertilizer application and thus higher nitrogen levels in a plant can also lead to higher organic acid concentrations which may act to kill herbivores (Brodbeck et al. 1990; Sarfraz et al. 2009 a) and/or change secondary chemistry and decrease the aucubin concentration of *P. lanceolata* (Fajer et al., 1992; Jarzomski et al., 2000). Jarzomski et al. (2000) assumed that the iridoid glycoside concentrations in *P. lanceolata* reflected a physiological response to nutrient availability. To avoid this kind of stress herbivores might prefer plants with lower nitrogen content (Fischer and Fiedler 2000).

Also higher trophic levels can respond to host plant quality, influenced by fertilization. A study by Kaneshiro and Johnson (1996) showed that the fecundity of the eulophid wasp *Chrysocharis oscinidis* (Ashmead) was enhanced at an optimum nitrogen level in bean foliage and was adversely affected at higher or lower levels.

Effects on the third trophic level mediated via the herbivores

Fertilization may either directly or indirectly (via the host species) influences also the third trophic level. The abundance of both herbivores in this study decreased with fertilization. At the same time we found an indirect negative effect of fertilization on parasitoid abundance. When fertilization effects were tested together with host abundances, *M. incultus* abundance only depended on the abundance of the herbivore *M. pascuorum* and no longer on fertilizer treatment. Although *M. incultus* is a generalist parasitoid, the wasp may prefer *M. pascuorum* as main host (Mohd Norowi et al. 2000; National History Museum: Universal Chalcidoidea Database) due to the higher abundance of this weevil species in our study. Our findings agree with other studies which found indirect effects of fertilization on parasitoids mediated via herbivores. Krauss et al. (2007) showed that also populations of natural enemies together with their prey seem to increase after fertilizer application. Furthermore Garratt et al. (2010) found that fertilizer treatments that improve cereal-aphid fitness will improve parasitoid fitness as well. The influence of fertilization in this study, however, is still quite important for the third trophic level by cascading up to higher trophic levels through the herbivorous hosts and affecting species interactions.

In summary we showed that experimental fertilization was beneficial to the vegetation structure, the above ground biomass of the host plant, and its nitrogen content, but it was unfavourable for the plant species richness, the host plant abundance and finally for the herbivore-parasitoid model system.

Fertilization negatively affected the two specialized herbivores, and its negative effect on herbivore density cascaded up to the third trophic level. Studies of the effects of experimental fertilization on plant-herbivore-parasitoid systems over such a large range of geographic regions are rare. Results of this study might therefore offer important insights into how land use intensification can affect higher trophic levels and species interactions in managed grassland ecosystems with implications for future landscape management and conservation of biodiversity in nutrient poor grasslands.

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Table 1: Results of the linear mixed effects models for the effects of experimental fertilization on the vegetation cover of grass and different host plant parameters. The values with standard errors (SE) and the degrees of freedom (df) are given. Significant P values are marked bold. 76 plots in 2009 and 81 in 2010 are included in the analysis.

		Host plant abundance[a]				Rosette diameter				Satlk number				Biomass[b]			
Year	Explanatory variables	value	SE	df	P	value	SE	df	P	value	SE	df	P	value	SE	df	P
2009	Intercept	25.246	4.857	75	< **0.001**	17.917	0.871	75	< **0.001**	2.023	0.159	75	< **0.001**	4.907	0.138	75	< **0.001**
	Treatment control	4.912	3.994	75	> 0.05	-1.264	0.532	75	< **0.05**	-0,066	0.121	75	> 0.05	-0,110	0.053	75	< **0.05**
2010	Intercept	2.161	0.126	80	< **0.001**	18.700	0.811	80	< **0.001**	1.373	0.434	80	< **0.01**	4.919	0.211	80	< **0.001**
	Treatment control	0.460	0.103	80	< **0.001**	-2.894	0.774	80	< **0.001**	0.426	0.179	80	< **0.05**	-0,191	0.072	80	< **0.01**

[a]: arcsin square root transformed; [b]: ln transformed for the years 2009 and 2010

Table 2: Number of plots (fertilized and control) with and without the incidence of *Mecinus labilis*, *Mecinus pascuorum* and *Mesopolobus incultus* for the years 2009 and 2010.

		2009			2010		
Treatment	Number of plots	*Mecinus labilis*	*Mecinus pascuorum*	*Mesopolobus incultus*	*Mecinus labilis*	*Mecinus pascuorum*	*Mesopolobus incultus*
Fertilized	with incidence	15	10	13	17	13	28
	without incidence	61	66	63	64	68	53
Control	with incidence	17	9	16	28	26	32
	without incidence	59	67	60	53	55	49

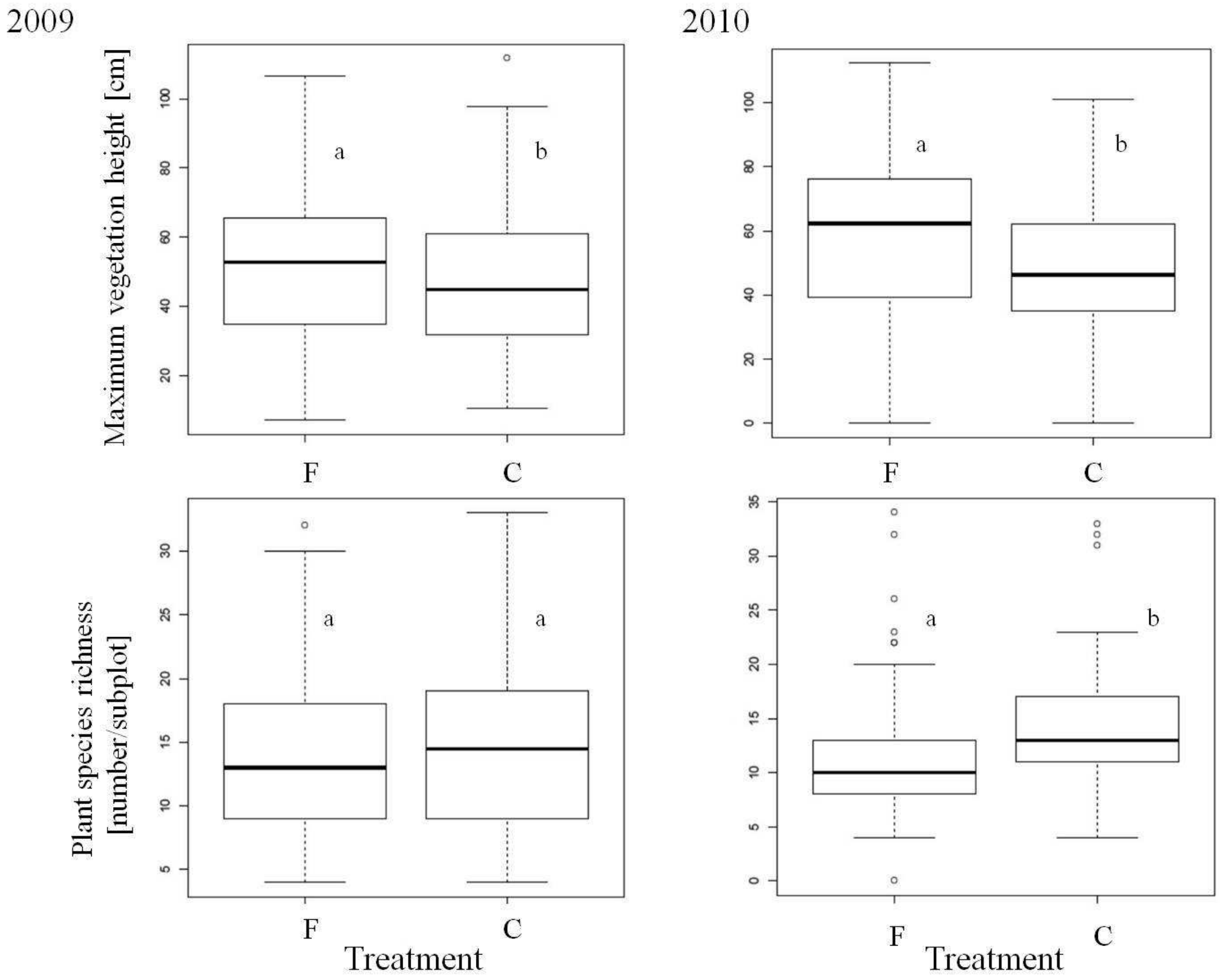
2009
2010
Maximum vegetation height [cm]
0
20
40
60
80
100
a
b
a
b
F
C
F
C
Plant species richness [number/subplot]
0
5
10
15
20
25
30
35
a
a
a
b
F
C
Treatment
F
C
Treatment

Fig. 1

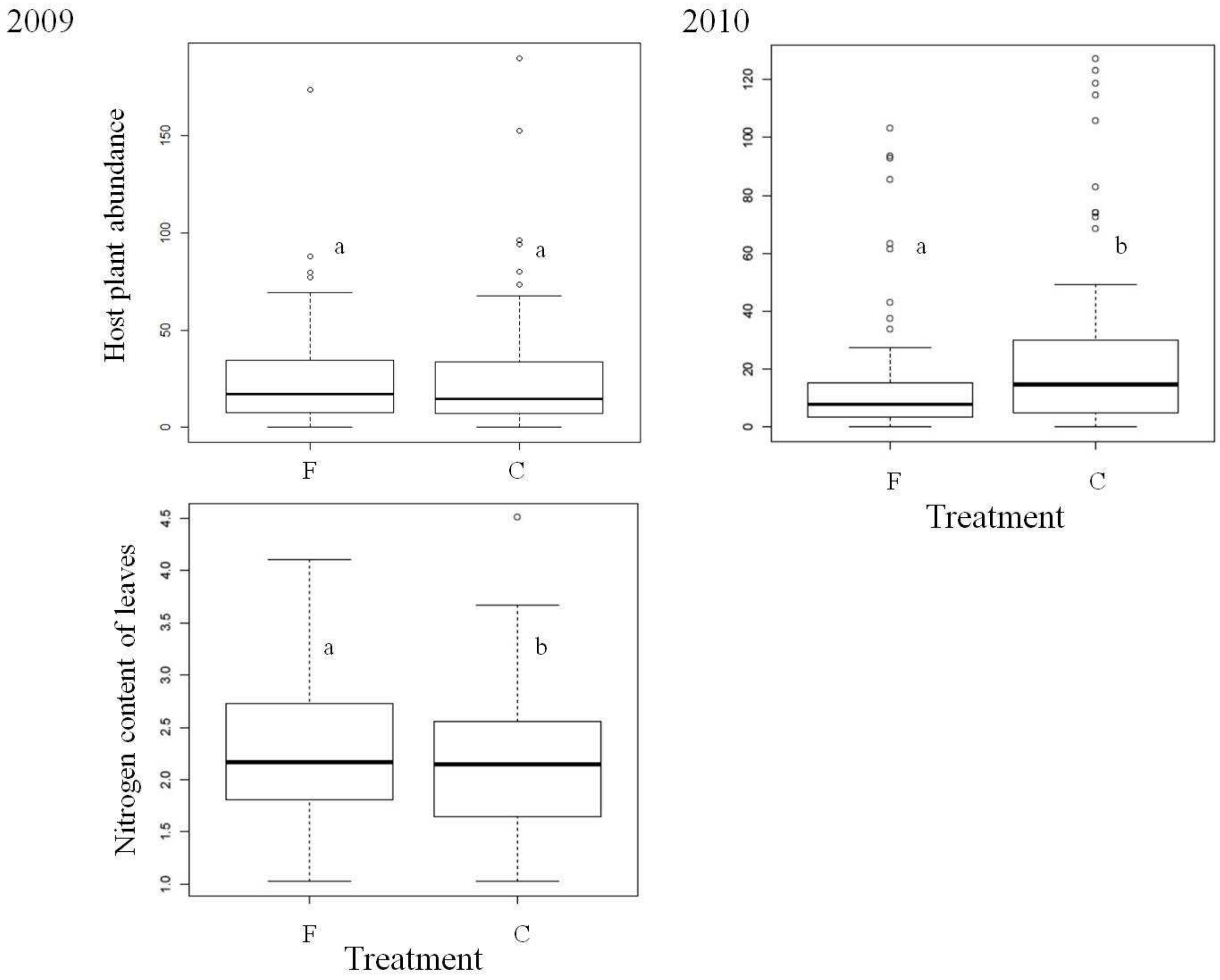
2009
2010
Host plant abundance
0
50
100
150
a
a
F
C
0
20
40
60
80
100
120
a
b
F
C
Treatment
Nitrogen content of leaves
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
a
b
F
C
Treatment

Fig. 2

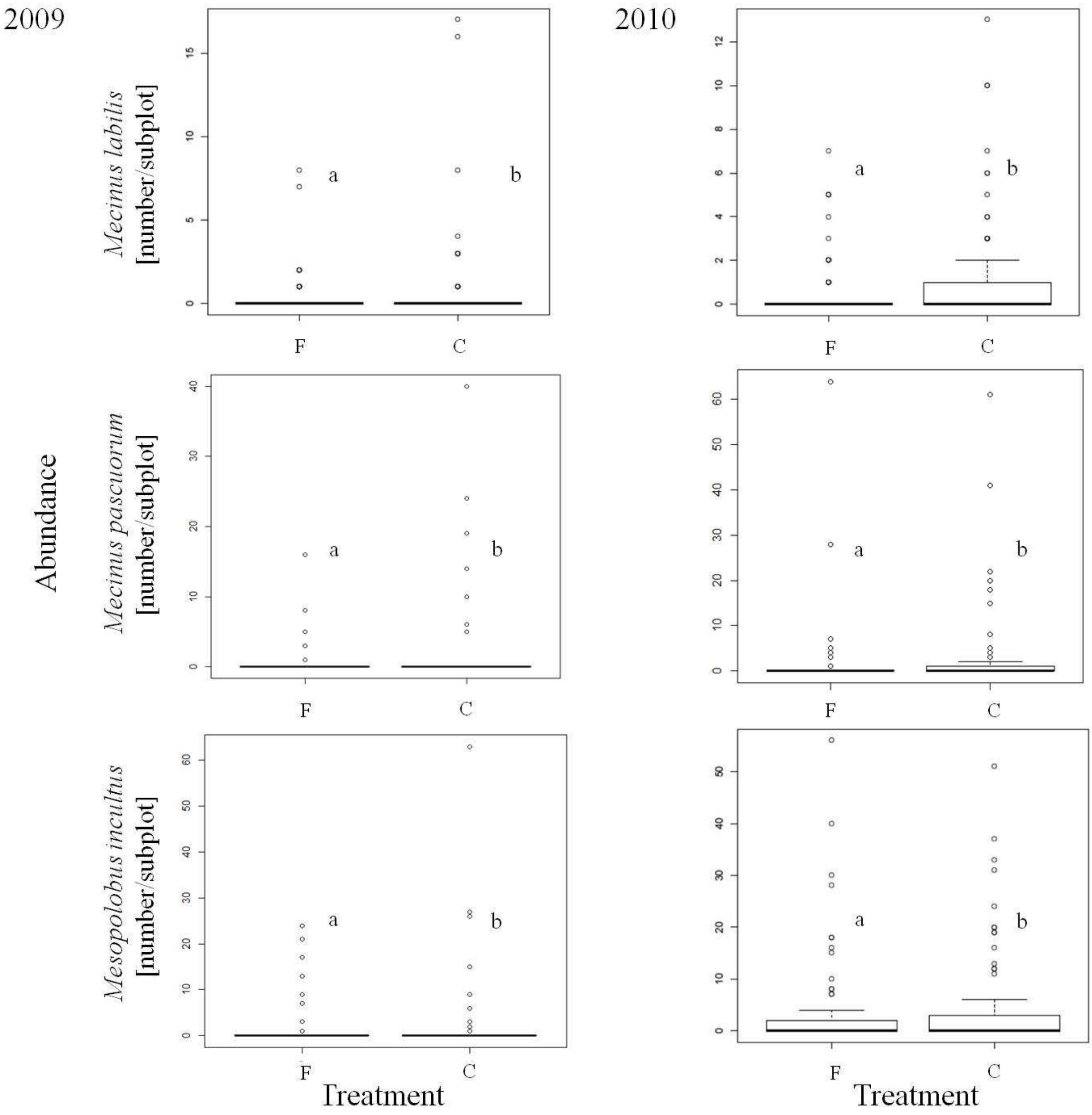
2009
2010
Abundance
Mecinus labilis [number/subplot]
Mecinus pascuorum [number/subplot]
Mesopolobus incultus [number/subplot]
0
5
10
15
0
2
4
6
8
10
12
0
10
20
30
40
0
10
20
30
40
50
60
0
10
20
30
40
50
60
0
10
20
30
40
50
a
b
F
C
Treatment

Fig. 3

Figure legend

Figure 1: Median, upper and lower quartiles and outliers for the maximum vegetation height (cm) and plant species richness per subplot combining all three exploratories for the years 2009 and 2010. Comparison between unfertilized (C: control) and fertilized (F) subplots. Boxplots with different letters were significantly different at $P \leq 0.05$.

Figure 2: Median, upper and lower quartiles and outliers for the host plant abundance and the nitrogen content of *Plantago lanceolata* leaves (%) per subplot combining all three exploratories for the years 2009 and 2010. Comparison between unfertilized (C: control) and fertilized (F) subplots. Boxplots with different letters were significantly different at $P \leq 0.05$.

Figure 3: Median, upper quartile and outliers for the abundance of *Mecinus labilis*, *Mecinus pascuorum* and *Mesopolobus incultus* per subplot combining all three exploratories for the years 2009 and 2010. Comparison between unfertilized (C: control) and fertilized (F) subplots. Boxplots with different letters were significantly different at $P \leq 0.05$.

Chapter VI

Landscape wide land use intensity determines local abundance of higher tropic levels in grassland habitats

Chapter VI

Landscape wide land use intensity determines local abundance of higher tropic levels in grassland habitats

Christine Hancock*[1], Sabrina Arnold[1], Michaela Bellach[1], Torsten Meiners[2], Marcell K. Peters[1], Christoph Rothenwöhrer[3], Juliane Steckel[1], Nicole Wäschke[2], Catrin Westphal[3], Elisabeth Obermaier[1,4]

[1] Department of Animal Ecology and Tropical Biology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

[2] Institute of Biology, Applied Zoology / Animal Ecology, Freie Universität Berlin, Haderslebener Strasse 9, 12163 Berlin, Germany

[3] Agroecology, Department of Crop Sciences, Georg-August-University Göttingen, Grisebachstrasse 6, 37077 Göttingen, Germany

[4] present address: Ecological Botanical Gardens, University of Bayreuth, 95440 Bayreuth, Germany

*Corresponding author. Present address:
Department of Animal Ecology and Tropical Biology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany
Tel.: + 49 (0) 171-76 49 916
E-mail address: christine-herbst@t-online.deKeywords: Landscape composition; land use intensity; mutitrophic interaction; spatial scales; Ribwort plantain

Abstract

Conversion of natural habitats to agricultural areas and intensification of land use are known as major reasons to cause worldwide decline in biodiversity. The quality of habitat patches within the landscape can substantially influence biodiversity, species abundances as well as trophic interactions. It has rarely been investigated, however, what influence land use intensification, as major factor determining habitat quality in central Europe, has on organisms of different trophic levels at the landscape scale. We studied the effects of land use intensity of grasslands in the landscape around focus habitats on species abundances and trophic interactions on different spatial scales using the higher trophic levels of a common herb as model system.

We examined the weevils *Mecinus labilis* Herbst, *M. pascuorum* Gyllenhal and their common parasitoid *Mesopolobus incultus* Walker on their host plant, the ribwort plantain, *Plantago lanceolata* L., on 76 sites. The effects of landscape composition on species abundances were analysed within the large scale project German Biodiversity Exploratories across multiple spatial scales (circular sectors around the plots with radii from 100 m to 2000 m). The abundance of the two herbivores was determined by the proportion of extensively managed semi-natural habitats in the landscape. In all three regions and across most spatial scales intensively managed grassland influenced herbivore abundance negatively and semi-natural habitat positively. The abundance of *M. labilis* was best explained at radii of r = 1500 to 2000 m by a low proportion of intensively managed grasslands. The abundance of *M. pascuorum* and *M. incultus* on the other hand was influenced strongest at smaller radii of r = 100 to 500 m by a high proportion of extensively managed semi-natural habitats. This can be explained with different host specificity of the insect species. The parasitoid, *M. incultus*, was exclusively influenced by the abundance of the two weevil species at all spatial scales investigated. Land use in the landscape affected the parasitoid indirectly via its hosts. The parasitoid was stronger linked to *M. pascuorum* than to *M. labilis*. Our findings show that even for an ubiquist like the common native plant species *P. lanceolata*, the land use intensity of grasslands in the surrounding landscape profoundly affects the abundance of its specialized herbivorous and entomophagous fauna. This may have important implications for the preservation of higher trophic level organisms and biodiversity of grassland habitats in agricultural landscapes.

Introduction

Recent advances in metapopulation theory, landscape ecology and macroecology (e.g. Rosenzweig 1995, Hanski 1999, Lawton 2000) have revealed the strong impact of landscape heterogeneity on local diversity and community structure. Though habitat quality may be the most important factor determining the presence of a species at a given site (Duelli 1997), diversity within a patch additionally depends on the heterogeneity of the surrounding landscape. The composition of a landscape is one of the key factors explaining species richness at different scales (Dunning et al. 1992, Dale et al. 2000, Wagner et al. 2000). Landscape diversity and percentage cover of certain land-use types might serve as useful indicators for species richness at the landscape scale (Dauber et al. 2003).

In Central Germany, insect populations face fragmented landscapes in which suitable habitat patches are interspersed between areas of intensive agricultural land use (Heisswolf et al., 2009). Species can respond in different ways to those landscape conditions. For example, Hendrickx et al. (2007) reported that the total species richness of all investigated groups (wild bees, carabid beetles, hoverflies, true bugs and spiders) was most strongly affected by increased proximity of semi-natural habitat patches. Bees likewise decreased in landscapes with a high intensity of farmland management. In a study by Weibull et al. (2003) species richness generally increased with landscape heterogeneity, but also habitat type had a major effect on the species richness for most investigated groups, with most species found in pastures and leys.

The number of species forming different trophic groups can decline in the order carnivores followed by phytophages and omnivores with increasing landscape simplification (Purtrauf et al. 2005). These findings seem to be partly due to increasing sensitivity to landscape changes with trophic rank, and partly to decreasing sensitivity of depauperate communities to environmental stress. Species loss can best be explained by the co-action of factors at local, landscape and regional scales (Purtrauf et al. 2005).

Since ecological interactions can depend much more on spatial scales than on single habitat patches, ecologists have become aware of the importance to include landscape scales into studies regarding ecological processes (Kareiva & Wennergren 1995, Wiegand et al. 1999). Changes in landscape composition can be characterized by the proportion of suitable habitat (Andrén 1994), the diversity of habitats, and the size and spatial scale of habitats in a landscape (Gustafson 1998). A few empirical studies provide an insight into how landscape composition determines the diversity and interactions of local communities and which spatial scale matters. Abensperg-Traun and Smith (1999) reported in their study of Eukalyptus remnants in grazed landscapes that predatory species of arthropods generally required larger habitat patches than did arthropods at lower trophic levels. Additionally, large-scale landscape effects can vary with the spatial scale and scale-dependency can differ between species (Roland & Taylor 1997, Steffan-Dewenter et al. 2001, 2002). However Thies et al. (2003) could not support that higher trophic levels experience the world at a larger spatial scale.

It has been suggested that biodiversity in agroecosystems depends on both landscape heterogeneity and farm management, but at the same time, studies of biodiversity in relation to landscape variables are rare (Weibull et al. 2003). Composition of ecosystems and habitat diversity have been shown to be interwoven with land-use intensity (e.g., Wrbka et al. 2004), and may influence, as landscape effects, important ecosystem functions such as biocontrol and herbivory (Gardiner et al. 2009) or pollination (Ricketts et al. 2008).

Intensification of land use is comprised by changes in landscape composition therefore different landscape parameters (arable land, forest, intensively managed grassland, and semi-natural habitats) were used. To analyze the influence of agricultural intensification by using the landscape composition at different spatial scales we selected a tritrophic system consisting of a common herb species typical for European grasslands as host plant and its specialized herbivore-parasitoid system living on the inflorescences. The ribwort plantain *Plantago lanceolata* L. (Plantaginaceae) is an index species for a cultivated landscape (Ellenberg et al. 1992). It grows in a very broad range of ground conditions (Dierschke & Briemle 2002), which makes *P. lanceolata* a perfect model for our study. It is very common in the three regions investigated and hosts two herbivorous weevils *Mecinus labilis* Herbst and *M. pascuorum* Gyllenhal (both Coleoptera: Curculionidae) and their natural enemy *Mesopolobus incultus* Walker (Hymenoptera: Pteromalidae) (Mohd Norowi et al. 2000).

Modifications in land use intensity can result in changes in the abundance and distribution of *P. lanceolata* and are likely to influence plant-herbivore and host-parasitoid interactions within the associated insect community, as known from other studies (Herbst et al. submitted). In this study we add the landscape perspective, investigating how agricultural intensification at landscape level affects the herbivore-parasitoid system in three regions. Therefore we focused on two habitat types to define land use intensity of grasslands on a landscape scale, the proportion of intensively managed grassland and extensively managed semi-natural habitat. The study was done in grasslands incorporating multiple spatial scales around each plot. We hypothesized that 1) the land use intensity at landscape scale has a negative influence on the abundance of the three insect species, 2) there are differences in the response of the second and the third trophic level, 3) the abundance of the two herbivores is more strongly influenced by the surrounding landscape at smaller spatial scales than at larger spatial scales to which the parasitoid should react more.

Material and Methods

Study sites and experimental design

Within the large-scale Biodiversity Exploratories project (http://www.biodiversity-exploratories.de) we investigated local and regional effects of land use intensity on the abundances of higher tropic level species in three different regions, Schwäbische Alb (Baden Württemberg), Hainich-Dün (Thuringia) and Schorfheide-Chorin (Brandenburg) (Fischer et al. 2010). The three regions differ in soil types, vegetation, precipitation and agricultural land use (Fischer et al. 2010). The three exploratories therefore enable the investigation of the influence of different habitat types on the same tritrophic system across a large geographical scale.

A total of 150 experimental grassland plots were established by the large-scale project (Fischer et al. 2010). According to the occurrence of our host plant (*P. lanceolata*), the number of investigated plots was 76 (Schwäbische Alb 33, Hainich-Dün 22, Schorfheide-Chorin 21). Every plot had a size of 50 m x 50 m and had a north-south alignment.

Study system

Plantago lanceolata, the ribwort plantain, is an ubiquitous herb that can grow up to 50 cm and forms a rosette. It blossoms from May to September (Schmeil & Fitschen 2003). According to Ellenberg (1996) and Opitz v. Boberfeld (1994) *P. lanceolata* grows both on wet and dry habitats.

Mecinus labilis lives oligophagous on *P. lanceolata* and *P. sempervirens* whereas *M. pascuorum* lives monophagous on the ribwort plantain. Both weevil species hibernate as adults and become active in May (Mohd Norowi et al. 1999). Females oviposit on newly-formed seedheads in June and July (Dickason 1968). The hatched larvae bore into a seedhead where they feed and develop in individual seeds. The larvae are fully developed by July and pupate in the seedheads (Mohd Norowi et al. 1999). Adults emerge from late August to September and move to the hibernation sites. *Mecinus labilis* can be found mainly on mown and dry pastures. Adults have a size of 1.7 to 2.2 mm (Lohse 1983). *Mecinus pascuorum* lives on dry grasslands and has a body length of 1.5 to 2.1 mm.

Mesopolobus incultus is a generalist parasitoid that parasitizes different insect orders (http://www.nhm.ac.uk/ research-curation/ research/ projects/ chalcidoids /[December 2008]). This pteromalid wasp is a solitary species and parasitizes the larvae of the two on *P. lanceolata* living weevil species described above (Mohd Norowi et al. 2000). *Mesopolobus incultus* hatches from the seedheads at the same time as the adult beetles (C. Hancock, pers. observ.). No further details of oviposition behavior are available and most parts of its life cycle are unknown.

Landscape composition

The land use in the landscapes surrounding the plots has been mapped and digitized for all 150 grassland plots based on an extensive field mapping campaign as well as on high resolution aerial photographs (resolution 40 cm, Hansa Luftbild Münster). The mapping included different land use types within a radius of 2 km around plots. For the baseline year 2009 eight general land use types (arable land, forest, intensively managed grassland, road, semi-natural habitats, urban, waterbodies, woodland) have been mapped.

In general, the surroundings of the grassland plots represent broad gradients in landscape composition, i.e. the proportions of the four major land use types, arable land, forest, intensively managed grassland, and semi-natural habitats.

Semi-natural habitats, like nutrient-poor grassland and meadows with scattered fruit trees, represent the most important land use type which generally harbors high species diversity in agricultural landscapes due to no or only extensive land use (Duelli & Obrist 2003; Billeter et al. 2008). Furthermore, semi-natural habitats represent a wide range of different habitat characteristics, for instance wet (fen) or dry (calcareous grassland) abiotic environments.

To analyse the influence of the surrounding landscape composition on the abundance of *Mecinus labilis, Mecinus pascuorum* and *Mesopolobus incultus* we calculated the proportions of intensively managed grassland (G) and extensively managed semi-natural (N) habitats. These two land use types can be considered as most important for the abundances of *M. labilis, M. pascuorum* and *M. incultus*. All other habitat types, e.g. woodland, urban areas or water bodies, were excluded because they are not qualified for potential usable habitat of the host plant and the three investigated insect species. Around the GPS based center points of each plot we applied eight circular buffers with radii of 100, 200, 300, 400, 500, 1000, 1500 and 2000 m that represent different spatial scales. The buffer areas were intersected with the underlying land use maps to calculate the percentage of intensively managed grassland and semi-natural area for the eight spatial scales. We used ArcGIS^{TM} 10 and FRAGSTATS (McGarigal et al. 2002) to calculate the landscape parameters.

Invertebrate sampling

100 inflorescences of *P. lanceolata* plants per plot were randomly collected for insect sampling. The collection was conducted between July and August 2008 in all three exploratories. The inflorescences were stored in plastic boxes (17.0 x 12.5 x 5.6 cm) with gauze covers (0.12 mm) under constant conditions (11:13 LD, temperature: 22°C, 50% rH). After hatching insects were identified and counted.

Statistical analyses

Our aim was to estimate how the abundance of *M. labilis*, *M. pascuorum* and *M. incultus* is influenced by the proportion (%) of intensively managed grassland (G) and extensively managed semi-natural habitats (N) in the surrounding landscape at different spatial scales. The statistical analyses were performed in R (Version 2.15.1; R Development Core Team, 2012) using generalized linear models (GLM) with the abundance of the three investigated insects as the dependent variable and the proportion of grassland or semi-natural habitats and region as predictor variables with assumed additive effects (Crawley 2007). As the proportion of grassland and semi-natural habitat was strongly negatively correlated (depending on the spatial scale) we did use the two variables in separate statistical models. We also tested if the effect of the proportion of G or N on species abundances differs among regions, i.e. by adding an interaction term proportion G or N: Region in models, but these models were in all cases less supported by the data (based on QAIC values) than simple models with additive effects of predictor variables. Due to the fact that the abundance of the parasitoid *M. incultus* may probably not only be influenced by landscape composition but also by the abundance of its hosts, we additionally calculated models in which we added the abundances of *M. labilis* and *M. pascuorum* as predictor variables. As the abundance data was of count type and the data showed signs of overdispersion, we modelled data assuming a quasipoisson distribution of errors. The influence of the proportion of G or N was analysed at seven different spatial scales, i.e. in radii of 100, 200, 300, 400, 500, 1000, 1500 and 2000 m around plots To test for spatial autocorrelation in the data we calculated Moran´s I values for the residuals of generalized linear models (Bivand et al. 2008). We tested the statistical significance of Moran’s I values for a distance class of 1 km using permutation tests.

Results

Influence of different land use types in the landscape on insect abundances

The proportion of intensively managed grassland (G) and extensively managed semi-natural habitats (N), had a profound effect on the abundance of the two herbivores and the parasitoid. Proportion of G influenced the abundance of all three insect species always negatively and proportion N always positively (Fig. 2). This could be shown at almost all spatial scales (100 – 2000 m) (Fig.1, Tab.1). The proportion of G decreased with increasing spatial scale and the proportion of N on the other hand increased with increasing scale. However the proportion of N was constant up to the 400 m radius. The proportion of G was always higher than of N (Tab.2) which means that other habitat types increase with larger spatial scales. Intensification of land use in the landscape had a negative influence on both higher trophic levels of the local seed feeder community of the host plant, *Plantago lanceolata*. This could be shown for a broad range of spatial scales in the landscapes. Moreover, models including the interaction term between proportion of G or N and Region were always less supported than models with simple additive effects of predictor variables, suggesting that the effect of the proportion of G or N on species abundances were consistent in the three different geographic regions of Germany.

Although the proportions of both habitat types in the landscape explain the abundances of all three insect species at almost all scales significantly. There are some differences in strength of interaction regarding the habitat type or the scale examined. The abundance of *M. labilis* is best explained by the habitat type intensively managed grassland (Tab.1A). Herbivore abundance decreased with increasing proportion of G. For *M. labilis* we found almost no differences between the three regions (Schwäbische Alb, Hainich-Dün and Schorfheide-Chorin). The extensively managed semi-natural habitats on the other hand best explained the abundance of *M. pascuorum* and the parasitoid *M. incultus* (Fig.1, Tab.1B, C). Abundance of the herbivore and the parasitoid increased with increasing proportion of N (Tab. 2B, C).

Dependence on the spatial scale

The two weevils and the parasitoid responded significantly to extensively managed semi-natural habitats at almost all spatial scales, but models differed in their fit to the data (in the coefficients of determination = R^2). The abundance of the herbivore *M. labilis* was best predicted by the proportion of G at radii of r = 1500 to 2000 m (Fig. 1, Tab. 1A). The abundance of the herbivore *M. pascuorum* and the parasitoid *M. incultus* was best predicted by the proportion of N at smaller spatial scales on radii of r = 100 to 500 m (Fig. 1, Tab. 1B, C). And the abundance of the parasitoid *M. incultus* was best explained by the proportion of N at smaller spatial scales (r = 100 to 500) (Fig. 1, Tab. 1C).

Differences between second and third trophic level

In a second step of data analysis the GLM model concerning the parasitoid was expanded and beside the proportions of G or N in the landscape, the local abundance of the two host species *M. labilis* and *M. pascuorum* were included as further explaining variables in models. We found that the abundances of the two herbivorous hosts had significant positive effects on the abundance of the parasitoid *M. incultus* (Tab. 3), while the effect of the proportion of N or G was not significant anymore in these models. Moreover, we found that the influence of the host *M. pascuorum* on the parasitoid was stronger, i.e. two to three times higher, than that of *M. labilis* (Tab. 3).

Discussion

Influence of land use on the landscape scale on higher trophic levels

It has been shown in many instances that landscape heterogeneity has a profound influence on local arthropod diversity and trophic interactions (Thies & Tscharntke 1999, Thies et al. 2003, Tscharntke & Brandl 2004, Gagic et al. 2011). In this study we investigate how intensification of land use of grassland habitats in the landscape influences specific herbivore-parasitoid interactions at different spatial scales. This was examined in a broad range of landscapes in different geographic regions. We focused on two habitat types to define land use intensity of grasslands on a landscape scale, the proportion of intensively managed grassland and extensively managed semi-natural habitat. Our study showed that the insect species of the investigated plant-insect community were affected by both habitat types in opposite ways. Increasing proportion of intensively managed grassland in the landscape negatively influenced the abundances of the two herbivores and the parasitoid whereas proportion of extensively managed semi-natural habitat had a positive effect on insect abundance. The negative effect of intensively managed grassland and the positive effect of semi-natural habitat in the landscape on the herbivores as well as the parasitoid could be shown for almost all spatial scales (i.e. for radii of 100 – 2000 m) and for all three geographic regions investigated. Results are supported by a study of Hendrickx et al. (2007) where the total species richness of all investigated groups (wild bees, carabid beetles, hoverflies, true bugs and spiders) was most strongly affected by increased proximity of semi-natural habitat patches. Wild bees likewise decreased in landscapes with a high intensity of farmland management. Neighboring anthropogenic habitats reduced the abundance of insects and their ecological functions in natural habitats (Holzschuh et al. 2011). In agricultural areas habitat diversity and composition can vary from structurally poor to rich landscapes. Thus it is very likely that such large-scale spatial patterns can influence local multitrophic interactions. This is supported by a few studies focusing on a plant-herbivore-parasitoid community (Kruess 2003), pollinators and seed set (Steffan-Dewenter et al. 2001, 2002) as well as parasitism rate of the rape pollen beetle (Thies & Tscharntke 1999).

The negative effect of land use intensity at the landscape scale on local abundance of the two herbivores is consistent over scales and regions although the host plant of the study system, *P. lanceolata*, is a common and ubiquitous plant with a broad range of habitats (Ellenberg 1996). Negative effects of land use intensity can on the one hand be due to the many mowing events on grasslands during the year. Here the lifecycle of the insects could be interrupted and no shelter for hiding is left. This is supported by a study of Braschler et al. (2009) where many orthopterans avoided the mown matrix in fragmented grassland, because of the lack of shelter. On the other hand landscape effects on local herbivore abundance could be explained by host plant availability or host plant quality, both potentially influenced by land use intensity. In an earlier study, we could show that host plant abundance was not affected by local land use intensity in the field. Experimental fertilization on the same plots, however, significantly decreased the abundance of *Plantago lanceolata* (Hancock et al., unpublished data) and increased host plant size. Potentially reduced host plant abundance at the landscape scale caused by land use intensification, might reduce herbivore abundances at the landscape as well as at the local habitat scale (Hancock et al. submitted). Thus, local interactions are triggered by factors such as resource availability at both the landscape and the habitat scale (Tscharntke et al. 2011). Further explanation for the observed patterns would be changes of patterns of nutrient quality of the host plant according to land use intensity and management at a landscape scale, with implications for the abundance of higher trophic level organisms. An analysis revealed a negative impact of total leaf nitrogen on the abundance of both weevil species investigated (Wäschke et al. unpublished data). Although many publications report on positive correlations between insect performance and host plant N concentrations (McNeill & Southwood 1978, White 1993, Obermaier & Zwölfer 1999), other studies found that high leaf nitrogen contents were not generally beneficial to herbivorous insect species (Joern & Behmer 1998, Fischer & Fiedler 2000, Cease et al. 2012). This might have implications on herbivore abundances also at the local habitat scale.

In a second step both, the effects of land use at the landscape scale (habitat types G and N), as well as of local host abundance of the two herbivores on the abundance of the parasitoid were analyzed together. It turned out that the parasitoid, *M. incultus*, was directly positively affected only by the density of its two host species and only indirectly, via its hosts, by land use intensity at the landscape scale. Land use intensity of grassland habitats in the landscape, however, stays an important parameter also for the parasitoid since as a change in proportion of intensively or extensively managed grassland habitats in the landscape profoundly affects abundance of the two host with a cascading bottom up effect also on the parasitoid. Heisswolf et al. (2009) showed that the occurrence probability of an egg parasitoid only depended (positively) on an increasing population density of its host, the leaf beetle *C. canaliculata* whereas the host was dependent on different landscape metrics like habitat size, isolation and quality. In a study of Kruess (2003) parasitoid species richness was at the same time influenced by habitat type and herbivore species richness. Not only habitat type and host abundance, but also landscape factors such as for example the percentage of non-crop area and the isolation of habitats (Kruess 2003) was shown to affect the abundance of herbivores and parasitoids.

In our study the proportion of semi-natural habitat as well as the proportion of intensively managed grassland were shown to be useful measurements of land use intensity at a landscape scale as they predicted very well the abundance of the two inflorescence feeders investigated. The abundance of the higher trophic level, the parasitoid was indirectly influenced by the land use at the different landscape sacles via his prey. Thies & Tscharntke (1999) as well as Steffan-Dewenter et al. (2001, 2002) showed that the percentage of non-crop area was a robust parameter to quantify the landscape context. Likewise Dauber et al. (2003) concluded that landscape diversity and percentage cover of certain land-use types might serve as useful indicators for species richness at the landscape scale.

Dependence on the spatial scale

Studies indicate that particular processes occur at particular spatial scales and that the spatial scale at which organisms interact may depend on both the landscape type and the species´ traits:

The three investigated species differed in the spatial scale at which they responded most deterministically to the landscape composition. While the abundance of the herbivore *M. labilis* was best explained by models in which landscape composition at larger spatial scales were integrated as predictor variables (r = 1500 – 2000 m), while the herbivore *M. pascuorum* and the parasitoid *M. incultus* responded most strongly to landscape composition at smaller spatial scales (r = 100 – 500 m).. The weevil *M. labilis* feeds oligophagous and may therefore have a larger range for host plant search (Lohse 1983). Körösi et al. (2012) showed that the percentage of grassland within 500 m around sampling sites affected species richness and abundance of leafhoppers negatively. *Mecinus pascuorum* responded in the same radius to the land use intensity. This weevil in contrast to *M. labilis* feeds monophagous on *P. lanceolata*. For the herbivore smaller ranges may be sufficient for food search due to host plant density. Maybe the weevil occurs more often at plots which are surrounded by areas with a high host plant density at smaller scales. *M. pascuorum* is positively influenced by increasing host plant density, as shown in other studies (e.g. Vanbergen et al. 2007). In contrast to our results studies based on observations from agricultural habitats show a decrease of specialized herbivores in more diverse habitat patches and conclude that specialists are negatively affected by plant diversity (Root 1973). For the parasitoid stronger effects at smaller spatial scales are contrary to other studies where higher trophic levels like parasitoids depend mostly on larger spatial scales. In our study the parasitoid depended on his main host *M. pascuorum* which was influenced at smaller spatial scales. In a geographic scale analysis, Thies et al. (2003) quantified the structure of the 15 landscapes for eight circular sectors ranging from 0.5 to 6 km diameter. Correlations between parasitism and non-crop areas as well as between herbivory and non-crop area were strongest at a scale of 1.5 km, thereby not supporting the view that higher trophic levels experience their environment at the largest spatial scales. Our findings support the general idea that higher trophic levels should be more susceptible to disturbance since they depend exclusively on the abundance of their two hosts as the second model shows. This is shown in literature by theoretical prognoses (Holt 1997, Holt et al. 1999) and empirical results (Kruess & Tscharntke 1994, Thies & Tscharntke 1999).

However the parasitoid depended only on the density of its hosts rather than on landscape configuration. *Mesopolobus incultus* especially was linked to the abundance of *M. pascuorum*. The impact of *M. pascuorum* on the parasitoid was twice as strong as of the second herbivore of the seed feeder system, *M. labilis*. Mohd Norowi et al. (2000) showed that the rate of parasitism of *M. incultus* was related to host density in different ways at different spatial scales. *Mesopolobus incultus* exhibited inverse density dependence at the finest (seedhead) scale, direct density dependence at the intermediate (plant) scale, and density independence at the large (habitat area 729 m^2) scale. In our study, in contrast, the parasitoid was positively density dependent on its two host species at the habitat scale in all three regions and across all spatial scales investigated. Likewise, the less specialized parasitoid *F. reptans* was more likely to be found on patches with high beetle density, while patch size and isolation seem to be less important (Heisswolf et al. 2009).

Land use intensity of grasslands at the landscape scale was the most important factor to influence the abundances of the higher trophic levels of the common native herb, *P. lanceolata*. The two weevil species, developing in the seeds of *P. lanceolata*, were negatively affected by proportion of intensively managed grassland and positively by proportion of extensively managed semi-natural habitat in the landscape at all spatial scales (100 – 2000 m) and in all regions examined. The parasitoid, *M. incultus*, was exclusively influenced by the local density of its two host species, *M. pascuorum* having twice as much influence on the abundance of the parasitoid than the other host, *M. labilis*. Land use intensity at the landscape scale had an important indirect influence on parasitoid abundance via the two weevil species. This is one of the few studies consistently showing the negative effect of land use intensity at different landscape scales on the higher trophic levels of a tritrophic system on the basis of the large data set of 76 landscapes, examined in different geographic regions. A general conclusion from this study is that both higher trophic levels, either directly or indirectly, depend strongly on the land use intensity of grassland habitats at the landscape scale (Holt et al. 1995, Thies & Tscharntke 1999, Kruess & Tscharntke 2000a). These findings might have important implications for the protection of trophic interactions and species diversity in nutrient poor grasslands of palaearctic regions.

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Figure legends

Figure 1: Effects of the proportion of intensively managed grassland (G) (red) and semi-natural habitat (N) (black) on the abundance of *Mecinus labilis* (radius = 2000 m) and the abundances of *Mecinus pascuorum* and *Mesopolobus incultus* (radius = 200 m). In grey: 95% confidence interval.

Figure 2: Influences of the proportion of intensively managed grassland (G) and semi-natural habitat (N) on the abundance of *Mecinus labilis*, *Mecinus pascuorum* and *Mesopolobus incultus.* White arrows indicate direct influences of land use type on the herbivores, grey arrows direct influences of the herbivores on the parasitoid and black arrows show the indirect influence of habitat types on the parasitoid. Plus signs show positive and minus signs negative influences.

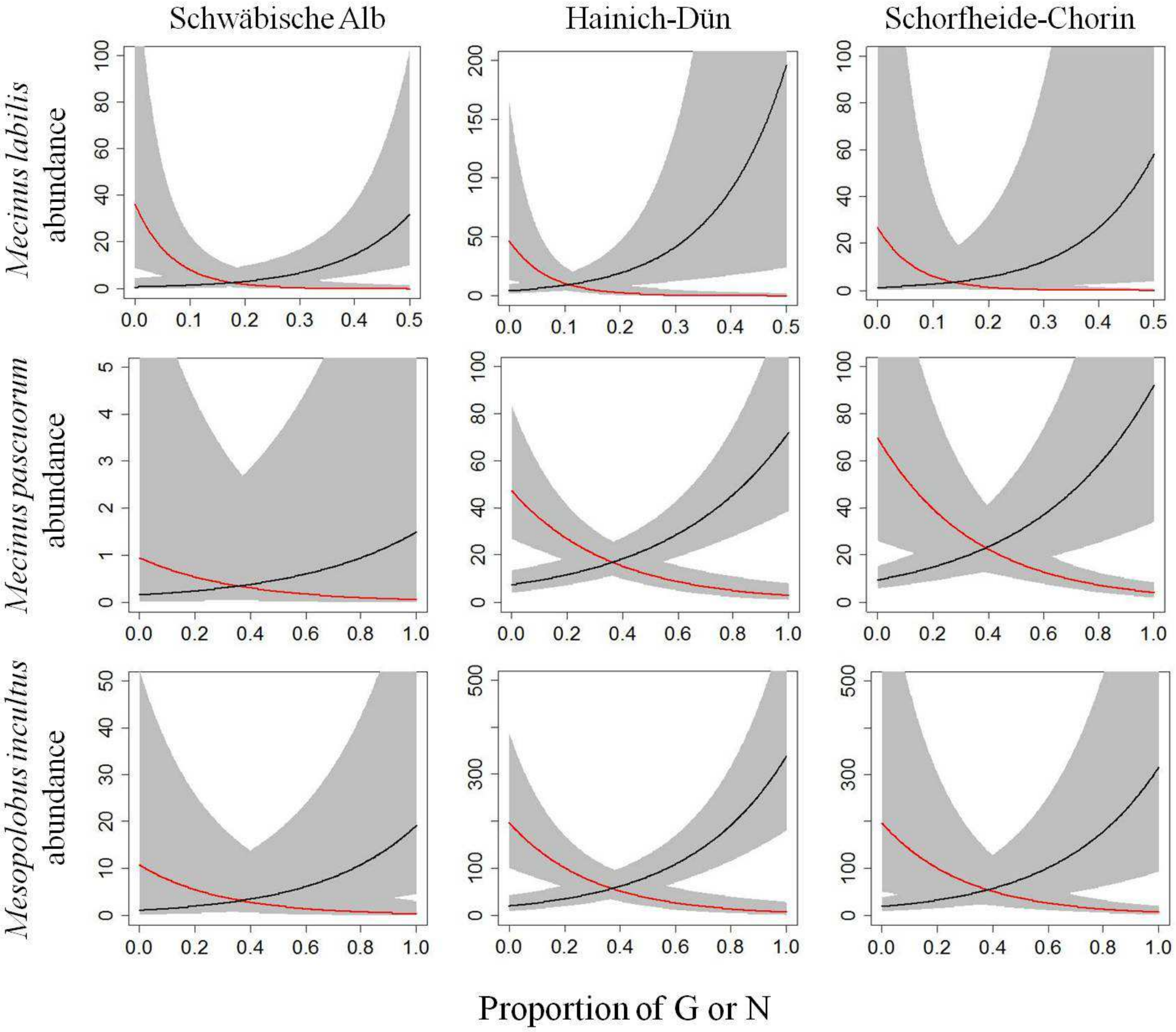
Schwäbische Alb
Hainich-Dün
Schorfheide-Chorin
Mecinus labilis abundance
Mecinus pascuorum abundance
Mesopolobus incultus abundance
0
20
40
60
80
100
50
150
200
1
2
3
4
5
10
30
300
500
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.8
1.0
Proportion of G or N

Figure 1

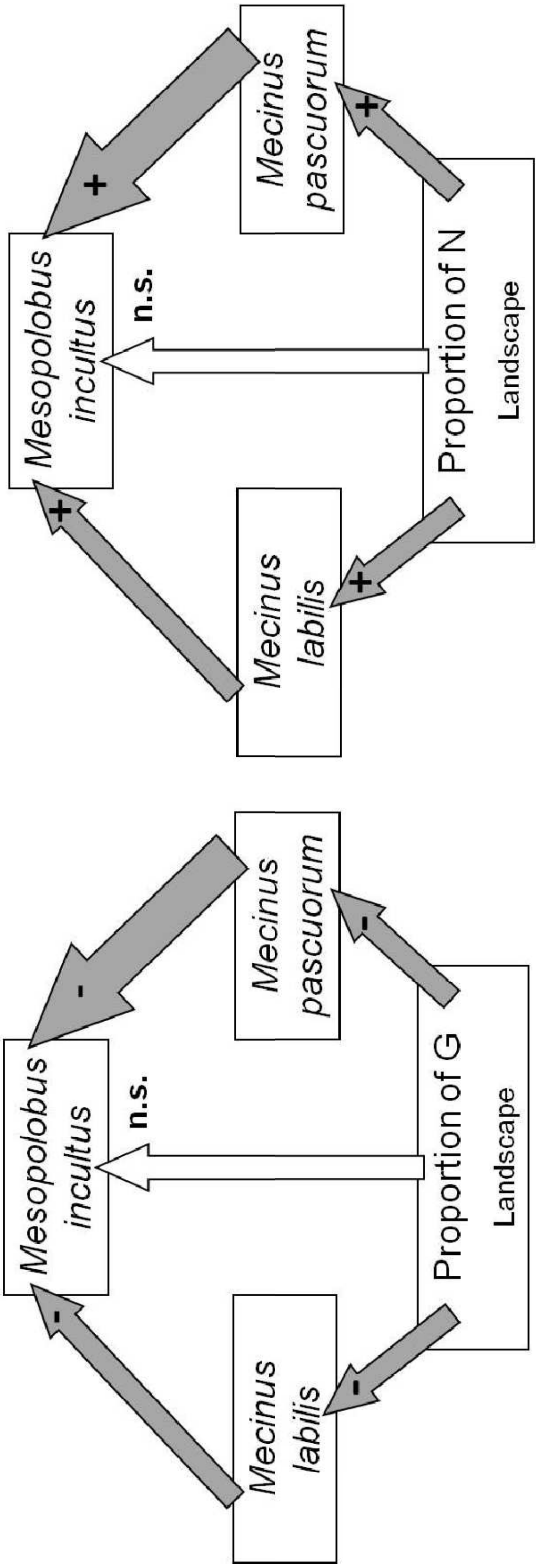
Mesopolobus incultus
n.s.
Mecinus labilis
Mecinus pascuorum
+
+
+
+
Proportion of N
Landscape
Mesopolobus incultus
n.s.
Mecinus labilis
Mecinus pascuorum
-
-
-
-
Proportion of G
Landscape

Figure 2

Table 1: Results of GLM models for the abundance of *Mecinus labilis* (A), *Mecinus pascuorum* (B) and *Mesopolobus incultus* (C) for spatial scales from 100 to 2000 with either the proportion of intensively used grassland (G) or semi-natural habitat (N) as explaining variables in models. The Region columns show the predicted model intercepts for the three different regions in which the study was conducted. An asterisk indicates a significant difference in the intercept of Hainich-Dün or Schorfheide-Chorin to the value of Schwäbische Alb. Proportion of G or N shows the strength and direction of the influence of G or N on the abundance of the measured species, R^2 is the coefficient of determination and indicates the model fit to the data. To check for spatial autocorrelation Moran´s I tests were performed for a distance of 1 km on model residuals.

A
Mecinus labilis

	Region					
	Schwäbische Alb	Hainich-Dün	Schorfheide-Chorin	Proportion of G	R^2	Moran's I
G_100	2.019	3.297	2.393	-2.024*	0.070	0.21 ns
G_200	2.346	3.425	2.949	-3.576**	0.094	0.11 ns
G_300	2.382	3.279	2.799	-3.986*	0.085	0.09 ns
G_400	2.411	3.262	2.808	-4.583**	0.088	0.08 ns
G_500	2.486	3.274	2.913	-5.511**	0.108	0.05 ns
G_1000	2.771	3.334	2.864	-8.938**	0.197	0.05 ns
G_1500	3.075	3.679	3.081	-12.565**	0.324	0.03 ns
G_2000	3.591	3.837	3.284	-14.975**	0.358	0.01 ns
				Proportion of N		
N_100	0.399	1.637	0.563	1.696*	0.075	0.23 ns
N_200	0.605	1.560	0.514	1.862*	0.077	0.19 ns
N_300	0.594	1.449	0.476	2.342*	0.081	0.13 ns
N_400	0.484	1.331	0.428	2.875**	0.091	0.03 ns
N_500	0.413	1.173	0.386	3.536**	0.096	0.004 ns
N_1000	-0.049	1.189	0.334	5.504**	0.134	-0.004 ns
N_1500	-0.270	1.403*	0.279	6.189*	0.151	0.07 ns
N_2000	-0.416	1.398*	0.182	7.762**	0.130	0.07 ns

B
Mecinus pascuorum

	Region					
	Schwäbische Alb	Hainich-Dün	Schorfheide-Chorin	Proportion of G	R²	Moran's I
G_100	-0.251	3.898**	4.098**	-1.959**	0.287	-0.13 ns
G_200	-0.055	3.856**	4.244**	-2.824**	0.296	-0.05 ns
G_300	-0.078	3.675**	4.031**	-2.950**	0.263	-0.002 ns
G_400	-0.212	3.502**	3.710**	-2.665**	0.221	0.02 ns
G_500	-0.323	3.345**	3.460**	-2.400*	0.188	0.03 ns
G_1000	-0.198	3.346**	3.450**	-3.730*	0.217	0.03 ns
G_1500	-0.483	3.091**	2.926*	-2.294	0.147	0.02 ns
G_2000	-0.471	3.056**	2.849*	-2.285	0.138	0.04 ns
				Proportion of N		
N_100	-1.993	2.182**	2.317**	1.890**	0.346	-0.16 ns
N_200	-1.872	2.004**	2.249**	2.275**	0.362	-0.19 ns
N_300	-1.755	1.990**	2.223**	2.556**	0.289	-0.22 ns
N_400	-1.787	1.946**	2.187**	2.911**	0.312	-0.17 ns
N_500	-1.796	1.849**	2.152**	3.410**	0.317	-0.15 ns
N_1000	-2.039	1.968**	2.135**	4.768**	0.212	-0.10 ns
N_1500	-2.838	1.940**	2.000**	6.908*	0.197	-0.02 ns
N_2000	-2.728	2.012**	1.934**	7.924	0.170	0.01 ns

C
Mesopolobus incultus

	Region					
	Schwäbische Alb	Hainich-Dün	Schorfheide-Chorin	Proportion of G	R^2	Moran's I
G_100	2.168	5.376**	5.216**	-2.427**	0.266	-0.14 ns
G_200	2.374	5.284**	5.280**	-3.343**	0.260	-0.15 ns
G_300	2.391	5.124**	5.105**	-3.651**	0.229	-0.13 ns
G_400	2.195	4.873**	4.595*	-3.039*	0.165	-0.08 ns
G_500	2.008	4.636**	4.184*	-2.426	0.122	-0.03 ns
G_1000	2.318	4.790**	4.453*	-4.885*	0.188	-0.02 ns
G_1500	1.838	4.373*	3.634	-2.273	0.084	0.06 ns
G_2000	1.708	4.239*	3.413	-1.539	0.076	0.09 ns
				Proportion of N		
N_100	-0.066	3.198**	3.009**	2.417**	0.360	-0.14 ns
N_200	0.120	2.993**	2.924**	2.833**	0.364	-0.18 ns
N_300	0.316	3.026**	2.900**	3.091**	0.256	-0.25 ns
N_400	0.296	3.005**	2.862**	3.448**	0.286	-0.20 ns
N_500	0.292	2.896**	2.820**	4.031**	0.294	-0.16 ns
N_1000	-0.104	3.036**	2.790**	5.827**	0.178	-0.10 ns
N_1500	-0.175	3.327**	2.757*	6.130*	0.129	0.04 ns
N_2000	-0.149	3.372**	2.690	7.178**	0.104	0.08 ns

Table 2: Median, minima and maxima (both in parentheses) of the proportion of intensively used grassland (G) and extensively managed semi-natural habitat (N) is shown for the radii from 100 to 2000 m. Additionally the Pearson correlation coefficients r for G and N are given. * P ≤ 0.05; ** P ≤ 0.01.

Scale [m]	Proportion G	Proportion N	Correlation r
100	0.81 (0.0-1.00)	0.01 (0.0-1.00)	-0.95**
200	0.57 (0.0-1.00)	0.05 (0.0-1.00)	-0.77**
300	0.45 (0.0-0.96)	0.06 (0.0-0.92)	-0.65**
400	0.37 (0.0-0.94)	0.07 (0.0-0.86)	-0.59**
500	0.33 (0.0-0.87)	0.07 (0.0-0.70)	-0.54**
1000	0.23 (0.0-0.64)	0.07 (0.0-0.58)	-0.53**
1500	0.21 (0.0-0.68)	0.07 (0.0-0.61)	-0.45**
2000	0.19 (0.0-0.56)	0.07 (0.0-0.09)	-0.39**

Table 3: Generalized linear mixed models (GLM) for the abundance of the parasitoid *Mesopolobus incultus* and additive effects of regions, the proportion of intensively used grassland (G) and extensively managed semi-natural habitat (N) and the abundance of the two host species *Mecinus labilis* and *Mecinus pascuorum*. Significant values of the second and third column indicate regional differences between the three exploratories. Proportion of G or N and the two following columns show the strength and direction of the prevailing variables on the abundance of *Mesopolobus incultus*. R² verifies these models. To check for autocorrelation Moran´s I test was performed for a distance of 1 km on the residuals of the model y~G/N_xxx+Region, glm (quasipoisson). We assume no autocorrelation because none of the values show a significant result. ns $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$

	Region							
	Schwäbische Alb	Hainich-Dün	Schorfheide-Chorin	Proportion of G	*Mecinus labilis*	*Mecinus pascuorum*	R^2	Moran's I
G_100	1.304	2.583	2.680	0.064	0.011*	0.027**	0.779	0.132 ns
G_200	1.430	2.778	2.927*	-0.251	0.011*	0.025**	0.777	0.121 ns
G_300	1.394	2.715	2.360*	-0.180	0.011*	0.026**	0.777	0.123 ns
G_400	1.026	2.238	2.183	0.957	0.011*	0.029**	0.776	0.172 ns
G_500	0.791	1.956	1.796	1.775	0.012*	0.030**	0.777	0.210 ns
G_1000	0.904	2.170	2.091	1.871	0.013	0.029**	0.776	0.176 ns
G_1500	0.899	2.221*	2.179*	2.021	0.014*	0.027**	0.777	0.163 ns
G_2000	0.609	2.040*	1.960*	3.291	0.015**	0.027**	0.777	0.171 ns
				Proportion of N				
N_100	1.192	2.617	2.761*	0.307	0.012	0.024**	0.782	0.121 ns
N_200	0.995	2.511*	2.763*	0.899	0.014*	0.020**	0.783	0.116 ns
N_300	1.025	2.482*	2.743*	1.051	0.014*	0.021**	0.782	0.113 ns
N_400	1.210	2.575	2.739*	0.538	0.012*	0.024**	0.781	0.117 ns
N_500	1.279	2.604	2.738*	0.281	0.011*	0.025**	0.780	0.123 ns
N_1000	1.181	2.547	2.716*	0.880	0.012*	0.025**	0.779	0.109 ns
N_1500	1.246	2.590	2.716*	0.566	0.011*	0.026**	0.777	0.115 ns
N_2000	1.091	2.523	2.659*	1.619	0.011	0.025**	0.777	0.115 ns

Chapter VII

Defensive plant compounds relate to plant species richness but plant nitrogen content mediates multitrophic interactions in cultural landscapes

Chapter VII

Defensive plant compounds relate to plant species richness but plant nitrogen content mediates multitrophic interactions in cultural landscapes

Nicole Wäschke[1], Christine Hancock[2], Monika Hilker[1], Elisabeth Obermaier[2], Torsten Meiners[1]

[1] Freie Universität Berlin, Institute of Biology, Applied Zoology / Animal Ecology, Haderslebener Str. 9, 12163 Berlin, Germany

[2] University of Würzburg, Department of Animal Ecology and Tropical Biology, Am Hubland, 97074 Würzburg, Germany

Corresponding author: Dr. Torsten Meiners
phone +49-30-83855910
fax +49-30-83853897
e-mail: meito@zedat.fu-berlin.de

Abstract

Anthropogenic land use may shape vegetation composition and affect trophic interactions by altering host plant metabolites. Here, we investigated the hypotheses (1) that plant nitrogen and secondary metabolite contents of the herb *Plantago lanceolata* are affected by land use intensity and surrounding vegetation composition (plant species richness and *P. lanceolata* density) and (2) that quantities of primary and secondary metabolites of *P. lanceolata* are correlated on the one hand with abundances of the herbivorous weevils *Mecinus pascuorum* and *M. labilis*, and on the other hand with abundance and parasitization success of their larval parasitoid *Mesopolobus incultus*. We determined plant species richness, *P. lanceolata* density, and abundances of the herbivores and the parasitoid on 77 grassland plots across Germany differing in land use intensity (LUI index). We also measured nitrogen and defensive secondary metabolite (iridoid glycosides (IGs): aucubin and catalpol) contents of *P. lanceolata* leaves. We showed (1) that plant species richness was positively correlated with IGs; the LUI index was positively correlated with leaf nitrogen content. Furthermore, (2) abundances of herbivores were correlated negatively with leaf nitrogen, and not related to IG content. Parasitoid abundance and parasitization success were not related to plant metabolite contents, but to host abundance. We conclude that plant species richness can lead to changes of the defensive metabolite content of plants, but these changes may be without effect on higher trophic levels. In contrast, land use intensification can have negative effects on multitrophic interactions by changing the nutritional quality of the host plant.

Key words: neighboring plants, plant species richness, plant metabolites, multitrophic interaction, *Plantago lanceolata*, land use

Introduction

Multitrophic interactions are important drivers of ecosystem stability and functioning, especially because they can influence nutrient and energy cycling (reviewed by Weisser and Siemann 2004, Schmitz 2010). Ecosystem stability is endangered by biodiversity loss (Balvanera et al. 2006) caused by the on-going process of land use intensification in numerous habitats (Foley et al. 2005). The loss of plant species and decrease in abundance of a plant species may affect higher trophic levels (Joshi et al. 2004, Petermann et al. 2010).

Vegetation composition in terms of plant species diversity and conspecific plant density can impact the performance of an individual plant by forming different abiotic environments or changing competition levels for this plant species (Barbosa et al. 2009); this may lead to changes of primary and secondary metabolite concentrations (Cipollini and Bergelson 2001, Broz et al. 2010, Scherling et al. 2010). For example, plants grown in the neighborhood of conspecifics (as compared to heterospecific neighbors) allocate less primary metabolite levels denoted as a decrease in amino acid amounts (Broz et al. 2010), whereas an increase in concentrations of defensive secondary metabolites indicates higher resource allocation towards defense (Barton and Bowers 2006, Broz et al. 2010). Furthermore, land use practice (mowing, grazing by cattle, and fertilizer application) influences the vegetation composition (Spiegelberger et al. 2006) as well as primary and secondary metabolite allocation in plants (Prudic et al. 2005).

Changes of metabolite allocation in a plant can shape multitrophic interactions in different ways by changing the performance and preference of the second (Schoonhoven et al. 2005) and third trophic level (Gols and Harvey 2009). In general, primary metabolites such as nitrogen are proposed to be correlated positively with herbivore performance (and thus herbivore abundance) (Mattson 1980, White 1993, Throop and Lerdau 2004). However, plants with high nitrogen content can also negatively affect the fitness of herbivores (Joern and Behmer 1998, Fischer and Fiedler 2000). In addition to a direct influence of the plant´s nutrient content on herbivore performance, plant nutrients may also affect herbivores by their indirect impact on the third trophic level *via* the trophic cascade (host or prey quality), thus leading to altered parasitism rates (Thompson 1999, Sarfraz et al. 2009a).

Secondary metabolites of plants may function as defense compounds against herbivores, which in turn have evolved different ways to handle secondary metabolites by sequestering or detoxifying them (reviewed by Opitz and Müller 2009). The secondary metabolite content of a host plant may also affect the third trophic level either indirectly by influencing host or prey quality or by direct interactions between the plant and enemies of the herbivores (Poelman et al. 2008, Gols et al. 2009). The nutritional quality and direct defense compounds of a single plant can be traced from the herbivore to further trophic levels in the trophic cascade (Kos et al. 2011).

Up to now, the effect of plant species diversity on allocation patterns of specific plant metabolites has been investigated only by Mraja et al. (2011); they showed that plant species richness affected concentrations of secondary plant metabolites of a perennial herb that is native to Europe and widespread in very different kinds of habitats, i.e. in *Plantago lanceolata* L. (Plantaginaceae) (Schubert and Vent 1990). Characteristic secondary metabolites of this plant are iridoid glycosides (IGs), especially aucubin and the more toxic catalpol (Bowers 1991). The IGs are monoterpene derivatives and toxic for many herbivores (Bowers 1991, Dobler et al. 2011); hence, they are considered as a direct defense system of *P. lanceolata*. Mraja et al. (2011) showed that concentrations of aucubin decreased and those of catalpol increased when plant species richness was raised. Their study was conducted on experimental grassland plots where plant species were sown and weeded to keep plant species richness constant. In order to investigate the observed patterns in a natural setting, field studies in different regions that vary in their landscape composition and abiotic parameters are needed (Fischer et al. 2010).

Here, we investigated the effect of land use intensity and the surrounding vegetation composition on the levels of primary and secondary metabolites of *P. lanceolata* under natural field conditions. The secondary metabolites investigated were major defensive compounds of this plant species, the IGs aucubin and catapol. In order to elucidate the effect of land use and vegetation composition on the primary plant metabolism, we determined the total nitrogen content as a proxy of the plant´s protein content (Schoonhoven et al. 2005) which is a crucial nutritional factor for insects and often limits insect growth. Furthermore, we investigated how the plant´s metabolism that is affected by land use and vegetation composition influences the abundances of herbivorous insects and parasitoids in the field. The herbivorous insects studied were *Mecinus pascuorum* (Gyllenhal) and *Mecinus labilis* (Herbst) (both Coleoptera: Curculionidae) which feed on *P. lanceolata* stalks and leaves during their adult phase; females oviposit into seeds and larvae develop inside the seeds. The parasitic insect species studied was the larval parasitoid *Mesopolobus incultus* (Walker) (Hymenoptera: Pteromalidae) which attacks *Mecinus* larvae inside the seeds (Mohd Norowi et al. 2000). Baden et al. (2012) could not detect IGs in adult *M. pascuorum* and concluded that this weevil does not sequester IGs. Detoxification of defensive compounds can imply fitness costs for an herbivore (e.g., Pankoke et al. 2012), and therefore, *M. pascuorum* may avoid IG-rich plants. As yet, the impact of the defensive IGs of *P. lanceolata* on *M. labilis* and the parasitoid *M. incultus* is unknown.

Our study was conducted in a cultural landscape in Central Europe and on a large scale in different geographic regions with varying environmental conditions. In summary, we tested the hypotheses that (1) land use intensity and vegetation composition (plant species richness and *P. lanceolata* density) affect nitrogen and IG (aucubin and catalpol) leaf contents of *P. lanceolata* and that (2) plant metabolite changes are correlated with the abundances of the herbivores *M. pascuorum* and *M. labilis*, their parasitoid *M. incultus* as well as the interaction between herbivores and parasitoids (parasitization success).

Material & Methods

Study sites

Sampling took place within the German priority project “Biodiversity Exploratories” which is described in detail by Fischer et al. (2010). We conducted our study in three geographic regions (exploratories) in Germany (from north to south), i.e. Biosphere Reserve Schorfheide-Chorin, National Park Hainich-Dün, and Biosphere Area Schwäbische Alb. The three regions across Germany, in the following referred to as Schorfheide, Hainich and Alb, differ in their environmental conditions like precipitation (from north to south: (500 - 600, 500 - 800, 700 - 1000) mm), altitude ((3 - 140, 285 -

550, 460 - 860) m) and annual mean temperature ((8 - 8.5, 6.5 - 8, 6 - 7) °C) (Fischer et al. 2010). In each region (exploratory), 50 grassland plots (50m x 50m) are established for biodiversity research. The grassland sites represent a land use gradient and concomitant differences in plant species diversity. Land use is classified by meadows, pastures and mown pastures which are either fertilized or unfertilized. According to Blüthgen et al. (2012) land use intensity can be calculated as a continuous variable consisting of different measures for land use: amount of fertilizer (kg N ha^{-1}), frequency of mowing (y^{-1}), and livestock density (livestock units × days ha^{-1}) and is summarized as the land use intensity (LUI) index. According to the occurrence of the host plant (*P. lanceolata*), the number of plots studied was 21 in Schorfheide, 22 in Hainich, and 34 in Alb.

Sampling of leaf material

Within each plot, we chose ten focus *P. lanceolata* plants in 4m x 4m subplots located in the corners of each plot. Plant species richness (r = 15cm) and *P. lanceolata* density (r = 100cm) around the chosen focus plant were surveyed from the end of May until the beginning of July 2008. Metabolite composition of leaves varies with factors like leaf age, genotype (Bowers and Stamp 1992), ecotype (Marak et al. 2000), and herbivory (Bowers and Stamp 1993, Darrow and Bowers 1999). Nevertheless, we sampled leaves from ten individual plants randomly along the plot regardless of leaf damage, leaf age and geno- or ecotype; thus, we obtained a group of leaves that was representative for the entire plot. Leaves were harvested and immediately stored on dried ice. In the laboratory leaves were kept at -30°C until sample preparation.

Determination of insect abundances and parasitization success

To determine the abundances of the herbivores *M. pascuorum* and *M. labilis* and the larval parasitoid *M. incultus,* we collected 100 randomly chosen *P. lanceolata* inflorescences per plot in July and August 2008. Inflorescences of *P. lanceolata* were kept in plastic boxes ((17.0 x 12.5 x 5.6) cm) with a fine-meshed gauze (0.12mm) top cover under constant conditions (11:13 LD, temperature: 22°C, 50% rH). Adult insects that emerged from the inflorescences in August and September 2008 were identified and counted. The parasitization success was defined as the ratio of parasitoids emerging from the inflorescences compared to the sum of the number of emerging parasitoids and the number of herbivores.

Chemical analysis

Leaf material that was sampled and frozen as described above was taken out the freezer and ground in liquid nitrogen. After grinding, the fine powder was lyophilized.

Leaf nitrogen content was used as a proxy for the nutritional quality of the host plant for the insects (Quintero and Bowers 2012). Nitrogen content of the dried and ground plant material was analyzed by an elemental analyzer (Euro EA 3000 Elementalanalyser, EuroVector, Milan, Italy) and is given in % dry weight (DW).

For IG analysis, we added 50µl phosphate buffer (20 mM KH_2PO_4) and 1ml methanol to 20mg lyophilized leaf material. The mixture was shaken at 40°C and 1500rpm for 4h in a rotary shaker (Thermomixer comfort, Eppendorf, Hamburg, Germany) and centrifuged for 3-4min at 10,000rpm. The supernatant (100µl) was concentrated to dryness under a N_2 stream. Finally, we reconstituted the concentrate in 100µl phosphate buffer (20mM KH_2PO_4) and analyzed the solution by HPLC-DAD analysis.

The Shimadzu HPLC system (Shimadzu Deutschland GmbH, Duisburg, Germany) consisted of two LC-20AD pumps, a CBM- 20A system controller, a manual injection system, a Spherisorb ODS-2 analytical column (3µm; 60mm x 4.6mm) with a guard column (same material, 10mm x 4.6mm). We used the solvents phosphate buffer (20mM KH_2PO_4) (A) and acetonitrile (B) and the following gradient: 0-5min, 5% of B; 5-9min, 5%-50% of B. The flow rate was 0.5ml min^{-1}. The injection volume was 20µl. The IGs were detected with a photo diode array detector SPD-M20A (190nm - 300nm). Retention times and UV spectra were compared with reference compounds (aucubin + catalpol: purity ≥ 99%, Phytoplan Diehm & Neuberger GmbH, Heidelberg, Germany) via the software Chromatographydatasystem LC-Solution (Shimadzu Deutschland GmbH, Duisburg, Germany). For quantification, peak areas of aucubin and catalpol were determined at 210nm. Amounts of catalpol and aucubin were calculated by calibration with the respective reference compounds. Quantities of the single IGs as well as the sum of both compounds (total IGs) are given in mg/g DW.

Statistical analysis

Field data were analyzed using linear mixed models and generalized linear mixed models. The models are based on mean values per plot for *P. lanceolata* density and mean number of plant species (= plant species richness) per plot. Furthermore, the total number of weevils (or parasitoids) per plot were used for the models. Additionally, the nitrogen and IG contents that were determined for leaf material samples collected per plot were implemented into the model as plot values. The region (exploratory) was used as a random effect. All calculations were performed using R (Version 2.12.1; R Development Core Team, 2010). Models were calculated with the lme (package nlme Version 3.1-97) and the lmer function (package lme4 Version 0.999375-37). Percentage response variables were arc sine transformed to fulfill model assumptions. Explanatory variables with non-normal distribution were ln transformed for stabilizing variance (Crawley 2007). A term (+1) was added before transformation if necessary. For plant metabolite parameters as response variables we used a Gaussian error distribution (link = identity) and fitted the models by maximum likelihood. For the insect abundance data as response we used a Poisson error distribution (link = log) and the Laplace approximation. For parasitization success as response, we applied the binomial error distribution (link = logit) and linked the number of successes (number of emerging parasitoids) and number of failures (number of emerging herbivores not parasitized by the parasitoid) as the response variable (Crawley 2007). Furthermore, to account for overdispersion we added an individual-based random effect (Elston et al. 2001). We started with the full model and dropped terms that were not significantly different from zero. Model comparison was done by Akaike Information Criterion (AIC, Burnham and Anderson 2002) until we ended up with a minimal adequate model (with the AIC not dropping anymore).

To elucidate the effect of the LUI index and vegetation composition on plant metabolite parameters (nitrogen, total IG, aucubin, and catalpol) we used the fixed effects “plant species richness”, “*P. lanceolata* density”, and “LUI index”. When asking for the effect of plant metabolite concentrations on the multitrophic interactions we added nitrogen, total IGs, ratio of catalpol to total IGs, and host abundance (*P. lanceolata* density for herbivores as response and herbivore abundance for parasitoids as response) as fixed effects in the model. The ratio of the more toxic catalpol to total IGs was included in the model since this variable might detect relationships between the parameters studied here more sensitive (Quintero and Bowers 2011). Host plant availability determines herbivore abundance (e.g., Root 1973) and host availability determines parasitoid abundance (e.g., Sheehan 1986); therefore, these parameters were included in the analysis as covariates.

Results

Effect of land use intensity and vegetation composition on P. lanceolata metabolites

To elucidate the effect of land use intensity and vegetation composition (plant species richness and *P. lanceolata* density) on concentrations of primary and secondary metabolites of a single plant we tested whether these variables were correlated with nitrogen and IG concentrations of *P. lanceolata*.

The analysis of the primary metabolites revealed that leaf nitrogen content of *P. lanceolata* was on average 2.3% DW in Schorfheide, 1.9% DW in Hainich, and 2.1% DW in Alb (Tab. S1, supplement). Leaf nitrogen content was not correlated with plant species richness and *P. lanceolata* density, but negatively correlated with the LUI index (Fig. 1a), indicating that a more intense land use leads to higher leaf nitrogen content.

The quantitative analysis of secondary metabolites of *P. lanceolata*, i.e. the IGs, showed the following mean values of the total leaf IG concentration: 36.3mg/g DW in Schorfheide, 41.3mg/g DW in Hainich and 41.5mg/g DW in Alb. The variation of this parameter ranged from 26mg/g DW to 51mg/g DW in Schorfheide, 20mg/g DW to 60mg/g DW in Hainich, and 20mg/g DW to 78mg/g DW in Alb

(Tab. S1, supplement). The IG concentrations in *P. lanceolata* were correlated positively with plant species richness (Fig. 1b, Tab. 1). This applies for the total IG concentration as well as the concentration of the single compounds aucubin and catalpol (Tab. 1). The LUI index and *P. lanceolata* density had no effect on the IG concentrations of *P. lanceolata* leaves.

Plant metabolites and further trophic levels

The abundance of the herbivore *M. pascuorum* was highest in Schorfheide with a median value of 8 individuals per plot. Similarly, the abundance of *M. labilis* was highest in Schorfheide, but the median value of individuals was much lower (1 individual per plot). *M. labilis* showed lower individual numbers than *M. pascuorum* except for Alb where 0-52 individuals of this herbivore species were found per plot as compared to 0-4 individuals of *M. pascuorum* (Tab. 2). The abundances of the herbivores *M. pascuorum* and *M. labilis* were negatively correlated with host plant nitrogen content (Fig. 2). Abundances of both herbivore species were neither correlated with the amounts of total IGs nor with the ratio of catalpol to total IGs (Tab. 3). Furthermore, the abundance of *M. pascuorum* was positively correlated with *P. lanceolata* density. The results indicate that the host plant nitrogen content may have affected the abundances of members of the second trophic level since the abundances of the two herbivore species decreased with increasing leaf nitrogen content.

The abundance of the parasitoid *M. incultus* followed the abundance pattern of its hosts with highest abundance in Schorfheide and the lowest in Alb. However, the parasitization success was highest in Hainich with median values of 49% compared to 43% in Schorfheide and 24% in Alb (Tab. 2). In order to elucidate the effects of plant metabolite contents on the parasitoid, we included herbivore abundance as a covariate in the model. Contents of primary and secondary host plant metabolites did not explain abundance and success of the parasitoid. Herbivore abundance correlated positively with parasitoid abundance and negatively with parasitization success (Tab. 4, Fig. 2).

Discussion

Our field study addressed the question whether changes in land use intensity and vegetation composition (plant species richness and *P. lanceolata* density) induce changes in the contents of host plant primary and secondary metabolites and, if so, whether these changes affect higher trophic levels. We found that (1) land use (as quantified by the LUI index) was positively correlated with leaf nitrogen content, and plant species richness was positively correlated with the concentration of secondary metabolites (IGs) in *P. lanceolata* leaves. Furthermore, we could show that (2) leaf nitrogen content negatively correlated with the abundances of the herbivores. Contents of host plant metabolites (N, IGs) did neither affect parasitoid abundance nor parasitization success. The relationships between land use intensity, plant species richness, host plant metabolites, and insect abundances were detectable over a broad range of environmental conditions throughout Germany.

Effect of vegetation composition on P. lanceolata secondary metabolites

Our results show that *P. lanceolata* density did not affect concentrations of IGs in leaves; these findings suggest that intraspecific competition of *P. lanceolata* has no effect on leaf IG quantities. Greenhouse experiments conducted by Barton and Bowers (2006) showed higher IG concentrations in *P. lanceolata* grown together with conspecifics than in those grown without conspecific neighbors. This effect was especially marked six weeks after seedlings had been planted together, whereas nine weeks later this effect decreased considerably. Since the plants we sampled might have been even older than nine weeks; differences in IG allocation due to different intraspecific competition levels might have been not detectable anymore at this stage of ontogeny.

In our field study, concentrations of both aucubin and catalpol in *P. lanceolata* leaves were correlated positively with plant species richness. These findings contrast with recent studies by Broz et al. (2010) and Mraja et al. (2011). Broz et al. (2010) analyzed in the field and in the greenhouse the concentration of an ubiquitous and very broad group of plant secondary metabolites (plant phenolic compounds) in dependence of vegetation composition; they found higher levels of total phenolics in methyl jasmonate induced *Centaurea maculosa* Lam. (Asteraceae) grown in monoculture than in plants grown with heterospecific neighbors. The treatment by methyl jasmonate was conducted to mimic herbivory. The authors concluded that the plant invests in defense when growing in monoculture, but in growth when the plant is surrounded by heterospecific neighbors. This conclusion is based on the idea that plants surrounded by conspecifics might suffer more from specialist herbivores (Root 1973) than plants surrounded by heterospecifics; and thus, enhanced levels of herbivory may lead to induced levels of total phenolics (Broz et al. 2010). The comparison of the relationship of plant secondary metabolite concentration and plant species richness in our study and the one by Broz (l.c.) suggests that the biosynthesis of different plant metabolite types is affected differently by the species richness in the surroundings; furthermore, invasive species (like *C. maculosa* in the USA) may respond differently to heterospecifics than a native plant in the field (like *P. lanceolata*) in our study.

Mraja et al. (2011) found a positive correlation of catalpol with increasing plant species richness and a negative one with aucubin in *P. lanceolata*. They performed their study within experimental grassland plots which were maintained to keep a certain plant species richness per plot constant. In contrast, our study was conducted in a cultural landscape at the naturally varying environmental conditions. Different methodological approaches applied to understand ecosystem functioning can result in different outcomes (cf. Scherber et al. 2006, Unsicker et al. 2006).

The observed positive correlation between IGs and plant species richness in our study could be a result of plant-plant interactions in terms of interspecific competition. For example, in *Solanum nigrum* L. (Solanaceae) plants that were grown under competition, genes for primary metabolism were downregulated, and genes involved in defense traits were upregulated (Schmidt and Baldwin 2006). Secondary metabolites maintaining plant defense against herbivores also can act allelopathically against non-host plant species neighbors (Siemens et al. 2002). This has also been shown for IGs inhibiting seed germination (Adam et al. 1979, Page et al. 1994). *Plantago lanceolata* roots and root exudates contain IGs (Wurst et al. 2010) and their concentrations correlate with IG concentrations in leaves (De Deyn et al. 2009). Those IGs exuded from the roots might defend the plant against heterospecific plant neighbors via the rhizosphere. Thus, the positive correlation between IGs and plant species richness could be a result of allelopathy due to induction by heterospecific plant neighbors and interspecific competition. Future studies need to elucidate how IGs in *P. lanceolata* affect other plant species and whether these compounds have a function in plant-plant interactions.

Plant metabolites and further trophic levels

The abundances of the herbivores *M. pascuorum* and *M. labilis* were not influenced by the secondary plant metabolites studied here. Bernays and Graham (1988) suggested that secondary metabolites might not be the driving force in plant-herbivore interactions. Herbivores might select their host plant also in response to the top-down pressure exerted by predators and parasitoids (e.g., Randlkofer et al. 2007). While IG concentrations had no effect on the abundances of herbivores, other toxic compounds that were not considered in this study or primary metabolites might have affected abundances of the herbivores studied here (Agrawal 2004).

While IG concentrations had no obvious impact on herbivore abundance, leaf nitrogen content correlated negatively with the abundances of both herbivores. Leaf nitrogen content of *P. lanceolata* and the LUI index correlated positively in our study. Furthermore, the LUI index correlated positively with several other plant response variables (Ellenberg nitrogen indicator values for the plant community, nitrogen and phosphorus concentration in plant biomass, phosphorus concentration in the soil, C/N ratio in the soil) (Blüthgen et al. 2012) that indicate higher availability of nutrients on plots with a more intense land use. Thus, our data suggest that both herbivore species are more abundant in nutrient-poor habitats (here indicated by lower leaf nitrogen content). According to the N limitation hypothesis (White 1993), nitrogen is the limiting factor in insect growth, and a better herbivore performance would be expected when leaf nitrogen quantities increase. However, some studies showed a negative correlation between herbivore abundance and plant nitrogen content (e.g., Agrawal 2004) or a worse insect performance when the plant´s nitrogen content increased (shown for *Lycaena tityrus* (Poda) (Lepidoptera, Lycaenidae) (Fischer and Fiedler 2000). In the following, we discuss different possibilities that might explain the higher abundances of the herbivores in nutrient-poor habitats:

1.) Predation risk for herbivorous insects can be enhanced on plants with a high nitrogen level (Prudic et al. 2005), and herbivores could try to escape parasitism by choosing plants with a low nutritional value (Fox and Eisenbach 1992, Sarfraz et al. 2009a). Herbivores feeding on a plant with a low nutritional value might provide a low quality host for a carnivore. A parasitoid needs to search for a high quality host since it cannot compensate the low nutritional host quality by consuming larger amounts of tissue like predators can do (Williams 1999). Hence, host acceptance by parasitoids is indirectly influenced by the host plant´s nutritional quality (Sarfraz et al. 2009a). The parasitoid might preferentially search for plants with high nutritional value, while the herbivore might try to escape parasitism by choosing plants with lower nutritional content. Even though such a scenario would explain the relationship between the abundances of weevils and plant nitrogen content in our study, it does not match our data with respect to the abundance of the parasitoid since the plant nitrogen content did not correlate with the abundance of the parasitoid *M. incultus*.

2.) Different ways of feeding adaptation have evolved among herbivores known as “flush feeders” and “senescence feeders”. “Flush feeders” have been suggested to be adapted to nitrogen-rich plant material, whereas “senescence feeders” are supposed to be adapted to nitrogen-poor plant material (White 1993, Schoonhoven et al. 2005). “Senescence feeders” are supposed to respond negatively to fertilizer application. In our study, the herbivores are suggested to belong to the “senescence feeders” and thus, may be adapted to plants with low nitrogen content and may avoid higher nitrogen availability and high nutrient habitats.

3.) Leaf nitrogen content was correlated with the LUI index that is concomitant with fertilizer application. High nitrogen availability may lead to unbalanced leaf amino acid profiles or to higher organic acid concentrations in plants which could stress herbivores or act toxic against them (Brodbeck et al. 1990, Sarfraz et al. 2009b). Furthermore, fertilization can lead to an imbalance of the protein / carbohydrate ratio by enhanced protein content (Cease et al. 2012). An herbivore can compensate the imbalanced nutrient profiles of one host plant by a more diverse food intake (Jonas and Joern 2008). Search for a more diverse diet requires high mobility by the herbivores; highly mobile herbivores may have to cope with high predation risks (Raubenheimer and Simpson 2003). To avoid these kinds of stress (imbalanced nutrient profile; high predation risk), herbivores might prefer plants with a low nitrogen content (Fischer and Fiedler 2000). This hypothesis is most congruent with the results in our study and also supported by a fertilization field study (Herbst et al. unpubl.) which found higher abundances of the herbivores *M. pascuorum* and *M. labilis* in unfertilized than in fertilized subplots.

Parasitization success

Host plant metabolite content did not affect the parasitization success of *M. incultus*. While the abundance of the parasitoid was positively correlated with herbivore abundance, parasitization success was negatively correlated with herbivore abundance. The relationship between parasitism and herbivore abundance can be positive, negative, or without correlation (Heimpel and Casas 2007). Negative density-dependent patterns of parasitism as shown here have been observed frequently in host-parasitoid interactions (Stiling 1987, Williams et al. 2001, Vanbergen et al. 2007), and can result from interference among parasitoids, higher probability of superparasitism, and handling time of the parasitoids (Heimpel and Casas 2007).

Conclusion

Our field experiments which were conducted in different geographic regions in Germany with varying environmental conditions showed that plant species richness can affect secondary metabolites of *P. lanceolata*, but this effect on the host plant did not influence higher trophic levels in a cultural landscape. However, high amounts of leaf nitrogen content in a host plant may negatively affect abundances of specialist herbivores that have evolved in a close interaction with the plant. The results of our study underline a negative impact of land use intensification on the abundances of specialist herbivores and their larval parasitoids.

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Table 1 : Effect of plant species richness, *Plantago lanceolata* density and the LUI index on total IGs (aucubin + catalpol), on aucubin and catalpol separately, and on nitrogen content of *P. lanceolata* leaves. Results of the linear mixed model are shown: *t* values, significant effects of the minimal adequate model (evaluated by Akaike information criterion: AIC), AIC, and denominator degrees of freedom (denDF) are given. P values are marked bold if significant. 77 plots are involved in the analysis.

	Total IG [a]			Aucubin [a]			Catalpol [a]			Nitrogen [b]		
Explanatory variables	*t* value	P	effect	*t* value	P	effect	*t* value	P	effect	*t* value	P	effect
Intercept	10.009	<**0.001**		7.462	<**0.001**		5.397	<**0.001**		11.880	<**0.001**	
Plant species richness	3.668	<**0.001**	+	3.523	<**0.01**	+	2.635	<**0.05**	–	-		
P. lanceolata density [c]	-			-			-			-		
LUI index	-			-			-			2.147	<**0.05**	+
AIC full model	593.2			531.8			537.7			-587.3		
AIC minimal model	590.8			523.0			534.5			-589.4		
denDF	73			73			73			73		

-: excluded by model simplification, [a] mg/g dry weight, [b] arc sine transformed, [c] ln transformed

Table 2: Medians (and interquartile ranges in parentheses), number of plots (N) and range of the abundances of the herbivores *Mecinus pascuorum* and *Mecinus labilis* and the parasitoid *Mesopolobus incultus* and their interaction (parasitization success) in each region.

Region		Herbivores		Parasitoid	
		Abundance of *M. pascuorum* (per plot)	Abundance of *M. labilis* (per plot)	Abundance of *M. incultus* (per plot)	Parasitization success of *M. incultus* (per plot)
Schorfheide (north)	Median	8	1	8	43
	(Interquartile)	(0-18)	(0-3)	(0-29)	(0-74)
	N	21	21	21	21
	Range	0-32	0-12	0-161	0-88
Hainich (central)	Median	1	0	6	49
	(Interquartile)	(0-19)	(0-3)	(0-25)	(0-82)
	N	22	22	22	22
	Range	0-137	0-110	0-618	0-100
Alb (south)	Median	0	0	1	24
	(Interquartile)	(0-0)	(0-4)	(0-5)	(0-100)
	N	34	34	34	34
	Range	0-4	0-52	0-31	0-100

Table 3: Effect of leaf nitrogen content and *Plantago lanceolata* (host) density on the abundances of the herbivores *Mecinus pascuorum* and *M. labilis*. Results of the generalized linear mixed model are shown: Estimates (β) with standard errors, *z* values and AIC are given for the minimal adequate model (evaluated by Akaike information criterion (AIC)). P values are marked bold if significant. 77 plots are involved in the analysis.

	Abundance *Mecinus pascuorum*				Abundance *Mecinus labilis*			
Explanatory variables	β	SE	*z* value	P	β	SE	*z* value	P
Intercept	1.2203	1.9882	-0.614	>0.05	2.3754	1.7061	1.392	>0.05
Nitrogen content [%]	-1.7581	0.6969	-2.523	**<0.05**	-1.8015	0.8445	-2.133	**<0.05**
P. lanceolata density[b]	0.7119	0.3174	2.243	**<0.05**	-	-	-	-
AIC full model	217.3				197.1			
AIC minimal model	213.4				194.0			

[b] ln transformed, -: excluded by model simplification

Table 4: Effect of herbivore (host) abundance on the abundance of the parasitoid *Mesopolobus incultus* and its parasitization success. Results of the generalized linear mixed model are shown: Estimates (β) with standard errors, *z* values and AIC are given for the minimal adequate model (evaluated by Akaike information criterion (AIC)). P values are marked bold if significant. 77 plots are involved in the analysis.

	Abundance *Mesopolobus incultus*				Parasitization success			
Explanatory variables	β	SE	*z* value	P	β	SE	*z* value	P
Intercept	-0.5121	0.2782	-1.841	<0.1	2.8153	0.5505	5.114	**<0.001**
Herbivore abundance[b]	1.1133	0.1216	9.159	**<0.001**	-0.9076	0.1728	-5.251	**<0.001**
AIC full model	251.6				150.9			
AIC minimal model	247.5				145.9			

[b] ln transformed

Fig. 1: The effect of (A) the LUI index on nitrogen content and (B) plant species richness on total IG content in *Plantago lanceolata*. Percentage data of nitrogen are arc sine transformed. Triangle symbols represent data of Schorfheide, circle symbols of Hainich, and square symbols of Alb. Lines show fitted values from the linear mixed model.

Fig. 2: The effect of plant species richness and the LUI index on plant metabolites (nitrogen and IGs) and further effects of metabolites and host density (*P. lanceolata* density for herbivores and herbivore abundance for the parasitoid) on abundances of higher trophic levels and their interaction (parasitization success). Solid arrows: positive relationship, dashed arrows: negative relationship, grey arrows: no significant relationship.

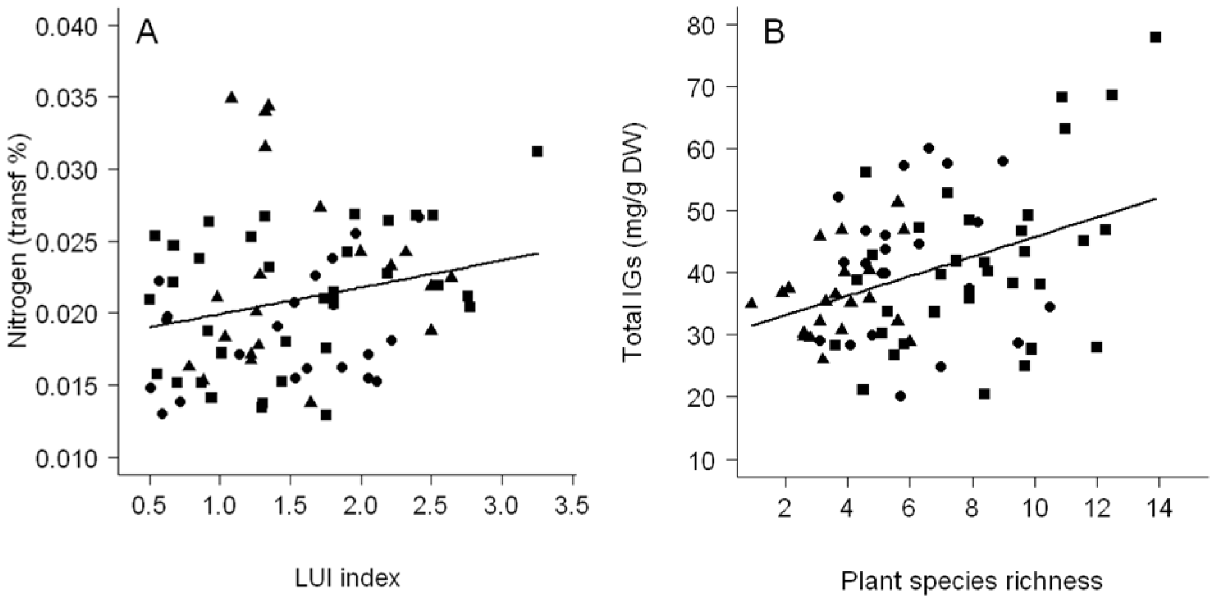
A
0.040
0.035
0.030
0.025
0.020
0.015
0.010
Nitrogen (transf %)
0.5
1.0
1.5
2.0
2.5
3.0
3.5
LUI index
B
80
70
60
50
40
30
20
10
Total IGs (mg/g DW)
2
4
6
8
10
12
14
Plant species richness

Fig. 1

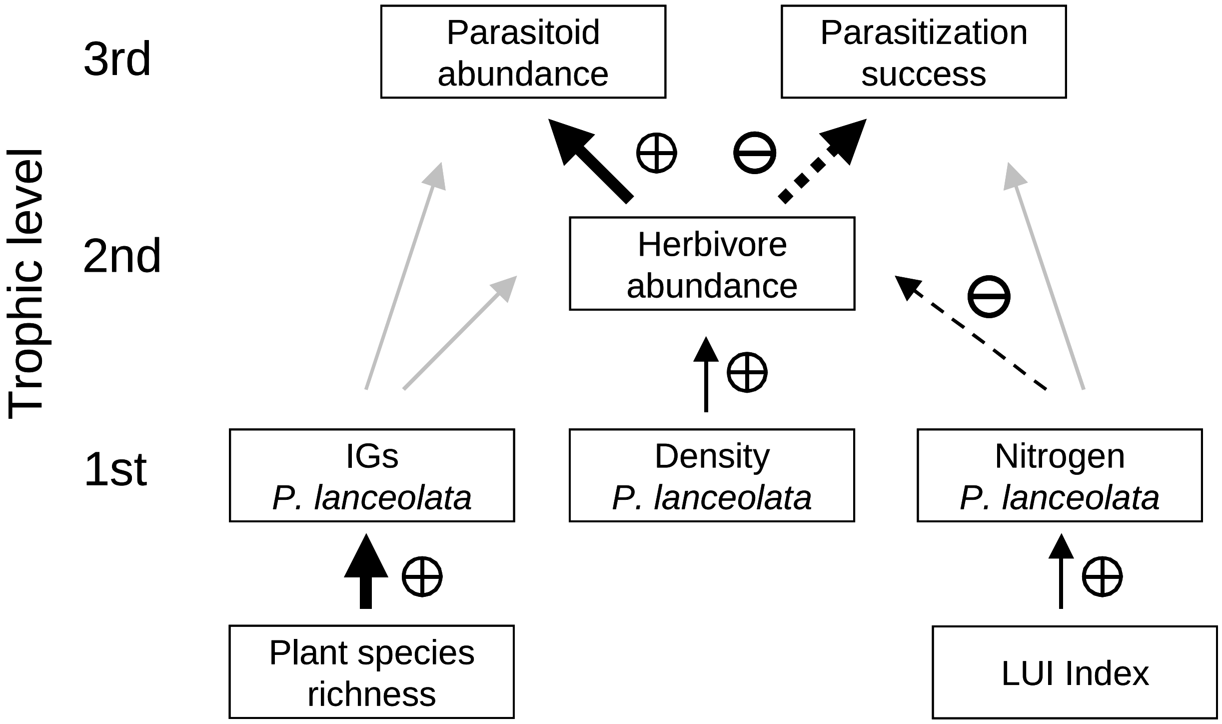
3rd
Parasitoid abundance
Parasitization success
Trophic level
⊕
⊖
2nd
Herbivore abundance
⊖
⊕
1st
IGs *P. lanceolata*
Density *P. lanceolata*
Nitrogen *P. lanceolata*
⊕
⊕
Plant species richness
LUI Index

Fig. 2

Supplemental Material:

Table S1 Medians and interquartile ranges in parentheses, number of plots (N) and ranges for the variables of the vegetation composition (plant species richness and *Plantago lanceolata* density) and *P. lanceolata* leaf metabolites (total IG and nitrogen contents) in each region.

Region		Plant species richness (average per plot)	*P. lanceolata* density (average per plot)	Total IG (mg/g DW)	Leaf nitrogen content (%)
Schorfheide (north)	Mean ± SE	3.7 ± 0.3	14.5 ± 3.1	36.3 ± 1.5	2.3 ± 0.1
	N	21	21	21	21
	Range	1-6	1-47	26-51	1.4-3.5
Hainich (central)	Mean ± SE	6.1 ± 0.4	17.4 ± 3.3	41.3 ± 2.5	1.9 ± 0.1
	N	22	22	22	22
	Range	3-11	1-52	20-60	1.3-2.6
Alb (south)	Mean ± SE	8.2 ± 0.5	22.9 ± 4.5	41.5 ± 2.3	2.1 ± 0.1
	N	34	34	34	34
	Range	4-14	2-111	20-78	1.3-3.1

Chapter - VIII

Habitats as complex odorous environment: How does plant diversity affect herbivore and parasitoid orientation?

Chapter VIII

Habitats as complex odorous environments: How does plant diversity affect orientation of insects?

Nicole Wäschke[1], Kristin Hardge[1], Christine Hancock[2], Monika Hilker[1], Elisabeth Obermaier[2,3], Torsten Meiners[1]

[1] Freie Universität Berlin, Institute of Biology, Applied Zoology / Animal Ecology, Haderslebener Str. 9, 12163 Berlin, Germany

[2] University of Würzburg, Department of Animal Ecology and Tropical Biology, Am Hubland, 97074 Würzburg, Germany

[3] present address: University of Bayreuth, Ecological-Botanical Gardens, 95440 Bayreuth, Germany

Corresponding author:

Dr. Torsten Meiners, Freie Universität Berlin, Institute of Biology, Applied Zoology / Animal Ecology, Haderslebener Str. 9, 12163 Berlin, Germany, phone: +49 30 83855910, email: meito@zedat.fu-berlin.de

Abstract

The effect of plant and plant odor diversity on non-pest insect species interactions under natural conditions has received attention in ecological research only recently. Here, we investigated the hypothesis that (a) plant diversity affects insect herbivore and parasitoid abundance, and (b) non-host plants which increase odor complexity in a habitat affect the host location ability of insect herbivores and their parasitoids. We chose the herb *Plantago lanceolata*, the herbivorous weevil *Mecinus pascuorum*, and its larval parasitoid *Mesopolobus incultus* as a model system. We surveyed vegetation and insect abundance data on 77 grassland sites in three different regions in Germany. Plant diversity was positively correlated with weevil abundance, whereas the parasitoid abundance was independent of plant diversity in the field. In laboratory olfactometer assays with weevils and parasitoids we offered combined host and non-host plant volatiles according to the field situation. Odors from non-host plant species did not affect the weevil´s detection capability of host plant odors in a newly established two-circle olfactometer. However, higher plant diversity enhanced the weevil´s foraging activity. A combination of host plant and host volatiles attracted the parasitoid both in the absence and presence of non-host plant volatiles in a Y-tube olfactometer. However, in choice-tests the parasitoid preferred the blend of host plant and host volatiles over its combination with non-host plant volatiles. Hence, both the weevils and parasitoids showed the sensory capacity to cope with complex vegetation odors during host search.

Keywords: tritrophic interactions, olfactory orientation, insects, non-host plants, complex odorous environment

Introduction

Host location is a crucial event in an insect's life history. It is a prerequisite for accessing food or oviposition sites (reviewed by Schoonhoven et al. 2005; Hilker and McNeil 2008). Herbivores as well as parasitoids use volatile cues of the host plant, the host, or the microhabitat, for locating their hosts at greater distances (reviewed by Visser 1986; Godfray 1994). However, multitrophic interactions take place in a heterogeneous and complex environment which is formed mainly by non-host plants (Casas and Djemai 2002). Non-host plants and high plant diversity may generate a complex odor bouquet (Randlkofer et al. 2010) which insects have to cope with when searching for their host by olfactory orientation.

Diverse plant patches affect host location behavior of herbivores (Root 1973; Unsicker et al. 2006; Randlkofer et al. 2010) and carnivores (Petermann et al. 2010; Randlkofer et al. 2010) in different ways. Negative effects may occur because of disruption of olfactory host location by non-host plant volatiles (Sheehan 1986; Finch and Collier 2000; Perfecto and Vet 2003; Randlkofer et al. 2007). Positive effects could occur indirectly due to an impact of plant diversity on adjacent trophic levels (Sheehan 1986; Theunissen 1994; Barbosa et al. 2009) or due to a higher food and nectar supply (Price et al. 1980; Andow 1991; Wäckers 2001). The availability of the host or the host plant also impacts insect performance and affects the occurrence and abundance of herbivores (Root 1973; Otway et al. 2005) and their parasitoids (van Nouhuys and Hanski 1999; Vanbergen et al. 2007). Only very few field studies have adopted a multitrophic approach when determining the importance of plant diversity on host location of insects (Aquilino et al. 2005; Petermann et al. 2010; Kostenko et al. 2012). However, the observed patterns of herbivore distribution might not only be the result of a bottom-up regulation by the host plant or of the olfactory capability of the herbivore, but also of an interaction of the herbivore with higher trophic levels resulting in a top-down regulation (Dicke 2000; Aquilino et al. 2005).

Both field and laboratory studies have to be taken into account when determining the impact of non-host plants on the orientation of herbivores and their antagonists. Field studies show correlative relationships between environmental factors and the presence of organisms, but controlled and simplified laboratory conditions are necessary in order to reveal the underlying mechanisms which determine successful performance of the organisms studied (Schmitz 2007). However, behavioral responses of insects to volatiles presented in the laboratory often differ from responses found in field studies (Randlkofer et al. 2007) since the impact of the complex odor bouquets present in the natural environment is often neglected in laboratory studies of insect olfactory behavior (Knudsen et al. 2008). Laboratory studies revealed that the effects of diverse odorous surroundings of a host plant or host may be manifold, e.g. positive for herbivores and their parasitoids, negative for both or for just one trophic level (reviewed by Schröder and Hilker 2008; Randlkofer et al. 2010). Non-host plant odors can mask the target odor (Thiery and Visser 1986; Gohole et al. 2003; Randlkofer et al. 2007) or may have a repellent impact (Hori and Komatsu 1997; Sanon et al. 2006). However, some insects are not disturbed by the diversity of odor released from other environmental sources present in the habitat where they are searching for a host (Dicke et al. 2003; Couty et al. 2006). Background (habitat) odor may indicate the presence of a host and even lead to the increased attraction of insects (Mozuraitis et al. 2002; Mumm and Hilker 2005).

So far, research of plant diversity effects on insects has focused mainly on crop plants and the orientation behavior of insect pest species (Finch and Collier 2000; Randlkofer et al. 2007). However, agricultural systems do not function like natural ecosystems, and insects might respond differently to environmental factors in the latter (Visser 1986). Since only a few studies investigated non-crop plant species and their interaction with higher trophic levels regarding volatile-based communication (Unsicker et al. 2009; but see Kessler and Baldwin 2001; Karban 2007; Pareja et al. 2007), the ecology of multitrophic interactions needs investigations under more natural conditions (Bezemer et al. 2010).

In the present study we combine both a field and a laboratory approach to reveal the impact of plant (odor) diversity on host location in a tritrophic system by using the perennial herb *Plantago lanceolata* L. (Plantaginaceae), the herbivorous weevil *Mecinus pascuorum* (Gyllenhal) (Coleoptera: Curculionidae), and its larval parasitoid *Mesopolobus incultus* (Walker) (Hymenoptera: Pteromalidae) as a model system. In the field we studied (1) the impact of plant diversity and host plant density on the abundance of the herbivorous weevil *M. pascuorum* and the parasitoid *M. incultus*; we surveyed vegetation data (number of plant species and their abundances) and abundances of the insect species studied here on grassland plots differing in plant diversity within a large scale project in Germany. In order to mimic natural odorous conditions in the lab we established a new olfactometer assay and tested (2) whether *M. pascuorum* is attracted by odor of its host plant, and if so, whether this attraction is affected by plant diversity. Furthermore, we investigated (3) whether the parasitoid *M. incultus* is attracted to odor of the host complex (host plant + host), and how plant diversity (presence of non-host plants) affects the parasitoid olfactory orientation to the host complex.

Material and Methods

Field

The ubiquitous herb *P. lanceolata* is native to Europe (Schubert and Vent 1990). It occurs on meadows and pastures and is widespread along different kinds of habitats differing in plant diversity. The weevil *M. pascuorum* oviposits in the seeds of *P. lanceolata* in June and July. Weevil larvae develop within the seeds and hatch from August to September. They are hosts of the parasitic wasp *M. incultus* (Mohd Norowi et al. 2000). The host plant *P. lanceolata*, the weevil and the parasitoid occur in all three geographical regions studied here.

The study took place within the German priority project Biodiversity Exploratories which is described in detail by Fischer et al. (2010). In three geographical regions (exploratories) in Germany (from north to south: Biosphere Reserve Schorfheide-Chorin, National Park Hainich-Dün, and Biosphere Area Schwäbische Alb) 50 grassland plots were assigned to biodiversity research. One plot is 50m x 50m. The three regions across Germany differ in their environmental variables like precipitation, altitude, and annual mean temperature (Fischer et al. 2010). The grassland sites are established within a land use gradient and thus show differences in plant diversity. Since land use intensity and plant diversity are negatively correlated with each other (Blüthgen et al. 2012), we discarded land use intensity and focused on plant diversity effects on insect abundance.

Because of the limitation given by the occurrence of the host plant (*P. lanceolata*), the number of plots studied was 21 in Schorfheide-Chorin, 22 in Hainich-Dün, and 34 in Schwäbische Alb. Within each plot we sampled ten focus *P. lanceolata* plants in 4m x 4m subplots located in the corners of each plot. The number of herbaceous plant species and the vertical coverage of each plant species (Braun-Blanquet 1964) (r=15cm) as well as host plant density (r=100cm) around the chosen focus plant were surveyed from May until July 2008. To determine the abundance of *M. pascuorum* and *M. incultus* we collected *P. lanceolata* inflorescences from July to August 2008. Since just a small number of insects might hatch from the ten focal plant inflorescences collected at each plot, we collected additionally 100 randomly chosen *P. lanceolata* inflorescences per plot. Inflorescences of *P. lanceolata* were kept in plastic boxes (17.0cm x 12.5cm x 5.6cm) with a fine-meshed gauze (0.12mm) top cover under constant conditions (11:13 LD, temperature: 22°C, 50% rH). Insects hatched in August and September 2008 and were identified and counted.

Laboratory assays

Herbivore. Weevils used for the olfactometer bioassays were collected from June to July 2009 when female *M. pascuorum* were searching for oviposition sites (Dickason 1968; Mohd Norowi et al. 2000). To reduce a possible impact of sampling in the studied regions, the weevils were not collected on the Biodiversity Exploratory plots, but at a site called Wuhletal (Berlin Marzahn-Hellersdorf, Germany; 52°32'2''N, 13°34'50''E). The weevils were reared at 23°C ± 1°C, 48% rH and 14:10 LD in plastic boxes (20cm long x 10cm wide x 6cm high) with a fine-meshed gauze top. Male and female weevils were kept together for at least one week to ensure mating before separating the sexes. Weevils were fed daily with fresh host plant material (inflorescences and leaves).

Plants used for bioassays with herbivores

Seeds were obtained from the seed collection of the Botanical Garden Berlin. Seeds were sown in soil (Einheitserde Typ T Topferde, Einheitserde- und Humuswerke Gebr. Patzer GmbH & Co. KG, Sinntal - Jossa, Germany), and individual plants were separated after 4 to 5 weeks. At the same time, pots were filled with soil and were later used as dummy plants. Seven- to nine-week-old plants were used in the bioassays. The *P. lanceolata* plants were in a flowering state at this age. All other plants used for bioassays were not bloomy. Two herbs (*Achillea millefolium* L. (Asteraceae), *Agrimonia eupatoria* L. (Rosaceae)) and two grasses *(Festuca rubra* L., *Poa pratensis* L. (both Poaceae)) were used for generating a complex odor blend. All plants occurred in the natural habitat of *M. pascuorum* and co-occurred with *P. lanceolata*. Plants were grown in a greenhouse at 24-30°C, 20-34 % rH, and 14:10 LD.

Olfactometer setup for bioassays with herbivores

We created a new type of olfactometer in order to mimic an odorous background around the host plant comparable to the field situation (Fig. 1). This static two-circle olfactometer consisted of a circular polyamide gauze (mesh width 0.12mm, diameter 180mm) that served as walking arena and was divided into a central (diameter 60mm) and an ambient circle. The walking arena was stabilized by metal stands of 40cm height. Test plants were placed below the walking arena either into the central chamber or into the ambient chamber. The wall of the chambers consisted of flexible polyethylene bags (Toppits ®, Cofresco Frischhalteprodukte GmbH & Co. KG, Minden, Germany) which were clipped to a glass plate (30cm x 30cm) at the bottom of the entire set-up. The ambient chamber provided space for four flower pots, and up to three flower pots could be placed in the central chamber. A light source (60 Watt, photosynthetic active radiation 6µmol m^{-2} s^{-1}) was located above the olfactometer in a distance of 50cm to the walking arena in order to illuminate the test arena uniformly. Bioassays were conducted from 10am to 6pm under laboratory conditions of 22 ± 1°C and 43-65% rH. To avoid diurnal biases, experiments with the same plant arrangement (treatment) were conducted on different days and at different day times. The experiments were conducted in June and July 2009 when females are ovipositing. After every tested female the walking arena was cleaned with 96% ethanol and allowed to dry before reuse. Odor from one plant arrangement was offered consecutively to five females. Afterwards the whole olfactometer was cleaned with ethanol. The polyethylene foil was changed for every treatment. Twenty females were tested separately for one treatment (plants: N=4).

Each female was observed for 300s. The females were allowed to acclimate for one hour in the test room without food. The plants were acclimated in the olfactometer setup for one hour. In order to evaluate the weevils´s host plant finding success and search activity, we recorded different behavioral components by using the software “The Observer 3.0” (Noldus, Wageningen, The Netherlands): time the weevil spent in the central field (duration of stay), time the weevil needed to enter the central field (target odor) for the first time (latency), number of switches between central and ambient field (frequency), total time walking of one weevil during the observation time (activity).

Herbivore olfactory orientation to the host plant

Dummy plants (here referred to as dummies) were built from a flower pot with soil and a green sheet of paper that was rolled up and plugged into the soil. In experiments with vacant zones in the ambient chamber, dummies were placed in the olfactometer to provide a consistent visual stimulation (Finch and Collier 2000). For control, we tested a dummy in the central and four dummies in the ambient chamber. In order to test the attractiveness of a non-host plant, *A. millefolium* was placed in the central chamber, and four dummies were placed in the ambient chamber. The attraction to the host plant was tested by placing a flowering *P. lanceolata* in the central chamber, and four dummies in the ambient chamber. We used only flowering host plants because these are the targets of gravid female weevils. We compared the duration of stay in these experiments.

Herbivore olfactory orientation to the host plant in an odorous environment

Odors from different plants that are placed in the central chamber might be perceived as a single blend by an insect since the odor sources are placed very closely together. In contrast, odor provided by plants in the central chamber and odor released from plants in the ambient chamber might be perceived as separate blends because the odor sources are further apart than those placed altogether in the central chamber. It is well known that successful host location may depend on whether the odor source of a host is detected separately from other odor sources (e.g. Bruce et al. 2005). Therefore, we tested the effect of plant diversity (a) by placing non-host plants (two herbs) with the host plant in the central chamber while four dummies were placed in the ambient chamber and (b) by placing the host plant in the central chamber and two herbaceous non-host plants plus two dummies in the ambient chamber. In a further bioassay, we tested (c) how the orientation of the weevil to odor of the host plant in the central chamber is affected by odor from four different non-host plants (two herbs and two grasses) placed in the ambient chamber (i.e. higher plant diversity than in bioassay (b)) (Tab. 1). In all three set-ups a flowering *P. lanceolata* plant was positioned in the central field.

Parasitoid

Parasitoids were obtained from *P. lanceolata* inflorescences collected in the Biodiversity Exploratories in July and August 2010. The inflorescences were kept under the same conditions as the ones obtained for the fieldwork data in 2008 (described above). Emerging unparasitized *M. pascuorum* as well as parasitoids emerging from inflorescences infested with *M. pascuorum* larvae were taken out of the boxes every two days. *Mecinus pascuorum* were reared at 23°C ± 1°C, 48% rH and 18:6 LD in plastic boxes (described above) and fed regularly with host plant material. The parasitoid *M. incultus* was kept at 10°C ± 1°C, 65% rH, 18:6 LD and fed with aqueous honey solution. The parasitoids were kept in this cool environment in order to enhance their life span. Both insect species were kept at long-day period to mimic summer time and to retard hibernation. Parasitoids were four to six weeks old when tested. In total 446 male and female parasitoids hatched. Both sexes were kept together for at least two weeks. Just female parasitoids were tested. Because of the high number of replications and a shortage of parasitoids, we had to test each parasitoid about 1.3 times. Parasitoids were pooled after testing. Female parasitoids were chosen randomly from this pool for the next test. Parasitoids could rest at least 2 days between two consecutive tests. Bioassays were performed in August and September 2010.

Plants used for bioassays with parasitoids

Seeds of plants for bioassay with parasitoids were obtained from Appels Wilde Samen GmbH

(Darmstadt, Germany). Sowing and planting was conducted as described above for the bioassay with weevils. Non-host plant species (*A. millefolium* and *A. eupatoria*, N = 7 each) were the same as those used for the bioassays with weevils. After each treatment we randomized these plant individuals and chose randomly plants for the next treatment. The non-host plants were about seven to nine weeks old when used for the bioassays with parasitoids.

Olfactometer setup for bioassays with parasitoids

We used a dynamic Y-tube olfactometer in order to test (i) whether the parasitoid is attracted by odors from the host complex and (ii) how olfactory orientation of the parasitoid is affected by the presence of non-host plants. The host complex consisted of flowering *P. lanceolata* and five female and five male *M. pascuorum* adults. Although the parasitoid *M. incultus* is a larval parasitoid, we were not able to conduct experiments with weevil larvae, since dissection of plant seeds would have injured the larvae and laboratory oviposition by weevils would not have been ensured an infestation. However, the weevils lay their eggs in *P. lanceolata* seeds in June and July and stay on the plants where they have oviposited (pers. observation). As parasitoids parasitizing inconspicuous hosts or host stages (here: larvae hidden within seeds) may use also cues from non-appropiate host stages (e.g. adults), the host complex was provided by adult weevils plus the host plant when testing the orientaion abilities of the parasitoid. The two-circle olfactometer used for testing the weevil could not be adopted for the parasitoid, since side or area preferences could not be excluded, and a faster movement of the parasitoids led to a long residence time in the central field in all setups. The Y-tube olfactometer consisted of a Y-shaped glass tube (one 20cm arm and two 14cm branched arms, diameter: 1.2cm). The open ends of the branched arms were connected by Teflon tubing to glass jars (2100 ml) containing the odor sources (host complex, plants). Air that entered the glass jars was charcoal-filtered and humidified. Air was pumped with a flow of 138 ml/min through the setup. The flow was controlled by flowmeters (Supelco, Bellefonte, PA, USA). Both, the tested odor sources and the parasitoids were acclimatized in the test room one hour before testing. Bioassays were conducted in a darkened room at 21° ± 1°C and 50-60% rH. One parasitoid was placed at the opening of the long arm of the Y-tube and was observed for maximal 300s. We recorded the number of parasitoids which entered one arm and crossed an imaginary border of 5cm within this arm. Ten parasitoids were tested for each odor source. After testing ten parasitoids, tubes and glass jars were cleaned with 96% ethanol, heated at 100°C for one hour, and odor source sides were exchanged. A blank test was conducted, and the parasitoids showed no side preference. As a control we used a pot filled with soil. The parasitoid´s olfactory response to the following combinations was tested: (a) odor of the flowering host plant *P. lanceolata* versus control (N = 30); (b) odor of five female and five male *M. pascuorum* adults versus a control (N= 30); (c) odor of the flowering host plant *P. lanceolata* with five female and five male *M. pascuorum* adults versus a control (N = 50); (d) odor of two non-host plants (*A. millefolium* and *A. eupatoria*) plus flowering *P. lanceolata* combined with five female and five male *M. pascuorum* adults versus a control (N= 50); and (e) odor of setup (c) versus setup (d) (N = 50).

Statistical analysis

Field

All calculations were performed by R (Version 2.12.1; R Development Core Team, 2010). Field data were analyzed using a generalized linear mixed model. Plant diversity was calculated according to the Shannon-Index $H = - \sum p_i \times \ln p_i$ where p_i is the ratio of the i^{th} species compared to the entire pool. We calculated mean values per plot for host plant density and plant diversity. The region was used as a random effect. Explanatory variables with non-normal distribution were ln transformed for stabilizing variance (Crawley 2007). A term was added (+1) before transformation if necessary. Models were calculated by the lmer function with Laplace approximation in R (package lme4 Version 0.999375-37) with a Poisson error distribution (link = log) for the abundance data as response variables. To account for overdispersion we added an individual based random effect (Elston et al. 2001). We started with the full model and dropped terms that were not significantly different from zero. Models were compared by Akaike Information Criterion (AIC, Burnham and Anderson 2002) until we ended up with a minimal adequate model with the AIC not dropping anymore or all terms included in the model being significantly different from zero. As fixed effects we added plant diversity and host plant density. To analyze the effect of vegetational parameters on the parasitoids´ abundance we corrected for the host abundance (Sheehan 1986) by including this variable in the model as a covariate. For calculating host abundance in addition to *M. pascuorum* another weevil (*Mecinus labilis* (Herbst) (Coleoptera: Curculionidae)) was included which co-occurs with *M. pascuorum* and is parasitized by *M. incultus*.

Herbivore olfactory orientation to the host plant

The two-circle olfactometer test data were analyzed by the Wilcoxon one sample-test when comparing the observed duration of stay in the central field with an expected value corresponding to the size of the field (33.3s). If the weevils´ duration of stay in the central field did not significantly differ from 33.3 sec, the weevils did not discriminate between odor in the central and the ambient field.

Herbivore olfactory orientation to the host plant in an odorous environment

We compared the weevils´ olfactory responses to the odors from the different odor sources combinations offered in the two-circle olfactometer (Tab. 1) by Kruskal-Wallis ANOVA followed by Mann-Whitney-U-Tests with Bonferroni correction (Sachs 1992). For these comparisons, we used the following parameters: duration of stay in the central field, latency, frequency, and walking activity. The behavioral component “latency” was the time the weevil needed to enter the central field for the first time during the observation period of 300s; the “latency” was set 300s when the weevil has not reached the central field at all. “Frequency” describes how often a field was visited and serves as indicator of switches between odor fields. Walking activity has been measured as the time during which the weevils were actively walking around rather than resting or cleaning themselves. We used a nonparametric test accounting for non-normality of the data. Variance homogeneity was checked by Levene-Test, and if necessary, logarithmic transformation was conducted.

Parasitoid

Data obtained from the parasitoid bioassay were analyzed by the Sign Test (MacKinnon 1964). Just parasitoids that made a decision were included in the analysis.

Results

Abundance of Mecinus pascuorum and Mesopolobus incultus in habitats with different plant diversity

To reveal the effect of plant diversity on abundance of the herbivore and its parasitoid we sampled vegetation data in three different regions in Germany. The field data revealed a positive effect of plant diversity and no effect of host plant density on the abundance of *M. pascuorum* (Tab. 2). The abundance of the parasitoid *M. incultus* was independent of plant diversity, and best explained by the abundance of its host (the weevils). Abundances of the parasitoids and the weevils correlated positively (Tab. 2).

Plant diversity was enhanced from north to south with a Shannon Index (H) of 0.9 in Schorfheide, 1.5 in Hainich, and 1.7 in Alb. Host plant (*P. lanceolata*) density in a radius of one meter around the chosen focus plant ranges in average from 14.5 ± 3.1 in Schorfheide, 17.4 ± 3.3 in Hainich, and 22.9 ± 4.5 in Alb. The abundance of the herbivore *M. pascuorum* was highest in Schorfheide with a median value of 8 individuals per plot and ranges from 0-32 individuals. In Hainich and Alb the individual numbers ranged from 0-137 and 0-4 with median values of 1 and 0 respectivley. The abundance of the parasitoid *M. incultus* followed the abundance pattern of its host with highest abundance in Schorfheide (median: 8 individuals per plot) and the lowest in Alb (median: 1 individuals per plot), and intermediate in Hainich (median: 6 individuals per plot). The ranges were highest in Hainich with 0-618 individuals, followed by Schorfheide with 0-161 and Alb with 0-31 individuals per plot.

Olfactometer assays

Herbivore

The two-circle olfactometer proved to be a suitable device for testing the orientation of the weevils in complex bouquets. There was no significantly longer duration of stay in the central field compared to the expected value (33.3s) for an equal duration of stay in the entire arena in the setup with dummies in the central and the ambient chamber (median duration of stay: 0 s; interquartile ranges: 0-4.8 s). Females of *M. pascuorum* stayed significantly longer in the central field with the host plant odor than expected (61.6 s; interquartile ranges: 7.1-124.8 s; Tab. 3) and thus were attracted and/or arrested by odors from the flowering host plant *P. lanceolat*a. In contrast, the weevils showed no response to odor of the non-host plant *A. millefolium* (no significantly longer duration of stay in the central field (median: 32.8 s; interquartile ranges: 0-103.8 s) than expected at equal durations of stay in the entire arena.

When comparing the weevil´s response to the different plant combinations, the duration of stay in the central field and latency of *M. pascuorum* to reach the central field did not differ between the treatments with the various plant arrangements tested here (Tab. 3). Neither odor from non-host plants offered in the ambient field nor that of non-host plants placed additionally to *P. lanceolata* beneath the central field hampered the host finding process. This indicates that there is no disrupting effect of non-host odors on host finding by the females.

However, when comparing the different treatments we observed significant differences in the frequency by which *M. pascuorum* crossed field borders as well as in its overall walking activity (Fig. 2). These behavioral components were enhanced when the weevils experienced odors from two herbs and two grasses in the ambient chamber additional to the host plant in the central chamber compared to the setup with only *P. lanceolata* placed in the central chamber and no other plants in the ambient chamber (Fig. 2).

Parasitoid

Dynamic Y-tube olfactometer studies were conducted for revealing the influence of non-host odors on the orientation of the parasitoid *M. incultus* in the laboratory. The parasitoid *M. incultus* was not attracted by odor of the host plant *P. lanceolata* (choices: 15 test / 11 control / 4 no decision; $p>0.05$) nor by host odor (choices: 11 test / 16 control / 3 no decision; $p>0.05$). However, the parasitoid was attracted to odor from the host complex consisting of flowering *P. lanceolata* and female and male weevils when tested against a control. Odor from non-host plants (i.e. increased plant diversity) did not affect the attractiveness of the host complex when added to the host complex odor and tested against a dummy. However, when offering the parasitoids a choice between odor from the host complex only (without non-host plant odor) and odor from the host complex with non-host plant odor added, *M. incultus* preferred the simpler odor bouquet (Fig. 3). The results indicate that *M. incultus* can distinguish between non-host and host odors.

Discussion

We used field surveys and laboratory assays to test the hypothesis that high plant diversity disturbs host location both by an herbivorous insect and a parasitic one attacking herbivorous larvae. We studied olfactory responses of the weevil *M. pascuorum,* a specialist on *P. lanceolata,* and of the pteromalid wasp *M. incultus,* a larval parasitoid of this herbivore. The field data showed that increased plant diversity is positively correlated with the herbivore´s abundance, but not with the parasitoid´s abundance; the latter was positively linked with the herbivore´s abundance. These findings indicate that successful resource location capacities of both insect species are not hampered by increased plant diversity. This suggestion is supported by our laboratory data. Both insect species showed excellent olfactory host finding capacity even in the presence of non-host plant odors; host location was hardly hampered by the presence of non-host plant odors. Hence, we suggest that increasing plant diversity does not (or only marginally) disturb olfactory host location by the non-crop species studied here.

Impact of plant diversity on a herbivore and its parasitoid in the field

Studies based on observations from agricultural habitats show a decrease of the abundance of specialized herbivores in more diverse habitats and conclude that specialists are negatively affected by plant diversity (Root 1973, reviewed by Finch and Collier 2000). In contrast, when considering plant - herbivore interactions of non-crop species in a natural or seminatural context, several studies found a positive effect of plant diversity on plant damage by herbivory (Mulder et al. 1999; Scherber et al. 2006), probability of herbivore occurrence (Randlkofer et al. 2007), and herbivore abundances (Siemann 1998; Unsicker et al. 2006), thus corroborating the results of our study.

In our field study the abundance of the larval parasitoid *M. incultus* was explained exclusively by host abundance. The positive correlation found between abundances of weevils and parasitoids might be explained by improved oviposition possibilities for the parasitoids in patches with high host density, thus leading to an aggregation of parasitoids in those patches (Janz 2002; Vanbergen et al. 2007). The higher the trophic level, the more difficult it is to determine the impact of specific environmental factors on this trophic level. The observed outcome is often not directly caused by a single factor, but is mediated indirectly *via* its effect on other trophic levels (Siemann et al. 1998; Petermann et al. 2010).

Impact of plant diversity on host location of a herbivore and its parasitoid in the laboratory

In the laboratory we tested the effect of different arrangements with varying plant diversity on the olfactory orientation of the weevil and its parasitoid. A new olfactometer setup was designed and presented in detail in the ‘Methods’ section above. The weevil´s orientation ability could be tested very well with this device, but not so that of the parasitoid. The searching behavior of these two insects differs substantially. The weevil is walking through vegetation rather than flying like the parasitoid species tested here (pers. observation). Flying insects orientate differently in the vegetation and use wind-borne signals. A dynamic olfactometer might be more appropriate for testing the orientation of insects which move fast and fly when searching for a host (Visser 1988; Schoonhoven et al. 2005).

The herbivorous weevil *M. pascuorum* was attracted by volatiles of flowering *P. lanceolata*. Females oviposit in the seedheads and feed on stalks and leaves. Our study shows that diverse plant bouquets did not hamper the weevil´s success in finding the host plant by olfactory cues, but induced increased activity of *M. pascuorum*. A suitable foraging habitat might be an environment which elicits intensified host searching behavior (Pettersson et al. 2008), i.e. increased locomotion activity. Since the ubiquitous herb *P. lanceolata* emits few volatiles (Fontana et al. 2009), background odor released from non-host plants might indicate the presence of a suitable habitat. The role of non-host plant odor for host foraging in insects is ambivalent. On the one hand, non-host odor has been shown to impede host foraging in many insect species (e.g. Zhang and Schlyter 2004, 2010), whereas on the other hand non-host odor or ubiquitous green leaf volatiles were found to have positive effects on host location of some insects (e.g. Müller and Hilker 2000; Mumm et al. 2005). The weevil *M. pascuorum* may need additional olfactory cues from the host plant habitat for activation of searching behavior. A rich odorous environment may stimulate weevils to search more intensively and may improve the likelihood of locating a host plant. Thus, our field and laboratory data suggest that odor of vegetation with greater plant diversity indicates a "patch of interest" for the weevil. Such patches may provide enhanced host plant quality or offer more refugia which allow the weevils to escape from natural enemies or competitors (Gilbert and Singer 1975).

The larval parasitoid *M. incultus* was attracted by the host complex odor (i.e. odor from the host plant *and* weevils) and was capable to discriminate between host complex odor offered with and without non-host odor. The parasitoid preferred the simpler bouquet without non-host odor. Parasitoids do not only use volatile cues from the host or the host-plant for host location, but also cues emitted by the host habitat (Vet et al. 1995). In the tritrophic system studied here the weevils lay their eggs in June and July and remain on the plants where they have oviposited (pers. observation). The host plant *P. lanceolata* emits mainly green leaf volatiles. The emission of volatiles from *P. lanceolata* was found to significantly increase after herbivory by generalist larvae which severely damaged the plant (Fontana et al. 2009). However, *M. pascuorum* adults only chew small parts of the flower stem. Since plant damage is usually proportional to plant volatile emission, at least for lipoxygenase pathway products (green leaf volatiles) (Copolovici et al. 2011), the weevils are not expected to induce *P. lanceolata* in such a way that it leads to a significantly higher volatile emission. However, for parasitoids searching for herbivorous hosts the quantity of emitted and perceived plant volatiles is important (Dicke et al. 2003). Thus, it might be beneficial for the parasitoid to take into account general cues from the habitat where the host with its host plant occurs. Gohole et al. (2005) showed that the presence of non-host plants does not hinder the close-range foraging activities of different parasitoids. In conclusion, *M. incultus* may use habitat odors for long-range orientation and might respond specifically to the pure host complex at a short-range distance.

In our laboratory bioassays we have shown that both insect players are not prevented from host location by greater plant diversity. In the field, the herbivorous weevil is positively associated with plant diversity, and odors from a plant-rich community enhanced its activity in the olfactometer. Diverse habitats may constitute high quality patches in grasslands when considering multitrophic interactions occurring in natural or semi-natural habitats. To understand the mechanisms behind the positive relationship between plant-rich communities and organisms taking part in multitrophic interactions, further studies are necessary to determine the factors being altered by plant diversity and impacting habitat quality.

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Figure legends:

Figure 1: Bioassay setup. The two-circle olfactometer consists of a central field (diameter 60mm) and an ambient field. The total diameter of the arena is 180mm. Plants and dummies are placed below the walking arena consisting of a gauze. The chamber walls are provided by polyethylene foil (here: cooking bag).

Figure 2: Orientation of the weevil *Mecinus pascuorum* to its host plant *Plantago lanceolata* in different odorous surroundings during 300s in the two-circle olfactometer (N=20 females per treatment). Number of switches between the fields (frequency) and walking time (in seconds) are shown as medians and quartiles for combinations of host plant (HO) and non-host plants (HE: herbs; GR: grasses) as well as dummies (DU). Different letters indicate significant ($P \leq 0.05$) differences (Kruskal-Wallis ANOVA followed by Mann-Whitney-U-Test and Bonferroni correction).

Figure 3: Response of the parasitoid *Mesopolobus incultus* (in % of total number of females responding) to odors offered in a Y-tube olfactometer. A pot with soil served as control. Different odor sources were used: HO: host plant *Plantago lanceolata*, weevil: five male and five female *Mecinus pascuorum*. HE: herbs *Achillea millefolium* + *Agrimonia eupatoria*. Just parasitoids making a decision were included in the analysis. Numbers of parasitoids making a decision (N_d) and numbers of tested parasitoids (N_t) are given. Data were analysed by the Sign Test according to MacKinnon: **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 1: Different treatments tested to study for the orientation of the weevil *Mecinus pascuorum* to the host plant in an odorous environment in the two-circle olfactometer. Position of plants in the central and in the ambient chamber is given. Dummies consisting of a pot filled with soil and a green sheet of paper. Data were analyzed by Kruskal-Wallis ANOVA.

Central field	Ambient field
P. lanceolata (HO)	Dummies (DU)
P. lanceolata (HO) + *A. millefolium* + *A. eupatoria* (HE)	Dummies (DU)
P. lanceolata (HO)	*A. millefolium* + *A. eupatoria* (HE) + Dummies (DU)
P. lanceolata (HO)	*A. millefolium, A. eupatoria* (HE) + *F. rubra, P. pratensis* (GR)

DU: dummy. HO: host plant. HE: herbs. GR: grasses.

Table 2: Results of a generalized linear mixed model describing the abundances of the herbivorous weevil *Mecinus pascuorum* and the parasitoid *Mesopolobus incultus* in the field.

	Abundance of *Mecinus pascuorum*				Abundance of M*esopolobus incultus*			
Explanatory variables	β	SE	*z* value	P	β	SE	*z* value	P
Intercept	-5.6026	2.1219	-2.640	**<0.01**	-0.5121	0.2782	-1.841	<0.1
Plant diversity (H)	2.5085	1.0397	2.413	**<0.05**	NA	NA	NA	NA
Host plant density ^{b}	0.5961	0.3311	1.800	<0.1	NA	NA	NA	NA
Host (weevil) abundance ^{b}	-	-	-	-	1.1133	0.1216	9.159	**<0.001**
AIC full model	214.7				250.7			
AIC minimal model	214.7				247.5			

Estimates (β) with standard errors (SE) are given for the minimal adequate model (evaluated by Akaike information criterion (AIC)). P values are marked bold if significant. 77 plots were involved in the analysis.

^{b}: ln transformed; NA: excluded from the model; -: not included in the full model

Table 3: Host plant odor finding by the weevil *Mecinus pascuorum* in the presence of different plant odors surroundings in the olfactometer.

Treatment		Duration of stay in central field [s]	Time to reach the central field [s]
Central field	Ambient field		
HO	DU	61.6 (7.1-124.8)	74.1 (30.2-293.0)
HO + HE	DU	87.9 (0.0-155.6)	122.9 (80.2-300.0)
HO	HE + DU	78.0 (12.8-139.5)	43.9 (17.9-279.6)
HO	HE + GR	71.2 (51.1-112.3)	65.2 (27.0-125.5)
	Statistics	n.s.	n.s.

Duration of stay in the central field and time to reach the central field (latency) in seconds are shown for 20 females tested per treatment observed for 300s. Herbs (HE; *Achillea millefolium* and *Agrimonia eupatoria*) and grasses (GR; *Festuca rubra* and *Poa pratensis*) were presented in different combinations in the ambient or additional to the host plant (HO; *Plantago lanceolata*) in the central field (see Tab. 1 for details). Medianes and interquartile ranges (parentheses) are given. n.s. indicates no significant ($P>0.05$) difference evaluated by Kruskal-Wallis ANOVA followed by Mann-Whitney-U-Test and Bonferroni correction. DU: Dummy.

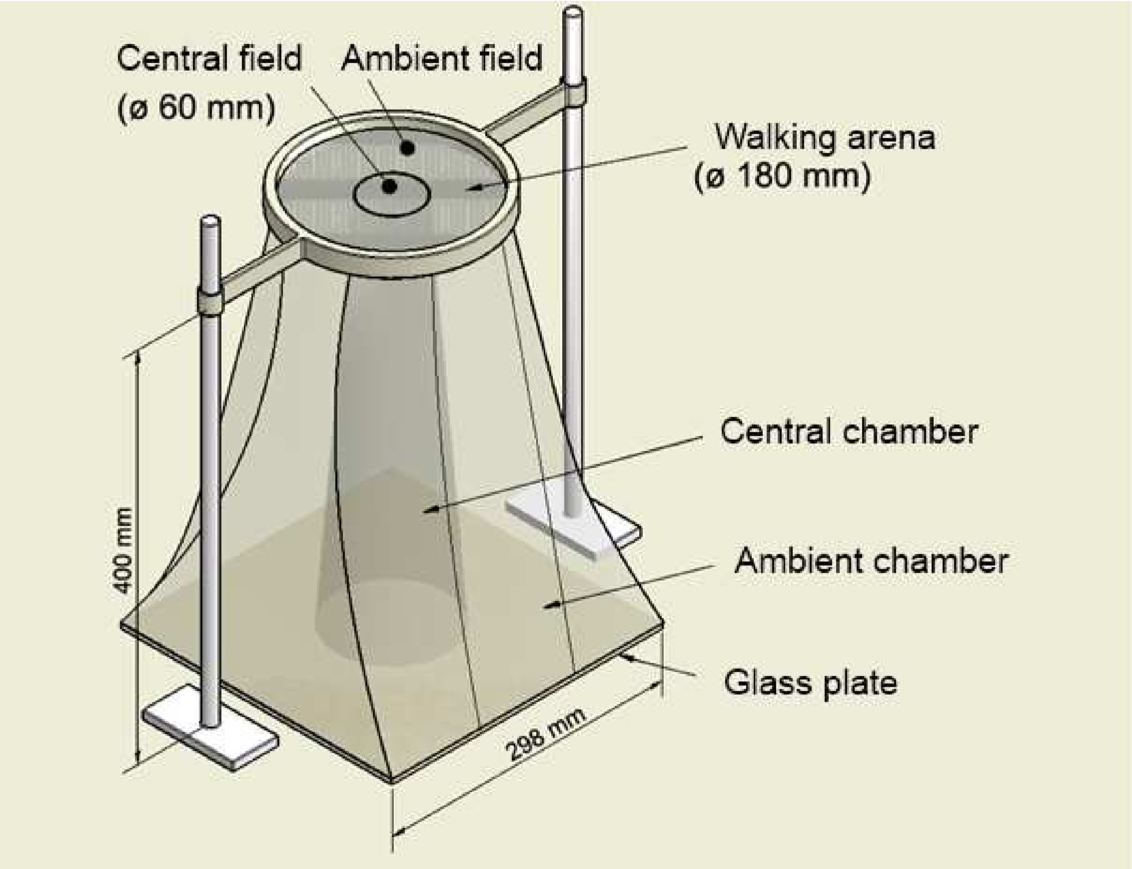
Central field
(ø 60 mm)
Ambient field
Walking arena
(ø 180 mm)
Central chamber
Ambient chamber
Glass plate
400 mm
298 mm

Fig.1

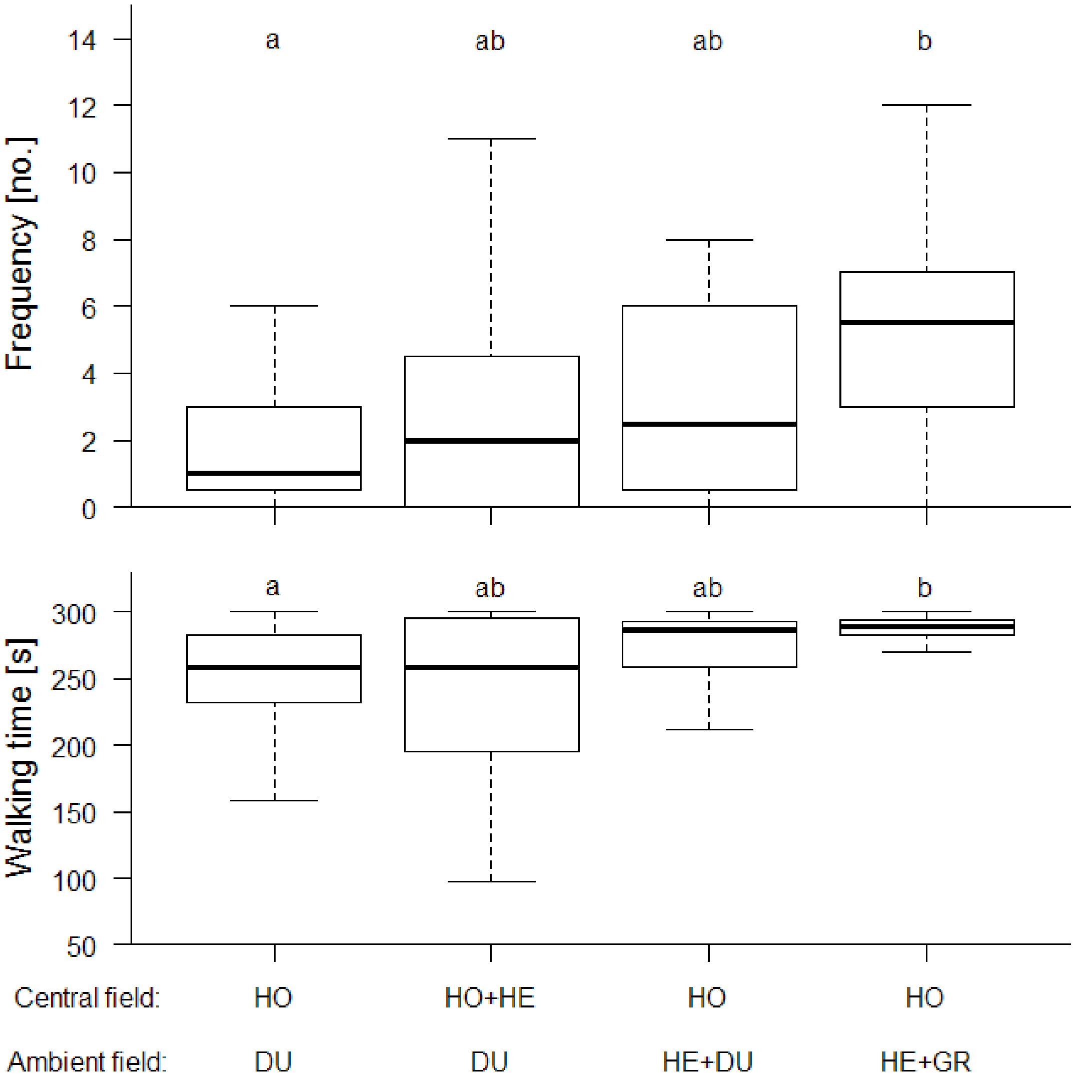
Frequency [no.]
14
12
10
8
6
4
2
0
a
ab
ab
b
Walking time [s]
300
250
200
150
100
50
a
ab
ab
b
Central field:
HO
HO+HE
HO
HO
Ambient field:
DU
DU
HE+DU
HE+GR

Fig. 2

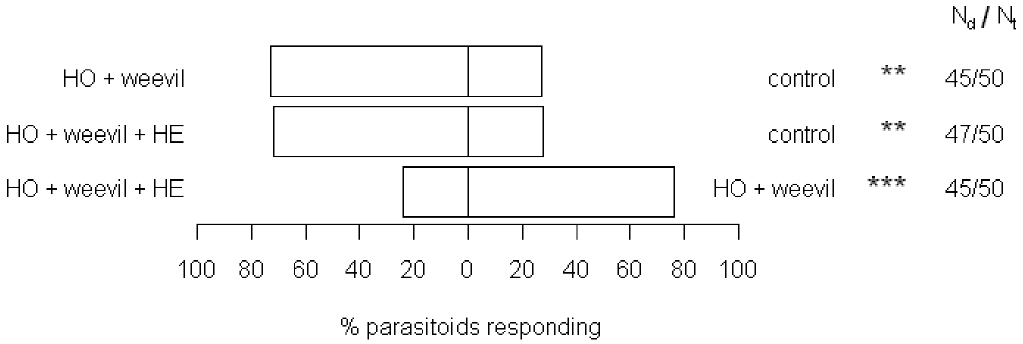
N_d / N_t
HO + weevil
control
**
45/50
HO + weevil + HE
control
**
47/50
HO + weevil + HE
HO + weevil
***
45/50
100
80
60
40
20
0
20
40
60
80
100
% parasitoids responding

Fig. 3

Chapter – IX

Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories

Chapter IX

Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories

E Kathryn Barto[1]*, Tancredi Caruso[1], François Buscot[2,7], Markus Fischer[3], Christine Herbst[4], Tanja S Maier[5], Torsten Meiners[6], Caroline Müller[5], Elisabeth Obermaier[4], Daniel Prati[3], Stephanie A Socher[3], Ilja Sonnemann[1], Nicole Wäschke[6], Tesfaye Wubet[2], Susanne Wurst[1], and Matthias C Rillig[1]

[1] Institute of Biology, Dahlem Center of Plant Sciences, Freie Universität Berlin, Altensteinstr. 6, 14195, Berlin, Germany

[2] UFZ- Helmholtz Centre for Environmental Research, Department of Soil Ecology, Theodor-Lieser-Strasse 4, 06120, Halle/Saale, Germany

[3] Institute of Plant Sciences, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland

[4] Department of Animal Ecology and Tropical Biology, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

[5] Department of Chemical Ecology, Bielefeld University, Universitätsstr. 25, D-33615 Bielefeld

[6] Institute of Biology, Applied Zoology / Animal Ecology, Freie Universität Berlin, Harderslebener Strasse 9, 12163 Berlin, Germany

[7] Institute of Biology, University of Leipzig, Johannisallee 21-23, 04103 Leipzig

* Corresponding author:
Telephone: 49 (0)30 838 53172
Fax: 49 (0)30 838 53886
e-mail: barto@zedat.fu-berlin.de

Chapter IX

Abstract

Biodiversity, a multidimensional property of natural systems, is difficult to quantify partly because of the multitude of indices proposed for this purpose. Indices aim to describe general properties of communities that allow us to compare different regions, taxa, and trophic levels. Therefore, they are of fundamental importance for environmental monitoring and conservation, though there is no consensus about which indices are more appropriate and informative. We used data collected around the focal plant *Plantago lanceolata* on 60 temperate grassland plots embedded in an agricultural landscape to explore relationships between the common diversity indices of species richness (S), Shannon's diversity (H'), Simpson's diversity (D_1), Simpson's dominance (D_2), Simpson's evenness (E), and Berger Parker dominance (BP). We calculated each of these indices for herbaceous plants, arbuscular mycorrhizal fungi, aboveground arthropods, belowground insect larvae, and *P. lanceolata* molecular and chemical diversity. Compound indices such as H', D_1, and D_2 incorporate information on richness and abundance, and are therefore predicted to outperform other more basic indices. We used principal components analysis (PCA) to determine whether compound indices were better at discriminating sites than more basic indices. We also used linear regressions to determine whether compound indices were more able to detect hypothesized negative effects of land use intensity (LUI; as a range of fertilization, grazing and mowing). Finally, we used path analysis to determine whether compound indices detected more relationships between diversities of different organisms and traits than more basic indices. The compound diversity measures D_1 and D_2 were the most effective at discriminating sites. Only plant diversity, as measured by S, H', and D_2, was affected, negatively, by LUI. In the path models more paths were significant when using H', even though all models except that with E were equally reliable. This demonstrates that while common diversity indices may appear interchangeable in simple analyses, when considering complex interactions the choice of index can profoundly alter the interpretation of results. Data mining in order to identify the index producing the most significant results should be avoided, but simultaneously considering analyses using multiple indices can provide greater insight into the interactions in a system.

Key words*:* diversity; Shannon index; Simpson’s index; Berger Parker; evenness; Hill’s powers; land use; grassland; arbuscular mycorrhizal fungi; arthropods; chemical diversity; molecular diversity; plant diversity; *Plantago lanceolata*

Chapter IX

Introduction

Biodiversity represents the variety and heterogeneity of organisms or traits at all levels of the hierarchy of life, from molecules to ecosystems. Typically the focus is on species diversity, but other forms of diversity, such as genetic and chemical diversity, are also important and informative. Even after deciding which form of diversity to measure, quantifying biodiversity remains problematic because there is no single index that adequately summarizes the concept (Hurlbert 1971, Purvis and Hector 2000). Richness (S), or the number of species or attributes present, is the simplest metric used to represent diversity (Whittaker 1972), and it remains the most commonly applied (Magurran 2004). Intuitively, species or trait abundance is also important for diversity, and the proportional abundance of species can also be incorporated into indices representing diversity. The simplest of these indices was proposed by Berger and Parker, has an analytical relationship with the geometric series of the species abundance model (May 1975, Caruso et al. 2007), and reports the proportional abundance of only the most abundant species in the population (BP, Table 1, Berger and Parker 1970).

There have been numerous attempts to create compound indices that combine measures of richness and abundance. Foremost among these are the Shannon's diversity (H') and Simpson's diversity (D_1) indices (Table 1), which differ in their theoretical foundation and interpretation (Magurran 2004). H' has its foundations in information theory, and represents the uncertainty about the identity of an unknown individual. In a highly diverse (and evenly distributed) system, an unknown individual could belong to any species, leading to a high uncertainty in any prediction of its identity. In a less diverse system dominated by one or a few species, it is easier to predict the identity of an unknown individual and there is less uncertainty in the system (Shannon 1948). This metric is common in the ecological literature, despite its abstract conceptualization (Magurran 2004). D_1 is the complement of Simpson's original index, and represents the probability that two randomly chosen individuals belong to different species (McCune and Grace 2002). D_2 is closely related to D_1, being the inverse of Simpson's original index (Simpson 1949).

Both of these transformations serve to make the index increase as diversity intuitively increases, and although both are used, D_2 is more common (Magurran 2004).

Finally, evenness represents the degree to which individuals are split among species with low values indicating that one or a few species dominate, and high values indicating that relatively equal numbers of individuals belong to each species. Evenness is not calculated independently, but rather is derived from compound diversity measures such as H’, D_1, and D_2, since they inherently contain richness and evenness components. However, evenness as calculated from H’ (J’) is of only limited use predictively because it mathematically correlates with H’ (DeBenedictis 1973). E, calculated from D_2 (Table 1), is mathematically independent of D_1 (Smith and Wilson 1996), and therefore a more useful measure of evenness in many contexts.

There is much confusion in the literature about the appropriateness of these various diversity measures, with S favored over more complex measures by Whittaker (1952, 1956, 1960, 1965, 1972). Among the compound diversity measures, D_1 is recommended by Pielou (1969) and Lande (1996), while D_2 is recommended over D_1 by Hill (1973), and yet Whittaker (1972) prefers H’. Several recent analyses have attempted to make sense of this confusion by exploring correlations between these indices to determine whether or not they actually convey the same information. Correlations between S, H’, D_2, E, and J’ are remarkably consistent across organisms (Stirling and Wilsey 2001, Ma 2005, Wilsey et al. 2005, Bock et al. 2007, Heino et al. 2008), lending further support to the idea that the relationships between many of these indices are based on mathematics instead of biology (DeBenedictis 1973, Hill 1973).

Strong correlations between diversity measures should not be surprising since they represent aspects of the same phenomenon. In fact, most of the measures analyzed here can be derived from the same basic generalized entropy formula $N_a = (\sum_{i=1}^{S} P_i^a)^{1/(1-a)}$, where N_a is the effective species number, S is total species number, P_i^a is the proportional abundance of species i, and a is the power (Table 1; Hill 1973). H’ is equally sensitive to rare and abundant species; sensitivity to rare species increases as a decreases from 1, and sensitivity to abundant species increases as a increases from 1 (Fig. 1; Jost 2007). Therefore, S is sensitive to rare species, D_1 and D_2 are sensitive to abundant species, and BP is sensitive to only the most abundant species. Since all the N_a's have species as the unit, the range of values can be interpreted as a continuum from effective number of the most rare species to effective number of the most abundant species.

Despite the strong relationships between these diversity measures, there is still some debate over which is appropriate in various contexts. Based on importance values (IVs) calculated from PCA scores, the importance of diversity measures for distinguishing riverine sites by macroinvertebrate diversity declined in the order H' > J' > S (Heino et al. 2008). Using the same technique with plant diversity data in temperate grasslands the importance of diversity measures was generally ranked D_2 > BP > E > S (Wilsey et al. 2005). These patterns are similar in the fact that a compound index was most effective, while S was least effective at distinguishing sites in both systems. This suggests that for purposes where ranking sites by their level of diversity is the primary goal, such as in conservation planning when selecting sites to be protected, compound indices are to be preferred over species richness (Magurran 2004). However, it remains unclear whether the same is true when the objective is to detect effects of external factors on diversity, such as when assessing anthropogenic impacts on the environment. There is some suggestion that simple indices may be more effective in these cases, since S correlated better with landscape parameters than either J' or H' for aquatic macroinvertebrates (Heino et al. 2008). As experiments and field surveys become ever more complex, an increasingly common objective in biodiversity studies is an understanding of how changes in biodiversity of one trophic level affect biodiversity of other trophic levels. In analyses such as these, additional insights into community dynamics can be obtained by including trait based diversity measures. For example, when modeling changes in species diversity throughout a community, knowledge of the genetic and chemical diversity of the primary producer (eg. a plant) in the system would provide mechanistic insights into any changes in herbivorous insect diversity that could be related to the complexity of herbivore defenses or attractants displayed by the plant. It is unclear which diversity index is most effective at this type of complex community level analysis.

We attempted to clarify these complex relationships, and develop guidelines for practical applications, using data collected in grasslands throughout Germany as part of the Biodiversity Exploratories research network, which consists of 150 plots in three regions that are managed with combinations of fertilization, mowing, and grazing (Fischer et al. 2010). We focused on 60 plots containing *Plantago lanceolata*, and collected data around focal *P. lanceolata* plants in each plot. Focusing data collection around one plant species allowed us to collect in depth data on the dynamics of similarly structured communities spread across a land use gradient. In addition to species diversity of the plants, arbuscular mycorrhizal fungi (AMF), aboveground arthropods, and belowground insect larvae, we also measured neutral molecular and chemical diversity of *P. lanceolata*. These neutral measures have not yet been included in analyses of this type, which have to date focused on species diversity components of biodiversity. Including them will allow us to determine whether changes in species diversity dynamics are reflected in other traits that also contribute to biodiversity.

We set out to test three hypotheses related to performance of diversity indices. (1) *Correlations between diversity indices, and which indices discriminate sites:* Compound diversity indices are predicted to outperform other indices at discriminating sites, since they contain information on both richness and abundance. This has been demonstrated previously, and we needed to verify that this trend was also observed in our dataset before testing novel, more complex, hypotheses. (2) *Dependence of effects of land use on diversity indices:* Land use intensity is expected to negatively impact diversities of all organisms/traits, with rare species/traits gradually disappearing in favor of dominant species/traits in the most intensively managed sites. Since rare species are often disproportionately impacted, an index providing greater weight to rare species (S) should perform the best. Performance of diversity indices will be judged by the number of significant effects of land use intensity identified, although if there is no effect of land use on diversity this method will actually identify poorly performing indices. (3) *Dependence of community dynamics on diversity index chosen:* Community dynamics, or interactions between species, can be modeled using path analysis to describe direct and indirect interactions between species, and to quantify the strength of these interactions. Including trait-based measures of diversity will provide insights into the mechanisms behind species interactions. The significance and strength of such interactions likely depends on the index used to represent diversity because the diversity indices differ in their emphasis on rare and abundant species, which are predicted to interact in different ways. As in the second aim, index performance will be assessed using the number of identified relationships. This is the first analysis to compare performance of diversity indices when quantifying complex community dynamics. Our results should provide guidelines for appropriate use and interpretation of diversity indices in future studies exploring biodiversity and community dynamics.

Methods

Field sites, measurements, and land use index

We sampled in 60 grassland plots spread across the three regions (Schorfheide Chorin, Hainich Dün, and Schwäbische Alb) of the German Biodiversity Exploratories (see Appendix for a list of sites, and Fischer et al. 2010 for site details). Ten focal *P. lanceolata* plants were marked on each plot in June and July of 2008, and future sampling was conducted around these focal plants. Interactions between plants, symbiotic fungi, above and belowground herbivores, and parasitoids in temperate grasslands are extraordinarily complex. Collecting data around only one consistent plant species on each plot allowed us to focus on a more manageable network of interactions, and to explore mechanisms driving interactions by including trait based measures of diversity. *P. lanceolata* was chosen as the focal plant because of its relative abundance in all three Exploratories, and because of its potential for mediating interesting interactions within and between aboveground (tritrophic interactions involving herbivores and parasitoids) and belowground biota (involving arbuscular mycorrhizal fungi and insect larvae; Gange and West 1994, Wurst and Van der Putten 2007). Furthermore, some target metabolites of *P. lanceolata* are well characterized (Fontana et al. 2009), and our expansion of this knowledge base using metabolic fingerprinting approaches allowed us to investigate how chemical diversity relates to diversity of other organism groups. Finally, *P. lanceolata* is a generalist species occurring in various grassland habitats and is known to exhibit genetic differentiation at the population level (Kuiper and Bos 1992).

Detailed methods used to assess diversity of all organisms/traits are given in the Appendix. Briefly, we quantified herbaceous plant diversity by estimating percent cover of each species in a 15 cm sampling radius around the focal plants. Arbuscular mycorrhizal fungal diversity was quantified using terminal restriction fragment length polymorphism analysis of DNA extracted from rhizosphere soil of focal plants. Aboveground arthropods were collected from plant surfaces, and belowground insect larvae were sorted by hand or heat extracted from soil cores collected beneath focal plants. *Plantago* molecular diversity was quantified for five loci, and *Plantago* chemical diversity was assessed by UHPLC-TOF-MS using metabolic fingerprinting techniques. All species/traits were sampled on small spatial scales around the focal plants so that we could focus on interactions involving *P. lanceolata*.

Land use intensity (LUI) on each site was quantified as an index incorporating three equally weighted variables: fertilization, mowing and grazing intensity. This index is conceptually similar to one proposed by Herzog et al. (2006) and has been found to predict soil aggregation, plant root length, and fungal hyphal length in our system (Barto et al. 2010). For each experimental plot i, land use intensity L[i] is defined as the sum of each variable divided by its mean over all experimental plots per Exploratory:

$$L[i] = F[i] / F[\text{mean}, E] + M[i] / M[\text{mean}, E] + G[i] / G[\text{mean}, E]$$

where F[i] is the fertilization level (kg nitrogen ha^{-1} year^{-1}), M[i] is the frequency of mowing per year and G[i] is the livestock density (livestock units ha^{-1} year^{-1}) on each site i. The mean L[i] across the years 2006-2008 was used in this study, where F[mean, E], M[mean, E] and G[mean, E] are defined as the mean value across all three years. LUI was square root transformed to improve normality and is dimensionless due to standardization by ratios. Land use data are based on interviews with farmers and landowners conducted each year by the management teams of each Exploratory (Nico Blüthgen, University of Würzburg, and the consortium of the Biodiversity Exploratories, unpublished data).

Statistical analyses

We calculated richness (S), Shannon's diversity (H'), Berger Parker dominance (BP), Simpson's diversity (D_1), Simpson's dominance (D_2), and Simpson's evenness (E) for each organism/trait group (Table 1). For plant, aboveground arthropod, and belowground insect larva data, abundance was quantified as number of individuals. For mycorrhizal fungi, terminal restriction fragments (TRFs) were used as surrogates for species, and abundance was quantified as peak height of each TRF. Measurement used as a surrogate for species with the population genetic data, and abundance was quantified. Also for population genetic data, D_1 is equal to the expected heterozygosity under Hardy-Weinberg equilibrium, whereas D_2 is known as the effective number of alleles, both of which are commonly used measures of genetic diversity (Frankham et al. 2002). Metabolites were used as surrogates for species in the chemical diversity data, and abundance was quantified. It is important to note that the formulas given in Table 1 are for complete populations, and that the actual formulas for calculating these indices from sample data are slightly more complex (Magurran 2004). However, in practice the difference between these two approaches is usually so small that the simpler formulas are generally acceptable (Magurran 2004). For organisms/traits where samples were taken around multiple focal plants per plot, the mean of each diversity index per plot was calculated.

In order to ensure that our estimates of S were reliable, we computed several estimates of total species/trait number based on extrapolations from species/trait accumulation curves, namely Chao 1, Jackknife 1, and Bootstrap for each organism/trait using R package 'vegan' and compared them with the observed total species/trait number (Magurran 2004, Oksanen et al. 2010).

Aim 1. We performed Pearson correlations between all metrics within an organism/trait group to assess relationships between the different diversity measures, after transforming data to improve normality where necessary. Each organism/trait group was analyzed separately because we could not be sure that the same pattern would be found for all groups and therefore did not want to pool data. In order to account for multiple comparisons we used Bonferroni corrected *P*-values for all correlations within each organism/trait group. We then used principal component analysis (PCA) of correlation matrices to determine which measures of diversity were most able to differentiate sites by calculating importance values (IV) for each index (Wilsey et al. 2005). The IVs synthesize information on the importance of each principal component axis and the score for each diversity index to generate one number representing the overall importance of each diversity index in distinguishing plots based on distances between plots in the ordination.

Aim 2. We also performed linear regressions of each measure of diversity within organism/trait groups on LUI in order to determine whether or not the effect of land use depended on the metric chosen, after transforming data to improve normality of residuals where necessary. As in Aim 1, each organism/trait group was analyzed separately because we could not be sure that each responded the same way to increases in land use intensity and therefore did not want to pool data. Indexes detecting the greatest number of significant effects were judged to be the most effective, although if land use did not affect diversity in our system these indices would actually be the least effective. We used Bonferroni corrected *P*-values within each organism/trait group to account for multiple comparisons.

Aim 3. Finally, we constructed a path model of hypothesized relationships between organism/trait groups (Fig. 2). Belowground insect larvae were not included in the path models because they were sampled on fewer sites than other groups and their inclusion would have reduced the sample size below acceptable limits given the complexity of our model. We ran the same structural model with each of the diversity indices, and we report model fit as χ^2 and its associated *P*-value, with *P*-values greater than 0.05 indicating an acceptable fit (Hooper et al. 2008). Since χ^2 can be influenced by sample size we also report the Root Mean Square Error of Approximation (RMSEA), where smaller values indicate more parsimonious models, and values less than 0.07 suggest an adequate model fit (Hooper et al. 2008). The Tucker Lewis Non-Normed Fit Index (TLNNFI) is less sensitive to sample size and accounts for model parsimony, with values close to one indicating good model fit (Hooper et al. 2008). Path analyses were performed using the ‘sem’ package version 0.9-16 in R (Fox 2006). All analyses were performed with R v2.11.1 and newer (R Development Core Team 2008).

Results

Robustness of S

Estimates of total species/trait number showed that our observed richness values likely underestimated total richness for many organism/trait groups (Table 2). Estimates of aboveground arthropod, chemical, and two loci of molecular richness overlapped our observed values, suggesting that these observations are robust. For the other organism/trait groups, compound diversity measures, especially D_1 and D_2, may be more appropriate than S because they are not as dependent on sample size (Magurran 2004).

Aim one: Correlations between diversity indices, and which indices discriminate sites

Correlations between diversity indices were generally strong within organism/trait groups, and BP correlated negatively with S, H’, D_1, and D_2 (Appendix, Table 1). E did not correlate in a consistent manner with any other index of diversity.

We used PCA to visually represent these correlations and determine if any metrics were better at differentiating plots, despite the strong correlations between all metrics (Fig. 3). The first principal component axis accounted for a large part of the variance for all organism/trait groups (60 - 72 %), and the first two axes accounted for almost all the variation (84 - 87 %). Generally, the compound diversity measures D_1 and D_2 loaded strongly on the first PC axis and had the highest IVs, with H’ also having high IVs. Evenness was often ineffective at discriminating sites, although it had a high IV for chemical diversity data. The simplest diversity index, S, was generally adequate at discriminating sites, but gave low IVs for aboveground arthropod and chemical diversity data. This suggests that the compound indices discriminate between plots better than more simple diversity measures.

Aim two: dependence of effects of land use on diversity indices

LUI generally did not affect diversity, with no evidence for stimulation or suppression of AMF, aboveground arthropod, belowground insect larvae, or *P. lanceolata* molecular or chemical diversity for any diversity index used (Table 3). This lack of effect of LUI is apparent in the PCAs (Fig. 3), where LUI is orthogonal (perpendicular) to most diversity indices, indicating that it is independent of them, and that there is little overlap in how plots are discriminated based on LUI or diversity. In contrast, we found evidence for effects of LUI on plant diversity for three of the six metrics (S, H', and D_2). Furthermore, the magnitude of this effect was similar for all six metrics. It is important to note that we used Bonferroni corrected *P*-values to account for multiple comparisons, but if only one diversity index had been chosen *a priori* for analysis all but E would have been significantly affected by LUI at the more typical $\alpha = 0.05$.

Aim three: dependence of community dynamics on diversity index chosen

As in the first two analyses, the path analysis also showed that E represents different information than that captured by the other diversity indices (Fig. 2, Table 4). Furthermore, the model using E fit the data poorly while the fit of the other models was excellent, as evidenced by low RMSEA values and high TLNNFI values. The χ^2 *P*-values increased slightly in the order $E << S < H' = D_1 < D_2 < BP$ (Table 4), suggesting that model fit may have improved slightly along this gradient from indices emphasizing rare species to those emphasizing dominant species. More significant paths were identified with H' than with other indices (Fig. 2), allowing for a deeper interpretation of the data than with the other indices. The only consistently significant path in all models (excluding E) was a negative effect of herbaceous plant diversity on chemical diversity of *P. lanceolata*.

Chapter IX

Discussion

We compared diversities of multiple organism/trait groups across a land use gradient in order to determine which diversity indices provided the greatest ability to discriminate sites, whether or not the effect of land use on diversity depended on the diversity index chosen, and how the choice of index affected results of path analyses. This is the first analysis of this type to include measures of molecular and chemical diversity along with the more traditional species diversity, and also the first to assess how the choice of diversity index influences detection of effects of an external factor on diversity, and detection and interpretation of community dynamics.

Aim one: Correlations between diversity indices, and which indices discriminate sites

As in other studies (Wilsey et al. 2005, Heino et al. 2008), we found that S provided a poor ability to discriminate sites, while the compound diversity measures, primarily D_1 and D_2, provided the greatest such ability. This shows that the compound diversity measures do indeed contain additional information beyond that supplied by S, notably the proportional abundances of each species. The failure of E to effectively discriminate sites shows that the synthesis of richness and abundance information is necessary for site discrimination, and that the individual components of the compound diversity measures (S and E) are much less informative when considered independently. The greater ability of measures derived from Hill's N_2 (D_1, D_2) to discriminate sites further suggests that site differences are largely based on differences in abundant species.

A further strength of compound diversity measures over species richness is their reduced dependence on sampling effort (Magurran 2004). As can be seen in Table 2, we likely underestimated diversity of plants, AMF, belowground insect larvae, and three molecular loci. This may be because we sampled multiple grasslands spread across Germany, and our sampling plan may not have been sufficient to adequately catalogue the diversity of some organisms/traits across such a broad area. For these groups, compound diversity indices are expected to be more robust than S, although they are still influenced to some extent by sample size (Magurran 2004).

Aim two: dependence of effects of land use on diversity indices

Diversity of organisms/traits in our system was remarkably uninfluenced by land use changes including increased fertilization, grazing, and mowing. We found no effect of land use on diversities of AMF, aboveground arthropods, belowground insect larvae, or *P. lanceolata* molecular or chemical diversity. Aboveground arthropods were identified to order while belowground insect larvae were identified to family. It is not entirely clear what level of resolution is achieved with the NS31-AM1 primers used in the AMF analysis, but it is almost certainly higher than species level. Any effects of LUI may only be apparent at finer taxonomic scales. In this analysis we focused on species associated with *P. lanceolata*, and it is possible that effects of LUI would be observed in broader communities. At least in this system, molecular and chemical diversity were less sensitive to land use than herbaceous plant species. Three of the six plant diversity measures (S, H’, D_2) were negatively affected by LUI. The differing sensitivities of diversity indices to LUI in our analysis were largely driven by our need to correct for multiple comparisons. In analyses using only one diversity index, similar significant effects of LUI would have been detected using any of the indices we included, expect E. Thus, when conducting simple statistical analyses of a specific effect of disturbance on diversity the choice of index does not appear particularly important.

Aim three: dependence of community dynamics on diversity index chosen

Our ability to detect relationships between diversities of organisms/traits was clearly influenced by the choice of diversity index, despite the fact that all path models (except that using E) fit the data extremely well. Model fit increased slightly as rare species/traits were excluded from the index used, suggesting that rare species/traits were behaving in ways deviating from model predictions. However, similar fit statistics using RMSEA and TLNNFI suggest that any such deviations were small. When using BP and focusing only on the most abundant species/trait we detected a negative dependence of *P. lanceolata* chemical diversity on herbaceous plant diversity. This shows that as the abundance of the most abundant plant species increases, the abundance of the most abundant chemical metabolite declines. This pattern also holds when using D_1 and D_2, in fact with a higher path coefficient, indicating that when other highly abundant species/traits are included the relationship between plant and chemical diversity is even stronger. When moderately rare species/traits are also considered by using H', even more relationships become apparent. The positive dependence of aboveground arthropod diversity on chemical diversity, and the negative dependence of aboveground arthropod diversity on molecular diversity may therefore be driven equally by rare and abundant species, while abundant species/traits do not seem important for these interactions. The positive dependence of aboveground arthropod diversity on plant diversity is apparent in the models using H' and S, suggesting that rare species are driving this interaction. The presence of a significant path from plant to chemical diversity for all indices (except E) suggests that changes in both rare and abundant metabolites are negatively affected by changes in rare and abundant plants.

The *a priori* choice of only one index for a path analysis could have profound consequences on interpretation of relationships between organisms/traits. Running models with a range of diversity indices along Hill's series allowed us to better understand interactions within our system. Abundance of rare to moderately rare arthropods was positively affected by abundance of rare to moderately rare plants. This may be due to increased niche availability for specialist insect species as plant diversity increased. The negative relationship we observed between plant and chemical diversity for all indices, except E, was also apparent in a separate analysis (only H' was calculated) using more extensive chemical and plant diversity data sets (Maier et al. *submitted*). This persistent trend could be explained by likely reductions in *Plantago* abundance as plant diversity increased and other plant species took up space in the system. In sites with low plant diversity, intraspecific *P. lanceolata* competition could affect chemical composition (Barton and Bowers 2006). Any decreases in *Plantago* abundance associated with increasing plant diversity would also be expected to lead to reduced attack of *Plantago* by specialist *Plantago* herbivores, and therefore reduced induction of defense responses, seen as reduced diversity in the metabolic profile of the plant. Positive relationships between chemical and aboveground arthropod diversity could easily be explained by increased production of compounds attracting and/or stimulating pollinators, herbivores, and parasitoids of herbivores, and by induction responses of the plant to different interacting species. These further hypothesized interactions between *Plantago* metabolites and different insect groups suggested by the current analysis could be specifically tested in future experiments or field sampling campaigns.

Conclusions

The importance of carefully deciding how to quantify diversity in multiple organism/trait groups is apparent from our analysis. The failure of any species/trait group other than herbaceous plants to detect effects of land use also calls into question the practice of using easy to measure indicator taxa to estimate effects on other taxa. At the very least, analyses such as this should precede selection of such indicator taxa to ensure that non-indicator taxa are in fact behaving as expected.

We could not identify one ideal diversity index. Simpson’s indices, D_1 and D_2, performed best when differentiating sites, but simpler indices were slightly preferable when detecting effects of land use intensity on diversity. All indices except E were equally effective when fitting path models to describe relationships between organisms/traits, although the greatest number of relationships was apparent when using H’. We assessed performance of each index largely as the significance of effects or number of relationships detected, with the inherent assumption that such effects and relationships did in fact exist. If effects of LUI or relationships between organism/trait groups are not strong, indices that did not detect effects may more accurately represent reality. Modeling approaches using artificial systems where relationships are predefined could help resolve this issue. While analyses of synthetic data would allow one to completely control community structure and avoid biases related to varying sample sizes, such an approach would also disallow the ecological realism obtained in the present analysis. It is clear that relationships between diversity indices do not always follow mathematically predicated patterns (Stirling and Wilsey 2001, Nagendra 2002), and it is therefore important to perform analyses such as these on real data to ensure that conclusions will be valid in the field.

Other attempts to identify an ideal diversity measure have failed to find one, and instead suggest reporting at least two measures (Whittaker 1972, Stirling and Wilsey 2001, Heino et al. 2008). Including multiple diversity measures, spread along Hill's continuum (Hill 1973), provided us with a more complete understanding of how shifts in rare and abundant species were driving interactions. Additional benefits of using the Hill series instead of the closely related more traditional indices include the simplified interpretation of results because units are always in effective number of species regardless of the position along the series (Jost 2006). Furthermore, effective species numbers behave as one would intuitively expect when diversity is doubled or halved, while other standard indices of diversity (H', D_1, D_2) do not (Jost 2006). Data mining to identify an index providing strong significant effects should be discouraged. We advocate *a priori* selection of, at most, a small number of diversity measures along Hill's series that are expected to capture the important aspects of diversity in the system under study. If effects are expected to be more apparent in rare species then S would be appropriate, whereas if dominant species are expected to be more important then D_1, D_2, and BP would be more appropriate. H' could be used in situations where rare and abundant species are expected to be equally important. Comparison of a few carefully chosen indices could greatly enhance understanding of the complex components driving diversity.

Chapter IX

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Table 1: Formulas used to calculate diversity measures analyzed.

Metric	Traditional formula*	Surrogate in Hill's Series, Hill's power**
Richness (S)	number of species	S, 0
Shannon's diversity (H')	$- \sum p_i \ln(p_i)$	exp(H'), 1
Simpson's diversity (D_1)	$1 - \sum p_i^2$	D_2, 2
Simpson's dominance (D_2)	$1/\sum p_i^2$	D_2, 2
Berger Parker dominance (BP)	p_{max}	BP^{-1}, ∞
Simpson's evenness (E)	D_2/S	--

*p_i is the proportion of individuals belonging to species i; p_{max} is the proportion of individuals belonging to the most abundant species. Formulas from McCune and Grace (2002), Shannon (1948), and Simpson (1949).

**Formulas from Hill (1973).

Table 2: Total observed species/order number, alleles per locus, and metabolites, and estimates of total species or trait number (mean ± SE) for each organism/trait.

	Observed species number	Chao	Jackknife	Bootstrap
Plant	177	239 ±20	240 ± 12	206 ± 6
AMF	60	71 ± 7	76 ± 4	68 ± 3
Aboveground arthropod [a]	14	16 ± 4	16 ± 1	15 ± 1
Belowground insect larvae [b]	23	30 ± 6	32 ± 3	27 ± 2
Molecular				
Locus 1	92	97 ± 4	102 ± 4	98 ± 2
Locus 2	54	59 ± 4	63 ± 3	59 ± 2
Locus 3	16	16 ± 0	16 [c]	16 ± 0
Locus 4	36	39 ± 3	42 ± 2	39 ±1
Locus 5	129	145 ± 9	153 ± 5	141 ± 3
Chemical	1449	1449 ± 0	1449 [e]	1449 ± 0

[a] Aboveground arthropods were identified to order

[b] Belowground insect larvae were identified to family

[c] No estimate of standard error possible because of the absence of singular alleles or metabolites

Table 3: Results of linear regression of richness (S), Berger-Parker dominance (BP), Shannon's diversity (H'), Simpson's diversity (D_1), Simpson's dominance (D_2), and Simpson's evenness (E) of various traits measured in grassland plots in and around *Plantago lanceolata* on land use intensity [F, P, r]. Values in bold indicate significance at Bonferroni corrected α of 0.05/6 = 0.0083.

	Plant (N = 60)	AMF (N = 60)	Aboveground arthropods (N = 60)	Belowground insect larvae (N = 20)	Molecular (N = 60)	Chemical (N = 59)
S	**9.68,**	0.004,	0.08,	0.09,	1.54,	0.69,
	0.0029,	0.947,	0.774,	0.771,	0.220,	0.411,
	-0.38	-0.01	0.04	0.07	-0.16	0.11
BP	7.40,	0.04,	1.21,	0.001,	2.19,	0.01,
	0.0086,	0.841,	0.276,	0.970,	0.144,	0.918,
	0.34	0.03	0.14	-0.01	0.19	-0.01
H'	**9.44,**	0.03,	0.55,	0.05,	2.84,	0.16,
	0.0032,	0.872,	0.462,	0.819,	0.097,	0.687,
	-0.37	-0.02	-0.10	0.05	-0.22	0.05
D_1	7.33,	0.11,	0.85,	0.03,	0.52,	0.005,
	0.0089,	0.742,	0.362,	0.868,	0.475,	0.946,
	-0.33	-0.04	-0.12	0.04	-0.09	-0.01
D_2	**8.86,**	0.13,	0.78,	0.002,	0.59,	0.04,
	0.0043,	0.720,	0.380,	0.989,	0.445,	0.842,
	-0.36	-0.05	-0.12	0.003	-0.10	0.03
E	1.27,	0.26,	1.35,	0.06,	0.04,	0.01,
	0.2650,	0.615,	0.251,	0.815,	0.841,	0.929,
	-0.15	-0.07	-0.15	-0.06	-0.03	0.01

Table 4: Model fit statistics for structural equation models.

Model	χ^2, P[a]	RMSEA[b]	TLNNFI[c]
S	1.18, 0.76	<0.0001	>0.99
H’	0.51, 0.92	<0.0001	>0.99
D_1	0.50, 0.92	<0.0001	>0.99
D_2	0.44, 0.93	<0.0001	>0.99
BP	0.15, 0.98	<0.0001	>0.99
E	6.39, 0.09	0.14	0.35

[a] For all models df = 3.

[b] RMSEA – root mean square error of approximation

[c] TLNNFI – Tucker Lewis non-normed fit index

Figure legend

Figure 1: Herbaceous plant diversity in sites with representative high and low land use intensity (LUI). The low LUI site (AEG07) was an unfertilized sheep pasture, while the high LUI site (AEG02) was a fertilized meadow that was mown three times a year. The effective species number decreases in both sites as Hill's power increases and increasingly abundant species are excluded.

Figure 2: Structural equation models of links between diversity of organisms or traits measured in and around *Plantago lanceolata*. S – richness, H' – Shannon's diversity, D_1 – Simpson's diversity, D_2 – Simpson's dominance, BP – Berger Parker dominance, E – Simpson's evenness, mol – *Plantago* molecular, chem – *Plantago* chemical, AMF – arbuscular mycorrhiza, plant – herbaceous plant, arth – aboveground arthropod. Solid lines indicate positive effects while dashed lines indicate negative effects. Black lines indicate significant paths while gray lines indicate non-significant paths at $\alpha = 0.05$. The magnitude of the path coefficient is indicated by line length.

Figure 3: Principal component analysis of diversity measures taken in and around *Plantago lanceolata*, and land use intensity (LUI). S – richness, H' – Shannon's diversity, D_1 – Simpson's diversity, D_2 – Simpson's dominance, E – Simpson's evenness, BP – Berger Parker dominance, plant – herbaceous plant diversity, AMF – arbuscular mycorrhizal fungal diversity, above – aboveground arthropod diversity, below – belowground insect larvae diversity, mol – *P. lanceolata* molecular diversity, chem – *P. lanceolata* chemical diversity. Numbers indicate importance values for each vector.

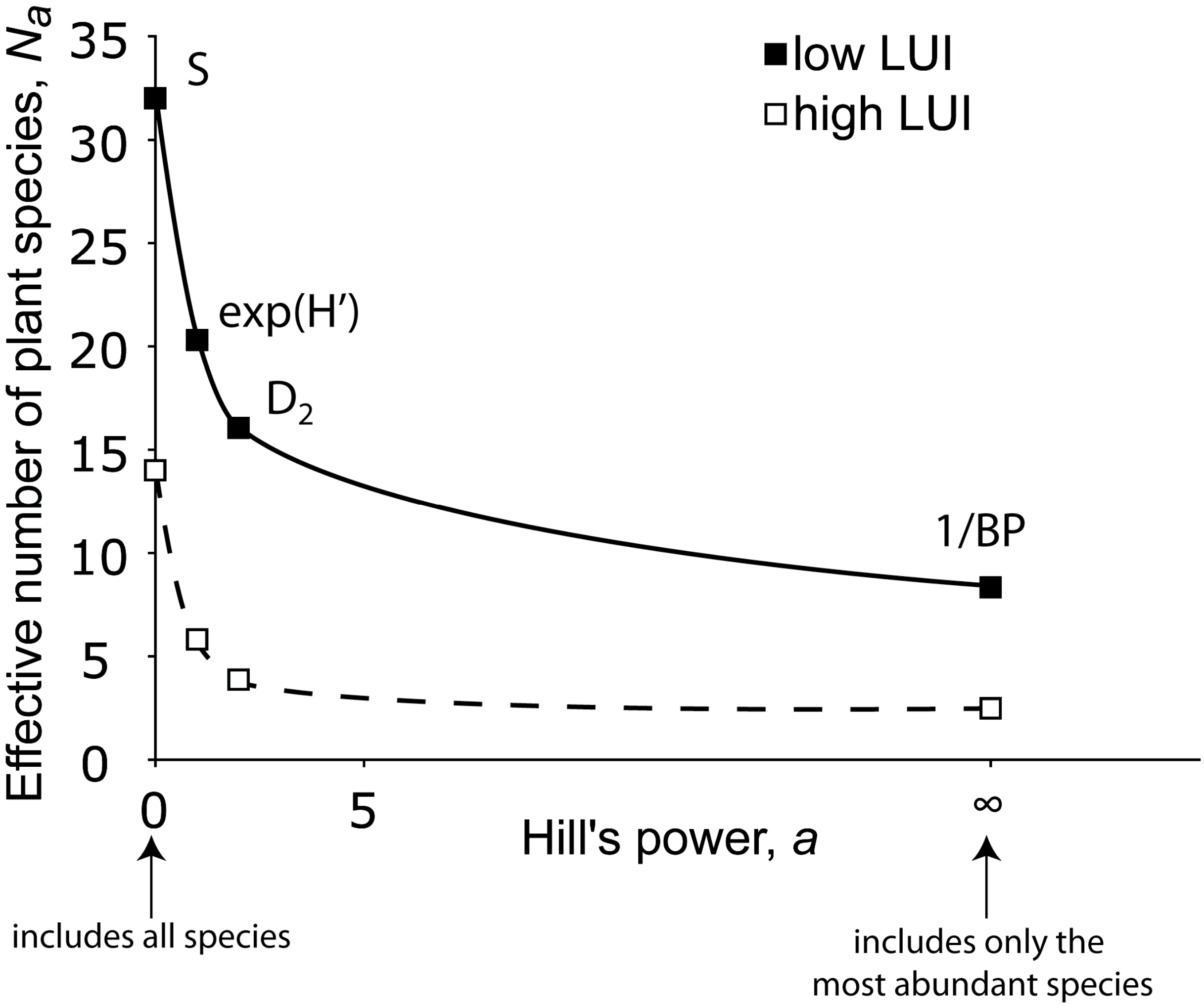
Effective number of plant species, N_a
35
30
25
20
15
10
5
0
S
exp(H′)
D_2
1/BP
■ low LUI
□ high LUI
0
5
∞
Hill's power, a
includes all species
includes only the most abundant species

Figure 1

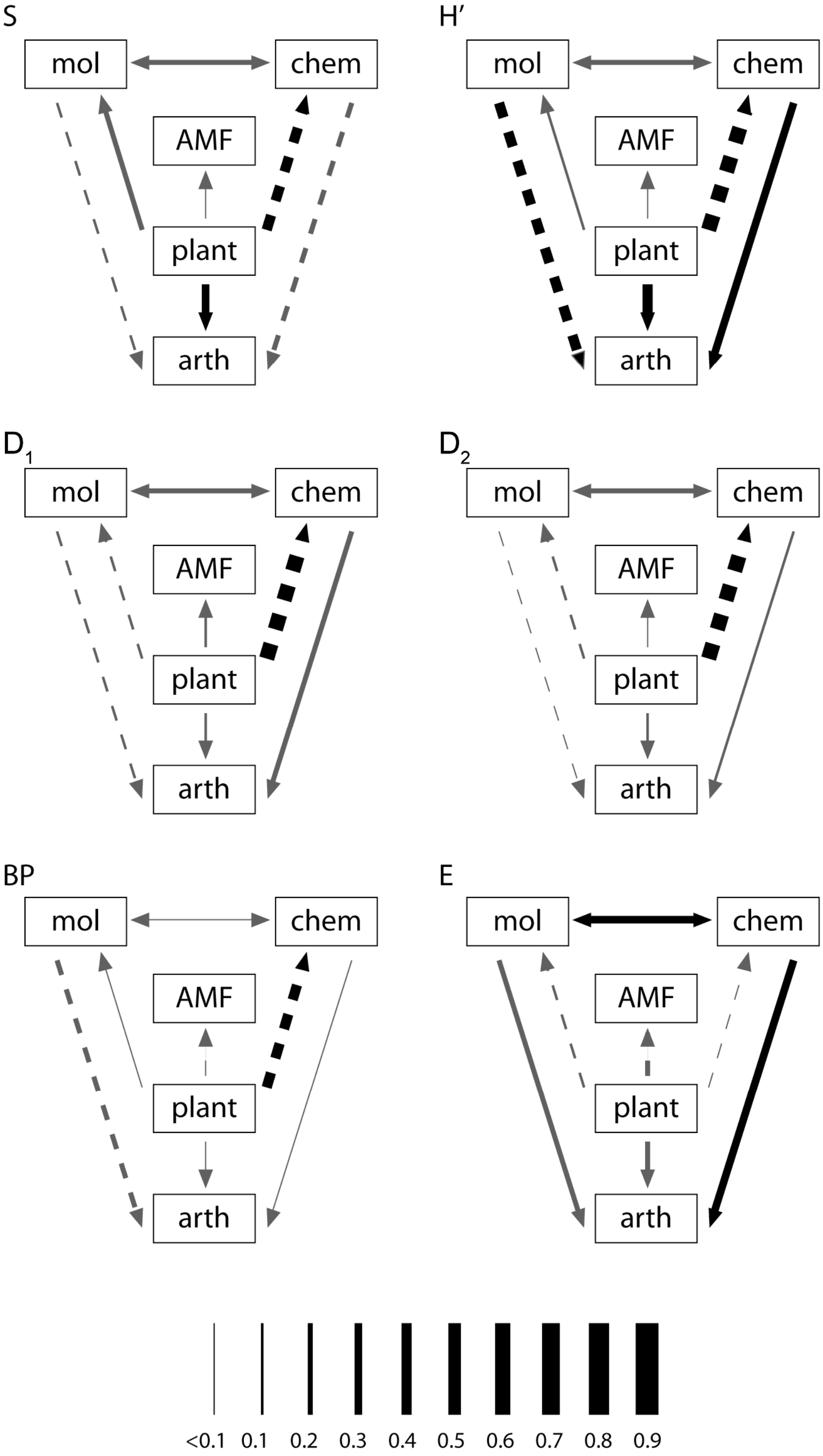
S
mol
chem
AMF
plant
arth
H′
mol
chem
AMF
plant
arth
D_1
mol
chem
AMF
plant
arth
D_2
mol
chem
AMF
plant
arth
BP
mol
chem
AMF
plant
arth
E
mol
chem
AMF
plant
arth
<0.1
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9

Figure 2

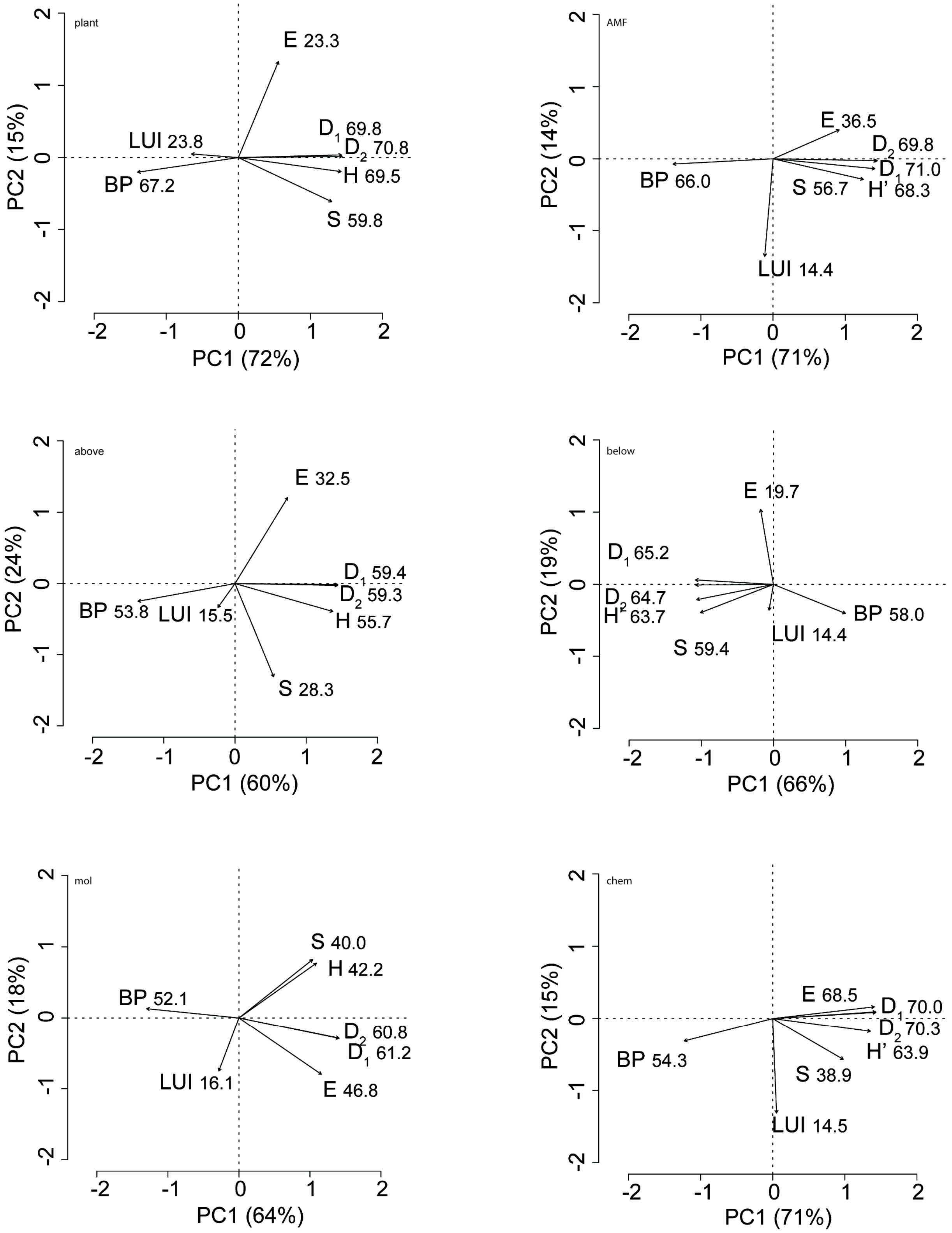
plant
E 23.3
D_1 69.8
D_2 70.8
LUI 23.8
BP 67.2
H 69.5
S 59.8
PC1 (72%)
PC2 (15%)
AMF
E 36.5
D_2 69.8
D_1 71.0
BP 66.0
S 56.7
H' 68.3
LUI 14.4
PC1 (71%)
PC2 (14%)
above
E 32.5
D_1 59.4
D_2 59.3
BP 53.8
LUI 15.5
H 55.7
S 28.3
PC1 (60%)
PC2 (24%)
below
E 19.7
D_1 65.2
D_2 64.7
H' 63.7
BP 58.0
LUI 14.4
S 59.4
PC1 (66%)
PC2 (19%)
mol
S 40.0
H 42.2
BP 52.1
D_2 60.8
D_1 61.2
LUI 16.1
E 46.8
PC1 (64%)
PC2 (18%)
chem
E 68.5
D_1 70.0
D_2 70.3
H' 63.9
BP 54.3
S 38.9
LUI 14.5
PC1 (71%)
PC2 (15%)
-2
-1
0
1
2

Figure 3

SUPPLEMENTARY METHODS

Sites sampled

In Schorfheide Chorin we sampled 15 sites; SEG06, SEG08, SEG31, SEG32, SEG33, SEG34, SEG35, SEG39, SEG40, SEG41, SEG43, SEG44, SEG45, SEG46, and SEG47. Similarly, 15 sites were sampled in Hainich Dün; HEG06, HEG08, HEG09, HEG11, HEG17, HEG18, HEG20, HEG30, HEG34, HEG36, HEG42, HEG43, HEG44, HEG46, and HEG48. Coverage was more extensive in Schwäbische Alb, with 30 sites covered; AEG02, AEG03, AEG06, AEG07, AEG09, AEG11, AEG12, AEG13, AEG15, AEG17, AEG18, AEG21, AEG22, AEG25, AEG26, AEG27, AEG28, AEG30, AEG31, AEG32, AEG33, AEG34, AEG36, AEG38, AEG40, AEG41, AEG42, AEG43, AEG47, and AEG49. Detailed site descriptions can be found in Fischer et al., (2010).

Plant diversity

We recorded plant species diversity by identifying herbaceous plant species and estimating the percentage cover of each species in a 15 cm radius around all 10 focal *P. lanceolata* plants in each plot.

Arbuscular mycorrhizal fungal diversity

Arbuscular mycorrhizal fungal diversity was assessed using terminal restriction fragment length polymorphism (T-RFLP) analysis. A 10 cm diameter soil core (0 – 10 cm depth) from immediately beneath a focal *P. lanceolata* plant in each site was collected between July and August 2008 and stored at -20 °C until analysis. Cores were thawed overnight at 4 °C, then split vertically down the center so that a soil sample (~ 3 g) could be collected from the *P. lanceolata* rhizosphere. These samples were again stored at -20 °C until DNA was extracted from 250 mg soil with a MoBio PowerSoil DNA Extraction Kit (96well) kit (Carlsbad, CA, USA), and AMF DNA was amplified with a nested PCR approach. We began with GLOMER WT0/GLOMER 1536 primers (0.5 μM each), 1X FIREPol 5xPCR Mix, 7.5mM $MgSO_4$ (Solis BioDyne, Tartu, Estonia), and 1 μL of template DNA in a final volume of 50 μL (Wubet et al.

2006). The PCR conditions were 98 °C for 30 sec, followed by 5 cycles of 94 °C for 30 sec, 60 °C for 45 sec, and 72 °C for 1 min with the 60 °C step decreasing by 1 C each cycle; then 25 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min, and finally 72 °C for 5 min in an Eppendorf thermocycler (Eppendorf, Hamburg, Germany). We performed two separate reactions for the second PCR using NS31-FAM and one of two AM1 primers: the original (Helgason et al. 1998), and a modified primer designed to capture more AMF genera than the original AM1 primer (AM1b: 5'- CTT TGG TTT CCC ATA RGG TGC C -3') (unpublished, Wubet). The second PCR was performed with the same recipe as the first PCR, but with PCR conditions of 98 °C for 1 min, 30 cycle of 94 °C for 30 sec, 63 °C for 30 sec, 72 °C for 1 min, followed by 72 °C for 5 min. PCR products were cleaned with a Nucleospin ExtractII kit (Machery Nagel, Düren, Germany), before quantification of DNA with a NanoPhotometer (Implen, Munich). We then combined 40 ng DNA from each AM1 primer reaction to regenerate complete samples. We used *HinfI* (Fermentas, St. Leon-Rot, Germany) in digests of 80 ng DNA, 2 µL buffer, and 0.3 units enzyme in 20 µL total volume. Digestions were incubated at 37 °C for 2 hours, then cleaned with a Nucleoseq kit (Machery Nagel, Düren, Germany) before analysis on an ABI 3730xl Genetic Analyzer with a custom made ROX size standard (BioVentures,
Murfreesboro, TN, USA).

Terminal restriction fragment (TRF) sizes and peak heights were determined using GeneMapper 3.7 software (Applied Biosystems, Carlsbad, CA, USA) with a threshold of 75 AU. Total fluorescence of each profile was standardized (Dunbar et al. 2001), then TRFs were aligned with T-REX (http://trex.biohpc.org/index.aspx) to combine fragments lengths differing by less than one base pair into the same TRF (Culman et al. 2009). TRF number was used as a surrogate for species richness, and TRF peak height as a surrogate for species abundance. The latter may not always be accurate due to the existence of the same TRF across different AMF species, PCR bias during amplification, and differences in gene copy number among different AMF (Corradi et al. 2007). However, the technique had been used to calculate diversity indices for AMF (Burke 2008), and it is instructive to do so in this context to allow for comparisons with diversity indices of other organism and character groups investigated in this study, while bearing in mind that for AMF specifically, richness remains the most reliable metric.

Above ground arthropod diversity

We used a modified hand-held vacuum cleaner (Rowenta AC 8818 Silencer 12V, Offenbach/Main, SEB Deutschland GmbH) to collect all arthropods resting on all 10 *P. lanceolata* plants in each plot. We began vacuuming over the inflorescences of each plant, moved down to the rosette, and then back up in order to prevent escape of the arthropods. Afterwards the arthropods were transferred with a small paintbrush to 70% ethanol and stored in the lab until individuals were identified to order level.

Below ground insect larval diversity

Only 20 sites were sampled for below ground insect larval diversity; SEG06, SEG08, SEG33, SEG34, SEG35, HEG06, HEG08, HEG11, HEG30, HEG48, AEG02, AEG03, AEG06, AEG07, AEG09, AEG11, AEG13, AEG22, AEG26, and AEG27.

Three soil cores of 5 cm diameter and 10 cm depth were taken randomly from a 1 m x 6 m subplot at each site in September/October 2009. Visible insect larvae were sorted out by hand and smaller larvae were extracted by subsequent heat extraction (MacFayden 1961). Larvae were stored in 70 % ethanol until identification to family level.

Plantago molecular diversity

To assess molecular diversity we sampled between five and eight individuals of *P. lanceolata* and extracted DNA from 50 mg dried leaf tissue. The tissue was homogenized with a mixer mill and DNA was extracted with a commercial extraction kit (NucleoSpin96 Plant, Macherey Nagel, Düren, Germany). The DNA content was measured with a fluorescence spectrometer and each DNA sample was diluted to 5 ng per 5 µl with nuclease free water.

We used the 5 polymorphic, dinucleotid, nuclear microsatellite loci (simple sequence repeats) developed by Hale and Wolff (2003). These microsatellite markers were labeled with fluorescent dye and multiplexed for PCR amplification. PCR reactions were carried out in 10 µl volumes containing 5 ng DNA, 5µl Qiagen HotStarTaq Master Mix Kit (Valencia, USA, consisting of multiplex PCR buffer with a final concentration of 3 mM $MgCl_2$, dNTP mix, and HotStarTaq DNA polymerase), 0.5 µl of locus-specific 5' fluorescent labeled forward primer (6-FAM ^{TM}, HEX ^{TM}: Microsynth, Balgach, Switzerland, and NED ^{TM} Applied Biosystems), and non-labeled reverse primer. PCR amplification was performed in a 96-well PTC-100 TM Programmable Thermal Controller (MJ Research, Inc.,Waltham, USA) by using the following cycling protocol: 15 min at 95°C; 30 cycles consisting of 30 sec at 52°C, 30 sec at 72 °C, 30 sec 95°C, followed by 1min at 52°C and 8 min at 72°C. Fluorescent PCR fragments were visualized by capillary electrophoresis on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) with GeneScan 500 ROX as a standard and analyzed by the GeneMapper® Analysis Software version 3.7 (Applied Biosystems). To ensure that the multiplexing had no effect on the size of the amplified DNA fragments, loci were amplified singly for several individuals and compared with the multiplex profiles. Furthermore, we re-analyzed several samples to test the reproducibility of band length and genotyping. We then assessed the number and frequencies of alleles at each locus, calculated the diversity indices for each locus separately and averaged the values across loci.

Plantago chemical diversity

Site HEG11 was not sampled for *P. lanceolata* chemical diversity. In May 2009, the oldest and the youngest leaf of one *P. lanceolata* plant per plot were cut and transferred to methanol:dichloromethane (2:1, pH 6). The sample was homogenized and stored at 4°C for one week. Then leaves were extracted in the laboratory three times at different pHs (pH 6, pH 2, pH 9) following the protocol by Maier et al., (2010). Supernatants from each extraction step were pooled and water was added (2:1:1 methanol:dichloromethane:water) for phase separation. The aqueous phases were stored in Eppendorff tubes at 4 °C until analyses. Remaining pellets were dried for determination of dry weight (Sartorius LA120S, precision +/- 0.1 mg, Göttingen, Germany).

Metabolic fingerprinting was performed on an UHPLC-TOF-MS (1290 Infinity UHPLC and 6210 TOF, Agilent Technologies, Santa Clara, U. S. A.) equipped with a Grom-Sil 120 ODS-4-HE-column (150 x 2 mm, 3 µm; Alltech Grom GmbH, Rottenburg-Hailfingen, Germany) using a gradient from water with 0.1% formic acid (98-100%, Co. Merck, Darmstadt, Germany; solvent A) to acetonitrile (LC-MS grade, Co. Fisher Scientific UK Limited, Loughborough, Great Britain; solvent B) with 0.1% formic acid at a flow of 0.75 ml and an oven temperature of 35 °C. The gradient started at 5% solvent B with a hold for 1 min, increased from 2-16 min to 95% B and was held for 1 min at 95% B, followed by a cleaning and column equilibration cycle. Measurements were done in positive mode with a Dual ESI source (gas temperature: 350 °C, drying gas flow: 11 l/min, nebulizer pressure: 55 psig). For further details see Maier et al., (2010).

Metabolite data were pre-processed with R (version 2.9.0 and newer) package “xcms“ (Smith *et al.* 2006; R Development Core Team 2008; Tautenhahn *et al.* 2008) (method = ”centWave”, ppm = 23, profmethod = ”bin”, peakwidth = c(5,12), snthresh = 10, prefilter = c(3,200), fitgauss = T; settings for ”group”: bw = 30, minfrac = 0.5, minsamp = 1, mzwid = 1, max = 50, sleep = 0). Peaks occurring in blanks were subtracted from samples, except peaks with mean intensities being 100 or more times intensive than in blanks. Intensities lower than $1 \cdot 10^3$ were not considered. Each peak was considered a metabolite, for simplicity. Data were log transformed for normalization.

Appendix Table 1. Correlations between richness (S), Berger-Parker dominance (BP), Shannon’s diversity (H’), Simpson’s diversity (D_1), Simpson’s dominance (D_2), and Simpson’s evenness (E) for each organism/character group (*P*, *r*) measured in grassland plots in and around *Plantago lanceolata*. All statistics refer to within group correlations. Values in bold indicate significance at Bonferroni corrected α of 0.05/15 = 0.0033.

Plant (*N* = 60)	BP	H’	D_1	D_2	E
S	**<0.0001, -0.80**	**<0.0001, 0.94**	**<0.0001, 0.86**	**<0.0001, 0.89**	0.7993, -0.03
BP		**<0.0001, -0.92**	**<0.0001, -0.97**	**<0.0001, -0.97**	**<0.0001, -0.48**
H’			**<0.0001, 0.98**	**<0.0001, 0.98**	0.0490, 0.26
D_1				**<0.0001, 0.99**	**0.0018, 0.40**
D_2					**0.0020, 0.39**
Arbuscular mycorrhizal fungi (*N* = 60)	BP	H’	D_1	D_2	E
S	**<0.0001, -0.72**	**<0.0001, 0.95**	**<0.0001 0.88**	**<0.0001, 0.87**	0.2102, 0.16
BP		**<0.0001, -0.88**	**<0.0001, -0.94**	**<0.0001, -0.93**	**<0.0001, -0.76**
H’			**<0.0001, 0.98**	**<0.0001, 0.97**	**0.0002, 0.47**
D_1				**<0.0001, 0.99**	**<0.0001, 0.61**
D_2					**<0.0001, 0.59**
Aboveground arthropod (*N* = 60)	BP	H’	D_1	D_2	E
S	0.1218, -0.20	**<0.0001, 0.59**	0.0037, 0.37	**0.00314, 0.38**	**<0.0001, -0.56**

BP		**<0.0001, -0.80**	**<0.0001, -0.92**	**<0.0001, -0.92**	**<0.0001, -0.61**
H’			**<0.0001, 0.95**	**<0.0001, 0.96**	0.0421, 0.26
D_1				**0.0001, >0.99**	**<0.0001, 0.50**
D_2					**<0.0001, 0.50**
Belowground insect larvae (*N* = 20)	BP	H’	D_1	D_2	E
S	**0.0005, -0.70**	**<0.0001, 0.98**	**<0.0001, 0.90**	**<0.0001, 0.92**	0.3735, -0.21
BP		**<0.0001, -0.81**	**<0.0001, -0.92**	**<0.0001, -0.90**	0.0219, -0.51
H’			**<0.0001, 0.96**	**<0.0001, 0.96**	0.8820, -0.04
D_1				**<0.0001, 0.97**	0.3374, 0.23
D_2					0.5616, 0.14
Plantago molecular (*N* = 60)	BP	H’	D_1	D_2	E
S	**<0.0001 -0.56**	**<0.0001, 0.87**	**<0.0001, 0.59**	**<0.0001, 0.59**	0.1462, 0.19
BP		**<0.0001, -0.56**	**<0.0001, -0.88**	**<0.0001, -0.84**	**<0.0001, -0.73**
H’			**<0.0001, 0.59**	**<0.0001, 0.60**	0.0399, -0.27
D_1				**<0.0001, 0.95**	**<0.0001, 0.87**

D_2					**<0.0001, 0.90**

Plantago chemical (*N* = 59)	BP	H’	D_1	D_2	E
S	0.0210, -0.30	**<0.0001, 0.79**	**<0.0001, 0.61**	**<0.0001, 0.60**	**<0.0001, 0.52**
BP		**<0.0001, -0.634**	**<0.0001, -0.86**	**<0.0001, -0.88**	**<0.0001, -0.90**
H’			**<0.0001, 0.92**	**<0.0001, 0.91**	**<0.0001, 0.88**
D_1				**<0.0001, 0.99**	**<0.0001, 0.99**
D_2					**<0.0001, 0.99**

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Chapter X - General discussion

Discussion

Intensive land use (like high fertilizer application and mowing) represents one fundamental impact for decreasing species richness and biodiversity loss. Grazing and mowing, for example, lead to changes in vegetation complexity, plant growth (Kruess & Tscharntke 2002) and vegetation composition which besides can influence the diversity of herbivorous arthropods (Köhler et al. 2004; Unsicker et al. 2006). These changes in the diversity of organisms of one trophic level can influence the diversity of the next level (Hartley & Jones 2003). Despite studies on agricultural influences we still know little about how intensification in land use influences species of higher trophic levels apart from simply reducing species numbers (van der Putten et al. 2004).

Therefore my study aims to investigate the influence of land use on different trophic levels of a model system (plant-herbivore-parasitoid) and on different spatial scales in three German regions. I want to give an overview of the effects of land use on the single plant, the vegetation to upper trophic levels like herbivores and their parasitoid and additionally considering different spatial scales from subplot over plot level up to the landscape scale.

I investigated the influence of land use (intensively vs. extensively) on the ribwort plantain (*Plantago lanceolata*) and upper trophic levels, like two herbivorous weevils (*Mecinus labilis* and *M. pascuorum*) and their common parasitoid (*Mesopolobus incultus*) living on it. Furthermore I took a closer look on the influence of additional fertilizer application by implementing a fertilizer experiment in all three regions. Additionally I wanted to know how the host plant itself and the surrounding vegetation are influenced by land use and how these changes in host plant quality and quantity as well as in the vegetation and the odor bouquet influence upper trophic levels. After investigating the system on smaller scales like the subplot (6m x 6m) and plot (50m x 50m) level I was interested in how the weevils and their parasitoid are influenced by land use on larger spatial scales. Taking these aspects into account I have structured my thesis and the discussion by dividing my studies on the one hand in investigating different trophic levels from plant over vegetation up to higher trophic levels and on the other hand in describing the results of different spatial scales like subplot, plot and landscape level.

Host plant level

For individual plant performance fertilization (land use intensity) normally results in an increase in leaf nitrogen content (plant quality), the biomass and plant growth (plant quantity) of the plant (McNeill & Southwood 1978; Chen, Dawn & Ruberson 2010). Interestingly, in my study, the usual fertilization of the farmers had mostly no influence on host plant size (*Chapter IV*), although fertilizer is known to support the plant growth (Ellenberg 1977). In my fertilization experiment, where I added additional fertilizer in the investigated grassland, on the other hand rosette diameter and above ground biomass increased, whereas the number of stalks and therefore the potential source for reproduction decreased with fertilization (*Chapter V*). *Plantago lanceolata* seems to invest in growth but not in reproduction when fertilized. Land use type, mowing vs. grazing, had a significant positive effect on the host plant size. In the experimental study, contrary to the plots treated only by farmers, where land use had no effect, host plant abundance decreased with fertilization. This could be due to the fact that *P. lanceolata* prefers to grow in less dense areas with a lower vegetation cover (Cavers et al. 1980). Possibly *P. lanceolata* is not sufficiently competitive against the rising cover of grasses. These findings show that land use intensity is not overall beneficial for the investigated model plant.

As I expected, *Plantago* leaf nitrogen content as a proxy for primary metabolites was higher in fertilized grassland, whereas carbon content was not influenced. Looking at the secondary metabolites of *P. lanceolata*, aucubin and catalpol (IG) were positively correlated with plant species richness (*Chapter VII*). This stands in contrast to a similar study of Mraja et al. (2011) and our study on calculated diversity indices (*Chapter IX*). Mraja et al. (2011) found opposite effects of plant species richness on aucubin and catalpol content in *P. lanceolata* on experimental grassland plots with catalpol increasing and aucubin decreasing with higher plant species richness. In our indices study we detected a negative dependence of *P. lanceolata* chemical diversity on herbaceous plant diversity (*Chapter IX*). This shows that as the abundance of the most abundant plant species increases, the abundance of the most abundant chemical metabolite declined. This result is in contrast to my investigation of the secondary metabolites aucubin and catalpol (IG) which I mentioned before.

In case of the metabolites of the ribwort plantain I showed that fertilization increased the leaf nitrogen, which can be an advantage for herbivore diet. Surprisingly secondary metabolites were not influenced by land use directly but indirectly via the surrounding vegetation which was influenced by the land use.

Vegetation level

Repeated application of fertilizer can lead to a reduction of plant species richness because only few forbs can handle the nutritional input leading to a dominance of these species (Foster & Gross 1998; Billeter et al. 2008). As I expected, both, experimental fertilization and fertilization by farmers decreased plant species richness in the investigated grassland (*Chapter IV, V*). Land use type in terms of mowing reduced plant species richness, too, which is supported by a study of Köhler et al. 2004. Here agricultural influences in form of intensive mowing represent one of the major negative impacts on plant species richness. In my study species richness was highest on extensively used pastures which points out that extensive land use (low life stock densities and moderate grazing by cattle) support plant species richness. Joern (2005) described a positive correlation between extensive grazing and plant species richness, too.

Like plant species richness, vegetation structure also was affected by land use. Intensive land use (fertilization and mowing) influenced the vegetation structure in the investigated grassland positively (*Chapter IV*). The highest and most dense vegetation was measured on intensively used plots like meadows and fertilized grassland. Taking a closer look on the fertilization experiment I could show that the vegetation already changed after one application of fertilizer. Fertilization supported the general growth of the vegetation. These findings are confirmed by the studies of Gratton & Denno (2003) and Willems & van Nieuwstadt (2009). The proportion of grass species increased with fertilization because it can use additionally offered nutrients faster than forbs and therefore dominate fertilized areas (De Deyn et al. 2004). I found this effect of higher vegetation density and height as well as a lower number of plant species on intensively used, too (*Chapter IV*).

In contrast to the plant species richness vegetation structure and density benefited from intensive land use like higher fertilizer application and a high frequency of mowing.

Upper trophic levels

Since herbivores and their natural enemies may be affected not only directly by land use, but also indirectly by changes in host plant quality and quantity, as well as vegetation diversity and structure I investigated these different factors in my study.

Plant diversity impacts plant-insect interactions by creating habitat heterogeneity and increasing the number of niches which can result in higher herbivore diversity and abundance (Siemann et al. 1998, Unsicker et al. 2006). Higher diversity on different trophic levels and thus a higher degree of herbivory might lead to induction of IGs in *P. lanceolata*. Field experiments showed a positive correlation between IGs in *P. lanceolata* and herbivore damage (Mraja et al. 2011). Secondary plant metabolites of *P. lanceolata* did not influence the abundances of the weevils *M. pascuorum* and *M. labilis* (*Chapter VII*). Maybe both herbivores can detoxify or eliminate these compounds somehow and higher amounts might be avoided by selecting the host plant. Besides the IGs I observed the nitrogen content of the leaves of *P. lanceolata*. Increased nitrogen content of the host plants in fertilized grassland did not support insect abundance as expected. This contrary effect of nitrogen is found by Fischer & Fiedler (2000) and Joern & Behmer (1998), too. Cease et al. (2012) showed that N fertilization and protein rich artificial diets can have consistent negative effects on the fitness of a locust. An explanation for a negative performance or avoidance of N-rich plants by herbivores could be that nutrients in the plant might be unbalanced due to nitrogen fertilization.

Besides indirect influences of land use on the plant compounds, the availability of the host plant, like I found in fertilized grassland, often determines herbivore abundance. *Mecinus pascuorum* was positively influenced by host plant density, as shown in several other studies (Joshi et al. 2004, Vanbergen et al. 2007), too. On the other hand abundance of the larval parasitoid *M. incultus* in the field was explained exclusively by weevil abundance as discussed later on. The higher the trophic level, the more difficult it is to determine the impact of specific environmental factors on this trophic level. The observed outcome is often not directly caused by a single factor, but is mediated indirectly via its effect on other trophic levels (Siemann et al. 1998, Petermann et al. 2010).

Vegetation structure can interfere with herbivore behavior in different ways as I showed in my study (*Chapter IV, V*). The structural complexity of habitats and of single plants influences the movement ability of herbivores and their host finding process (Goodwin & Fahrig 2002, Hannunen 2002). Dense vegetation like I found in fertilized grassland can reduce the motility of individuals walking on the ground (Jopp 2006), crawling through the vegetation, or on conducting flights as proposed by Coll and Bottrell (1994). The structure of non-host plants can also indirectly affect herbivores *via* their natural enemies. Non-host plant structure may provide shelter for the enemies of herbivores (Langellotto & Denno 2004) or, in contrast, may render host search by the enemies more difficult (Tschanz et al. 2005). Complex vegetation structure (Coll & Bottrell 1996, Tschanz et al. 2005), like I found in fertilized grassland, has a negative influence on the foraging efficiency of enemies. Intensive land use not only had a negative direct and indirect impact on both herbivores, but also on the parasitoid.

In the laboratory the searching behavior of the herbivore and parasitoid differed substantially (*Chapter VIII*). *Mecinus pascuorum* was attracted by volatiles of flowering *P. lanceolata* and was not hampered by diverse plant bouquets in finding the host plant via olfactory cues. However, higher plant diversity leaded to an altered searching behavior and caused increased activity. A suitable foraging habitat might be an environment which supports intense host-searching behavior (Pettersson et al. 2008). An odorous environment may stimulate the weevils to search more intensively and may improve the likelihood of locating a host plant. Thus, my field and the laboratory data indicate that greater plant diversity may be a preferred habitat for the weevils. This is reflected by my results that the herbivores were more abundant in extensively used grassland with high plant species richness. This shows that extensive land use supports the abundance of insects.

Mesopolobus incultus was attracted by the host complex odor (combination of host plant and weevil) but was capable of perceiving non-host odors in the laboratory (*Chapter VIII*). Experiencing greater plant diversity still allows a successful host location. However, the parasitoid preferred the simpler bouquet when testing the presence of non-host plants against the host complex alone. During host location parasitoids do not only use volatile cues from the host or the host-plant, but also cues emitted by the host habitat (Vet et al. 1995). Plant damage can be proportional to volatile emission, at least for green leaf volatiles (Copolovici et al. 2011). Indeed, the weevils may not induce *P. lanceolata* in such a way that it leads to higher volatile emissions by the plant. However, for host location the quantity of emitted and detectable volatiles is essential (Dicke et al. 2003). Thus, it might be beneficial for the parasitoid to take general cues from the surrounding habitat into account (*Chapter VIII*). Gohole et al. (2005) showed that the presence of non-host plants does not hinder the close-range foraging activities of different parasitoids. *Mesopolobus incultus* might use habitat odors for long-range orientation and respond to the pure host complex at a short-range distance.

We already know that increasing land use intensity and other human influences on environments lead to a reduction in species diversity in different habitats (Unsicker et al. 2006) but how and in which way we still do not understand completely. I found out that land use did not only influence insects directly but also indirectly via the host plant and the vegetation. Although the host plant quality increased with intensive land use the herbivores preferred grassland with extensive land use which resulted in smaller host plants and higher plant species richness. The parasitoid on the other hand was only indirectly influenced by land use via his hosts.

Subplot level

My study was integrated into the long-term and large scale project "Biodiversity Exploratories". There I investigated three different regions from north to south Germany (Schorfheide-Chorin, Hainich-Dün and Schwäbische Alb). In the three regions 50 experimental plots (50m x 50m) were provided. In these plots I made my investigations on the subplot (6m x 6m) level. There I performed my fertilization experiment and found interesting results concerning the influence of land use intensity (fertilization) on my study system.

Fertilization in the subplots decreased plant species richness and host plant abundance, whereas it enhanced total vegetation growth. *P. lanceolata* prefers to grow in less dense areas with a lower vegetation cover (Cavers et al. 1980) what could explain the lower abundance in fertilized subplots. Application of fertilizer at the same time reduced forbs in favor of grasses. Grass species may handle the nutritional input better than forbs which I have discussed already. Biomass and rosette diameter of *P. lanceolata* increased with fertilization. To be competitive the host plant seems to invest in growth when fertilized as mentioned in the host plant section. Nitrogen content, as expected, increased with fertilization in the subplot level whereas the carbon content was not influenced at all. However the increased size and heightened leaf nitrogen content did not influence the herbivores positively. On the contrary the abundance of the two herbivores was strongly negative influenced by fertilization at the subplot level. Land use intensity (fertilisation) decreased the weevil abundance directly and indirectly via their host plant and the surrounding vegetation. The parasitoid however only depended on the abundance of one of its hosts, *M. pascuorum* (positively density-dependent).

The reduced herbivore abundance due to fertilization, which was the most important factor influencing the weevils, can additionally be explained by indirect effects via a lower abundance of the host plant and by changed patterns of host localisation within higher vegetation. Fertilization indirectly affected the third trophic level negatively by cascading up via host abundance. Summing up land use intensity had a negative effect on the upper trophic levels of my study system.

Plot level

In every region the plots were differently treated by farmers. It was differentiated between three different land use types (meadows, mown pastures and pastures) and the land use intensity (fertilized or not fertilized plots). On the plot level I focused on the investigation of the influence of land use type (mowing vs. grazing) and land use intensity (fertilization), which was established and handled by farmers who use the grassland in extensive or intensive ways, on the tritrophic study system.
Land use intensity (fertilization) and type (mowing vs. grazing) negatively affected the plant species richness like in the subplot level and known from other studies (Gratton & Denno 2003; Baessler & Klotz 2006). In contrast, land use had a mostly positive effect on host plant size and vegetation structure in the plot. I found that vegetation composition (plant species richness), which is influenced by land use, was positively correlated with IG content in *P. lanceolata* leaves but these changes in secondary metabolites did not cascade up through higher trophic levels (*Chapter VII*). Abundances of the weevils were negatively correlated with leaf nitrogen content (Joern & Behmer 1998, Fischer & Fiedler 2000, Cease et al. 2012) like in the subplot level. Host plant quality and vegetation composition did not affect the parasitoid and thus the herbivore-parasitoid interaction.
Additionally we compared diversities of organism groups across a land use gradient in order to determine which diversity indices provided the greatest ability to discriminate sites (*Chapter IX*). Compared to the results measured on the plot the calculated diversity of organisms was astonishingly little influenced by land use changes like increased fertilization, grazing and mowing. In this analysis we found no effect of land use on aboveground arthropods, belowground insect larvae, *P. lanceolata* chemical (compound) diversity (*Chapter IX*).
Like on the subplot level I found the same consistent effects of land use on the observed model system. Intensive land use always had a negative effect on the weevils and their parasitoid, showing that extensive land use is essentially to preserve biodiversity in an agricultural influenced land.

Landscape level

Until today investigations on how anthropogenic land use on the landscape scale, like I investigated in my study, influences herbivore-parasitoid interactions on different spatial scales and in different geographic regions, are rare. Therefore I took a closer look on the influence of land use at the landscape scale on my study system. The three insect species investigated were affected by extensively and intensively managed grassland, in opposite ways. Increasing proportion of extensively managed grassland had a positive effect on insect abundances (radii of 100 – 2000 m). Intensively managed grassland on the other hand had a negative effect on the two herbivores and their natural enemy (radii of 100 – 2000 m). This result is supported by Hendrickx et al. (2007) where the species richness of different investigated groups was most strongly affected by increased proximity of seminatural habitat (e.g. extensively used habitats like nutrient poor grassland and meadows with scattered fruit trees) patches. Seminatural habitats are important environments where a high number of species occurs and a biodiversity of species is supported by this habitat type compared to intensively used grasslands where species richness is less.

Looking at the parasitoid, *M. incultus*, was directly positively affected only by the density of its two host species and only indirectly, via its hosts, by land use intensity like we found for the smaller subplot level, too. Heisswolf et al. (2009) supported my findings. They showed that the occurrence probability of an egg parasitoid only depended (positively) on an increasing population density of its host, the leaf beetle *C. canaliculata* whereas the host was dependent on different landscape metrics like habitat size, isolation and quality.

Interestingly, the three insect species differed in the spatial scale at which they responded to the landscape diversity. While the abundance of the herbivore *M. labilis* was best explained by larger spatial scales (r = 1500 – 2000 m), the herbivore *M. pascuorum* and the parasitoid *M. incultus* responded most strongly to landscape diversity at smaller scales (r = 100 – 500 m). *Mecinus labilis* feeds oligophagous on *P. lanceolata* and *P. sempervirens* and may therefore have a larger range for host plant search. In contrast *M. pascuorum* feeds monophagous on *P. lanceolata*. For the herbivore smaller ranges may be sufficient for food search due to host plant density. Looking at the plot level or a smaller spatial scale *P. lanceolata* density is higher compared to larger scales where suitable growing habitats are less abundant due to a higher proportion of forest and paved areas. At smaller scales *M. pascuorum* does not need to search very long for his host plant. For the parasitoid stronger effects on smaller spatial scales are contrary to other studies where higher trophic levels like parasitoids depend in most cases on larger spatial scales. *Mesopolobus incultus* abundance is influenced by the abundance of his main host *M. pascuorum* which is more abundant at smaller scales as described before. Likewise, in another study, the less specialized parasitoid *F. reptans* was more likely to be found on patches with high beetle density, while patch size and isolation seem to be less important (Heisswolf et al. 2009).

Summing up the results of my study, I found a negative influence of intensive land use and fertilization on the upper trophic levels of *P. lanceolata* at all spatial scales investigated. Furthermore I found out that land use can influence insects indirectly via the surrounding vegetation (vegetation density, plant species richness, odorous environment). Land use, vegetation and host plant in intensively used subplots and plots are able to decrease insect abundance and on extensively used subplots and plots to increase the abundance. The same results I found for different landscape levels. Here the herbivores and the parasitoid preferred habitats with a higher proportion of extensively used and lower of intensively used grasslands.

Chapter XI - References

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Land use intensification in grasslands: higher trophic levels are more negatively affected than lower trophic levels

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C. Herbst

N. Wäschke

E. K. Barto

S. Arnold

D. Geuß

I. Halboth

S. Reschke

J. Sänger

T. Meiners

E. Obermaier

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All authors contributed to the ideas of this manuscript. E. Obermaier and T. Meiners originally formulated the idea of the investigations. C. Hancock, N. Wäschke and U. Schumacher developed the methodology and the sampling design. C. Hancock and N. Wäschke conducted the fieldwork in the different exploratories. C. Hancock and N. Wäschke performed the statistical analyses. This manuscript was written by C. Hancock and revised mainly by K.E. Linsenmaier and E. Obermaier. The language of this paper has been edited by the native speaker Karen Voss.

C. Hancock

N. Wäschke

U. Schumacher

Karl Eduard Linsenmaier

Torsten Meiners

Elisabeth Obermaier

Chapter XIII - Author´s contribution

Landscape wide land use intensity determines local abundance of higher tropic levels in grassland habitats

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The percentage workload carried out by the authors is distributed as follows:

All authors contributed to the ideas of this manuscript. E. Obermaier and T. Meiners originally formulated the idea of the investigations. C. Hancock, N. Wäschke and C. Westphal developed the methodology and the sampling design. C. Hancock, N. Wäschke, M. Bellach, C. Rothenwöhrer and J. Steckel conducted the fieldwork in the different exploratories. S. Arnold, MK. Peters and J. Steckel performed the statistical analyses. This manuscript was written by C. Hancock and revised mainly by E. Obermaier. The language of this paper has been edited by the native speaker Karen Voss.

C. Hancock

S. Arnold

N. Wäschke

M. Bellach

MK. Peters

C. Rothenwöhrer

J. Steckel

C. Westphal

T. Meiners

E. Obermaier

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This chapter is submitted to Oecologia as:

Wäschke N, Hancock C, Hilker M, Obermaier E and Meiners T (submitted) Defensive plant compounds relate to plant species richness but plant nitrogen content mediates multitrophic interactions in cultural landscapes

The percentage workload carried out by the authors is distributed as follows:

All authors contributed to the ideas of this manuscript. T. Meiners and E. Obermaier originally formulated the idea of the investigations. N. Wäschke and C. Herbst developed the methodology and the sampling design. N. Wäschke and C. Herbst conducted the fieldwork in the different exploratories. N. Wäschke conducted the chemical analysis. N. Wäschke performed the statistical analyses. This manuscript was written by N. Wäschke and revised mainly by T. Meiners and M. Hilker. The language of this paper has been edited by the native speaker Spence Behmer.

N. Wäschke

C. Hancock

M. Hilker

E. Obermaier

T. Meiners

Habitats as complex odorous environment: How does plant diversity affect herbivore and parasitoid orientation?

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All authors contributed to the ideas of this manuscript. T. Meiners and E. Obermaier originally formulated the idea of the investigations. N. Wäschke and C. Herbst developed the methodology and the sampling design. N. Wäschke and C. Herbst conducted the fieldwork in the different exploratories. N. Wäschke and K. Hardge developed the experimental design and conducted the experiments. N. Wäschke performed the statistical analyses. This manuscript was written by N. Wäschke and revised mainly by T. Meiners and M. Hilker. The language of this paper has been edited by the native speaker Martin McLean.

N. Wäschke

K. Hardge

C. Hancock

M. Hilker

E. Obermaier

T. Meiners

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E. K. Barto

T. Caruso

F. Buscot

M. Fischer

C. Herbst

T. S. Maier

T. Meiners

C. Müller

E. Obermaier

D. Prati

S. A. Socher

I. Sonnemann

N. Wäschke

T. Wubet

S. Wurst

M. C. Rillig

Curriculum Vitae

Persönliche Angaben:

Name:	Christine Hancock, geb. Herbst
Adresse:	Kolpingstr. 14 74254 Offenau
Geboren am/ in:	31.08.1981/ Heilbronn
Familienstand:	verheiratet

Ausbildung:

Seit 2008	Promotion an der Universität Würzburg im Rahmen der Biodiversity Exploratories (www.biodiversity-expolratories.de) bei Prof. Dr. Elisabeth Obermaier Thema der Dissertation „ Influence of land use on *Plantago lanceolata* L. and its higher trophic levels at different spatial scales and in three geographic regions”
2002 – 2007	Biologiestudium, Universität Hohenheim Diplom Zeugnis – Note 1,4 Hauptfach: Zoologie 1. Nebenfach: Tierökologie 2. Nebenfach: Parasitologie Diplomarbeit zum Thema „Chemische Kommunikation bei Schwimmkäfern (Familie: Dytiscidae)“
1992 - 2001	Hohenstaufen-Gymnasium, Bad Wimpfen Abiturzeugnis – Note 2,4 Leistungskurse: Englisch und Erdkunde
1988 – 1992	Grundschule Offenau

Praktika:

2006 - 2007	Hiwi in der Arbeitsgruppe Limnologie von Dr. Beate Baier an der Universität Hohenheim
2006 - 2007	Hiwi in der Arbeitsgruppe Tierökologie von Prof. Dr. J.L.M. Steidle an der Universität Hohenheim
2006	Generalitat de Catalunya, Departament de Medi Ambient i Habitatge, Direccio General del Medi Natural, Spanien
2005	Firma Nacken Landschaftsbau
2004	Fischereiforschungsstelle des Landes Baden-Württemberg, Langenargen

Qualifikationsprofil

Biologiestudium in 11 Semestern:

Zoologie (Hauptfach mit Schwerpunkten Systematik, Physiologie und Versuchstierkunde)
Parasitologie (Nebenfach mit Schwerpunkten Systematik, Laborstudien)
Tierökologie (Nebenfach mit Schwerpunkten Verhalten und Chemische Ökologie)

Zusatzqualifikationen:
Säugetier- und Versuchstierkunde
Bildanalytik und Elektronenmikroskopie
Systematik, Taxonomie, Evolution
Aquatische Ökologie, Limnologie

Sprachen:
Deutsch (Muttersprache)
Englisch (verhandlungssicher in Wort und Schrift)
Spanisch (fortgeschrittene Kenntnisse)
Portugiesisch (Grundkenntnisse)

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(Christine Hancock)

List of publications

Poster:

Tagung der Deutschen Gesellschaft für Ökologie (GFÖ), Leipzig
Impact of different land use intensity on chemical odour diversity, structural complexity of vegetation and host-parasitoid interactions.
(Wäschke N; Herbst C; Obermaier E; Meiners T), poster
Leipzig, September 2008

Multitrophic Interactions workshop, Göttingen
Influence of land use on the parasitoid Mesopolobus incultus (Chalcidoidea: Pteromalidae) via plant associate and vegetation parameters.
(Sänger J; Herbst C; Meiners T; Obermaier E), poster.
Göttingen, March 2010

ISCE, Vancouver 2011
Vegetation diversity and its impact on tritrophic interactions: a case study of Plantago lanceolata, weevils and larval parasitoids
(Wäschke N; Herbst C; Obermaier E; Hilker M; Meiners T), poster.
Annual Meeting of the International Society of Chemical Ecology,
Canada, July 2011.

14. Symposium of Insect Plant Interactions (SIP14), Wageningen, Netherlands
Multitrophic interactions in complex habitats: role of plant odour diversity and vegetation structure.
(Meiners T; Wäschke N; Herbst C; Obermaier E) poster.
Wageningen, Netherlands, August 2011

96th ESA Annual meeting, USA
Choosing and using diversity indices: perspectives for ecological applications from a large scale field experiment.
(Caruso T; Barto K; Buscot F; Fischer M; Herbst C; Maier TS; Meiners T; Mueller C; Obermaier E; Prati D; Socher S; Sonnemann I; Waeschke N; Wubet T; Wurst S; Rillig MC), poster.
Austin, USA, August 2011.

Talks:

Tagung der Deutschen Gesellschaft für Ökologie (GFÖ), Bayreuth

Impact of increasing land use intensity on vegetation structure and diversity and the functionality of a host-parasitoid interaction taking place within.
(Obermaier E; Herbst C; Wäschke N; Meiners T), talk.
Bayreuth, September 2009

ISCE, Tours (France)

Orientation in complex odorous environments: Does plant species diversity affect plant chemical complexity and a weevil-parasitoid interaction?
(Meiners T; Wäschke N; Herbst C; Hilker M; Obermaier E), talk.
France, August 2010

Tagung der Deutschen Gesellschaft für Ökologie (GFÖ), Gießen

Orientation in complex odorous enviroments: Does plant species diversity affect complexity of vegetation odor and arthropod orientation?
(Meiners T; Wäschke N; Herbst C; Hilker M; Obermaier E), talk.
Gießen, August 2010

Land use and neighbor identify influence arbuscular mycorrhizal fungal community composition.
(Barto EK; Herbst C; Meiners T; Obermaier E; Wäschke N; Wubet T; Rillig M), talk.
Gießen, August 2010

Multitrophic Interactions workshop, Göttingen

Influence of land use on the tritrophic system of Plantago lanceolata (L.) and the surrounding vegetation in three different landscapes.
(Herbst C; Wäschke N; Meiners T; Obermaier E), talk.
Göttingen, March 2010

Influence of habitat odour diversity on multitrophic interactions.
(Wäschke N; Herbst C; Obermaier E; Meiners T), talk.
Göttingen, March 2010

DGAAE conference, Berlin
Tritrophic interactions in complex odorous environments: Do land use, plant species and odor diversity affect orientation of insects?
(Meiners T; Wäschke N; Hardge K; Herbst C; Hilker M; Obermaier E), talk.
Berlin, March 2011.

Tagung der Deutschen Gesellschaft für Ökologie (GFÖ), Oldenburg
Influence of land use on a tritrophic system in three landscapes.
(Obermaier E; Herbst C; Wäschke N; Meiners T), talk.
Oldenburg, Germany, September 2011

Paper:

Published:

Herbst C, Baier B, Tolasch T, Steidle JLM (2010) Demonstration of sex pheromones in the predaceous diving beetle *Rhantus suturalis* (MacLeay 1825) (Dytiscidae). Chemoecology. DOI 10.1007/s00049-010-0061-3

Accepted / in press:

Hancock C., Wäschke N., Schumacher U., Linsenmair K.E., Meiners T. and Obermaier E. (2012) Fertilizer application decreases insect abundance on *Plantago lanceolata*: a large-scale experiment in three geographic regions. Arthropod-Plant Interactions. DOI 10.1007/s11829-012-9237-9

Submitted:

Herbst C, Wäschke N, Barto EK, Arnold S, Geuß D, Halboth I, Reschke S, Sänger J, Meiners T and Obermaier E (submitted to Entomologia Experimentalis et Applicata) Land use intensification in grasslands: higher trophic levels are more negatively affected than lower trophic levels.

Wäschke N, Hancock C, Hilker M, Obermaier E and Meiners T (submitted to Oecologia) Defensive plant compounds relate to plant species richness but plant nitrogen content mediates multitrophic interactions in cultural landscapes

Wäschke N, Hardge K, Hancock C, Hilker M, Obermaier E and Meiners T (submitted to Oikos) Habitats as complex odorous environment: How does plant diversity affect herbivore and parasitoid orientation?

Barto EK, Caruso T, Buscot F, Fischer M, Herbst C, Maier TS, Meiners T, Müller C, Obermaier E, Prati D, Socher SA, Sonnemann I, Wäschke N, Wubet T, Wurst S and Rillig MC (submitted to Journal of Applied Ecology) Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories

In preparation:

Hancock C, Arnold S, Wäschke N, Bellach M, Meiners T, Peters MK, Rothenwöhrer C, Steckel J, Westphal C and Obermaier E (in prep.) Landscape wide land use intensity determines local abundance of higher tropic levels in grassland habitats

Ehrenwörtliche Erklärung

Gemäß § 4, Absatz 3, Ziffer 3, 5 und 8 der Promotionsordnung für die Fakultät für Biologie der Bayerischen Julius-Maximilians-Universität Würzburg vom 15. März 1999, zuletzt geändert durch Satzung vom 12. August 2009, erkläre ich hiermit ehrenwörtlich, dass ich die vorliegende Dissertation selbstständig angefertigt habe und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe. Die Dissertation hat keinem anderen Prüfungsverfahren vorgelegen, weder in gleicher noch in ähnlicher Form. Die Universität Hohenheim hat mir am 12.12.2007 den Titel „Diplom-Biologin“ verliehen. Weitere akademische Grade habe ich weder erworben noch zu erwerben versucht.

Würzburg, November 2012

(Christine Hancock)