Assessing the effects of pesticides and fertilizers on a natural plant community of a field margin: An experimental field study

von

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„Leben ist nicht genug, sagte der Schmetterling.

Sonnenschein, Freiheit und eine kleine Blume

gehören auch dazu."

Hans Christian Andersen, 1805-1875 – dänischer Schriftsteller

Overview of this dissertation

The present thesis is a **cumulative dissertation** based on the peer-reviewed publications listed below.

The dissertation begins with an introduction that presents the general topic of this thesis and its overall relevance (chapter 1). In the second chapter, the objectives of this thesis, the study design and the thesis structure are described (chapter 2) and subsequently, the publications are presented, which constitute different chapters (chapter 3, 4, 5 and 6).

The publications are followed by a general discussion that puts the key results of the thesis into a broader context (= a summary and discussion of the major results of this thesis) (chapter 7). In the last chapter, the conclusions and the outlook are presented (chapter 8).

Publications:

Paper I **Schmitz, J.**, Hahn, M., Brühl, C.A. (2014): Agrochemicals in field margins – An experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community. *Agriculture, Ecosystems & Environment*, 193: 60-69.

Paper II **Schmitz, J.**, Schäfer, K., Brühl, C.A. (2013): Agrochemicals in field margins – Assessing the impacts of herbicides, insecticides, and fertilizer on the common buttercup (*Ranunculus acris*). *Environmental Toxicology and Chemistry*, 32 (5): 1124-1131

Paper III **Schmitz, J.**, Schäfer, K., Brühl, C.A. (2014): Agrochemicals in field margins – Field evaluation of plant reproduction effects. *Agriculture, Ecosystems & Environment*, 189: 82-91.

Paper IV **Schmitz, J.**, Stahlschmidt, P., Brühl, C.A.: Assessing the risk of herbicides to terrestrial non-target plants using higher-tier studies. Manuscript.

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Thesis Abstract

English Abstract

Field margins are often the only remaining habitats of various wild plant species in agricultural landscapes. However, due to their proximity to agricultural fields, the vegetation of field margins can be affected by agrochemicals applied to the crop fields. The aim of this thesis was to investigate the individual and combined effects of herbicide, insecticide and fertilizer inputs on the plant community of a field margin. Therefore, a 3-year field experiment with a randomized block design including seven treatments (H: herbicide, I: insecticide, F: fertilizer, H+I, F+I, F+H and F+H+I) and one control was conducted on a low-production meadow. Each treatment was replicated 8 times in 8 m x 8 m plots with a distance of 2 m between each plot. The fertilizer rates (25 % of the field rate) and pesticide rates (30 % of the field rate) used for the plot applications were consistent with realistic average input rates (overspray + drift) in the first meter of a field margin directly adjacent to a wheat field.

The study revealed that fertilizer and herbicide misplacements in field margins are major factors that affect the natural plant communities of these habitats. In total, 20 of the 26 abundant species on the study site were significantly affected by the fertilizer and herbicide treatment. The fertilizer promoted plants with high nutrient uptake and decreased the frequencies of small species. The herbicide caused a nearly complete disappearance of three species directly after the first application, whereas sublethal effects (e.g., phytotoxic effects and reduced seed productions of up to 100 %) were observed for the other affected species. However, if field margins are exposed to repeated agrochemical applications over several years, then such sublethal effects (particularly reproduction effects) also reduce the population size of plant species significantly, as observed in this study.

Significant herbicide-fertilizer interaction effects were also detected and could not be extrapolated from individual effects. The fertilizer and herbicide effects became stronger over time, leading to shifts in plant community compositions after three years and to a 15 % lower species diversity than in the control. The insecticide significantly affected the frequencies of two plant species (1 positively and 1 negatively). The results of the experiment suggest that a continuous annual agrochemical application on the study site would cause further plant community shifts and would likely lead to the disappearance of certain affected plants. A clear trend of increasing grass dominance at the expense of flowering herbs was detected. This finding corresponds well with monitoring data from field margins near the study site.

Although herbicide risk assessment aims to protect non-target plants in off-field habitats from adverse effects, reproduction effects and combined effects are currently not considered. Furthermore, no regulations for fertilizer applications next to field margins exist and thus, fertilizer misplacements in field margins are likely to occur and to interact with herbicide effects.

Adaptations of the current risk assessment, a development of risk mitigation measures (e.g., in-field buffers) for the application of herbicides and fertilizers, and general management measures for field margins are needed to restore and conserve plant diversity in field margins in agricultural landscapes.

German Abstract (Zusammenfassung)

Feldsäume gehören zu den letzten verbliebenen Lebensräumen für Wildpflanzenarten in der Agrarlandschaft. Aufgrund ihrer unmittelbaren Nähe zu den bewirtschafteten Flächen kann jedoch ihre Vegetation durch den Eintrag von Agrarchemikalien beeinträchtigt werden. Das Ziel dieser Arbeit war es die Einzel-, und Kombinationseffekte von Herbizid-, Insektizid- und Düngereinträgen auf die Pflanzengemeinschaft eines Feldsaums zu untersuchen. Es wurde ein 3-jähriges Freilandexperiment mit einem randomisierten Blockdesign, bestehend aus 7 Behandlungen (H: Herbizid, I: Insektizid, D: Dünger, H+I, D+I, D+H, D+H+I) und einer Kontrolle mit jeweils 8 Replikaten (= Parzellen), auf einer Wiese durchgeführt. Die Parzellen hatten je eine Größe von 8 m × 8 m und waren durch 2 m breite Wege voneinander getrennt. Die für die Behandlung der Parzellen verwendeten Dünger- (25 % der Feldrate) und Pestizidraten (30 % der Feldrate) entsprachen realistischen Eintragsraten (Überspritzung + Abdrift) in den ersten Meter eines Feldsaums in Nachbarschaft zu einem Getreidefeld.

Die Studie zeigte, dass Dünger- und Herbizideinträge wesentliche Faktoren darstellen, welche die natürliche Pflanzengemeinschaft in Feldsäumen beeinflussen. 20 der 26 häufigsten auf der Wiese vorkommenden Arten zeigten signifikante Effekte durch die Dünger- und Herbizidbehandlung. Die Düngung förderte stickstoffliebende Pflanzenarten und reduzierte das Vorkommen von kleinwüchsigen Arten. Durch das Herbizid wurden drei Pflanzenarten bereits im ersten Jahr fast vollkommen verdrängt, während andere Arten hauptsächlich subletale Effekte (z.B. phytotoxische Effekte, eine bis zu 100 % reduzierte Samenproduktion) vorwiesen. Werden Feldsäume allerdings über mehrere Jahre Agrarchemikalien ausgesetzt, führen auch diese subletalen Effekte (insbesondere Effekte auf die Reproduktion) zu einer Reduzierung der Populationsgröße, wie in dem Feldversuch beobachtet werden konnte. Die Kombinationsbehandlung von Dünger und Herbizid führte zu signifikanten Interaktionseffekten, welche sich nicht von den Effekten der Einzelbehandlungen extrapolieren ließen. Die Dünger- und Herbizideffekte intensivierten sich über den Untersuchungszeitraum, führten nach 3-jähriger Anwendung zu einer Veränderung in der Pflanzengemeinschaft, und reduzierten die Pflanzendiversität um 15 % im Vergleich zur Kontrolle. Das Insektizid wirkte sich signifikant auf das Vorkommen von zwei Pflanzenarten aus (1 positiver, 1 negativer Effekt). Die Ergebnisse des Feldversuchs lassen darauf schließen, dass eine fortführende Behandlung zu weiteren Gemeinschaftsveränderungen und wahrscheinlich auch zum Verschwinden bestimmter Pflanzenarten führen würde. Es war eine Tendenz zur Ausbildung von Gras-Dominanzbeständen zu erkennen, welche einen Verlust von Blütenpflanzen mit sich brachte. Dies konnte auch in eigenen Monitoringstudien in Feldsäumen beobachtet werden.

Zwar zielt die Risikobewertung von Herbiziden darauf ab Nichtziel-Pflanzen in Habitaten außerhalb des bewirtschafteten Feldes vor nachteiligen Auswirkungen zu schützen, Reproduktionseffekte und Kombinationseffekte werden bisher jedoch nicht berücksichtigt. Zudem gibt es keine Regelungen zur Düngeranwendung in Nachbarschaft zu Feldsäumen, weshalb Düngereinträge in Feldsäume und deren Interaktion mit Herbizideffekten sehr wahrscheinlich sind.

Anpassungen der derzeitigen Risikobewertung, eine Entwicklung von Risikominderungsstrategien für die Herbizid- und Düngerapplikation, sowie generelle Managementmaßnahmen für Feldsäume sind daher dringend notwendig, um die Pflanzendiversität in Feldsäumen zu erhöhen und zu schützen.

1 Introduction

1.1 Background - Agriculture in Europe

Agriculture has a long history, dating back to approximately 10,000 years ago (Stoate et al. 2001). Traditionally, agricultural landscapes have developed over centuries, and a wide variety of farming practices have been implemented. However, the second half of the 20th century saw a revolution in agricultural practice that surpassed any previous agricultural change (Benton et al. 2003). Due to the increased human population growth after the Second World War and the belief that food demand would increase faster than food production, the intensification of crop production began. The mechanization of agriculture increased rapidly and the use of agrochemicals, such as synthetic pesticides and fertilizers, has become common practice. This intensification allowed an unprecedented increase in agricultural productivity but was also connected with dramatic landscape transformations (Freemark & Boutin 1995; Stoate et al. 2001). Farmers were urged to increase their output and thus, small, extensively managed farmlands with high landscape heterogeneity and diverse wildlife habitats (e.g., hedgerows, field margins, wetlands, ditches, and grasslands) have been removed and converted to intensively farmed areas (e.g., monocultures) (Flohre et al. 2011; Benton et al. 2003). Fields have been amalgamated and enlarged to enhance farming efficiency (Firebank et al. 2008; Stoate et al. 2009). For example, in Germany, from 1970 to 2010, the average farm size increased from approximately 17 ha to 56 ha, whereas the total number of farms decreased by over 50 % (BMELV 2013; Statistisches Bundesamt 2011). Currently, agriculture is the most dominant land use in Germany, and other European countries, accounting for almost half of the total area (approximately 160 million ha in the EU, and 17 million ha in Germany) (BMELV 2013; Stoate et al. 2009).

1.2 Field margins in agricultural landscapes

Landscape transformations and intensified land use management unquestionably contributed to the impoverishment of European farmland biodiversity (Geiger et al. 2010; Stoate et al. 2009). The fragmentation and destruction of natural and semi-natural habitats (e.g., hedgerows, field margins, wetlands, ditches, grasslands, and fallow land) caused population isolations and negatively affected the population dynamics and biodiversity of agroecosystems (Tscharntke et al. 2002; Stoate et al. 2009; Benton et al. 2003; Baessler & Klotz 2006; Robinson & Shuterland 2002). For example, long-term monitoring studies have revealed that agricultural intensification caused reduced farmland bird populations due to a loss of suitable breeding sites and diminished food supplies such as insects and plants (Chamberlain et al. 2000 and references therein; Freemark & Boutin 1995). Today, the most common habitat types remaining for wild animal and plant species within farmlands are **field margins**. These structures are semi-natural habitats along the boundaries of agricultural fields (Marshall & Moonen 2002). Field margins are basic components of agricultural landscapes; however, their types can vary among countries (Marshall & Moonen 2002; Tarmi 2002). In Germany, the term *field margin* describes linear, permanent vegetation strips of primarily grassy and herbaceous off-crop habitats directly adjacent to agricultural fields (Kühne & Freier 2001). These habitats are outside the treated area (off-field) and can be referred to as terrestrial non-target areas. Due to the reduced number of natural and semi-natural habitats in agricultural landscapes, field margins are ecologically important (Boutin et al. 2012; Marshall & Moonen 2002). These habitats provide corridors for the movement of flora and fauna between crops and off-crop habitats (Dover et al. 1994; Sparks & Paris 1995; Marshall & Moonen 2002; Nentwig 2000). However, the maintenance of biodiversity depends not only on habitat availability, but also on habitat quality (Bäckman et al. 2002; Tarmi 2002). The habitat quality of field margins primarily depends on plant species diversity and plant community composition (Tarmi 2011). Plants are the primary producers and form the basis of any food web in a terrestrial ecosystem. Several studies have demonstrated that high plant species diversity and productivity generally increase the diversity of higher trophic levels (e.g., Siemann et al. 1998; Wilson et al. 1999; Smart et al. 2000). Many herbivorous insects (e.g., grasshoppers, caterpillars, and cicadas) consume various parts of plants. In turn, these insects represent food for predatory arthropods, such as spiders, parasitoid flies, and wasps. In addition, wildflowers in field margins offer valuable sources of nectar and pollen for bumblebees, solitary bees, wasps, and butterflies (Bäckmann & Tiainen 2002; Carreck & Williams 2002; Holzschuh et al. 2009). Most arthropods are food for insectivorous birds, nestlings and mammals. Thus, plants in field margins provide not only shelter and an environment to reproduce but also essential food sources for many farmland organisms (Aebischer & Blake 1994; Tew 1994; Vickery et al. 2009). Moreover, a high abundance and diversity of arthropods in field margins have beneficial effects on agroecosystems because these arthropods move into adjoining arable fields and provide ecosystem services, such as natural pest control (Dennis & Fry 1992; Pfiffner & Luka 2000) and pollination (Pywell et al. 2004; Power et al. 2010).

Although, it is currently known that field margins are crucial for the conservation of biodiversity at the landscape level, these structures have nevertheless a limited width (Kleijn & Verbeek 2000; Hahn et al. 2014). A study using digital orthophotos and geographical information systems assessed the sizes of field margins in agricultural landscapes and demonstrated that field margins with a width of 1 to 2 m are the main and typical margins remaining in the German agricultural landscape (Hahn et al. 2014). Together with the linear structure of field margins, this limited width results in a high edge to area ratio. Consequently, field margins are extremely susceptible to disturbances from the surrounding agricultural land use, which can result in considerable effects on the plant and animal diversity of field margins at a local scale (Kleijn & Verbeek 2000; Deckers et al. 2004).

1.3 Factors influencing the plant diversity of field margins

In recent decades, large-scale monitoring studies have detected a reduction in plant diversity in field margins (Bunce et al. 1994; Jobin et al. 1997; Kleijn & Verbeek 2000; Roß-Nickoll et al. 2004; Hovd & Skogen 2005). For example, Roß-Nickoll et al. (2004) surveyed the vegetation composition in German agricultural landscapes and observed that field margins tend to develop vegetation that is dominated by grasses with a resulting loss of dicotyledonous plants. Similar observations were made in other parts of Europe, e.g., in the Netherlands (Kleijn & Verbeek 2000), Norway (Hovd & Skogen 2005), Finland (Tarmi et al. 2002) and Britain (Smart et al. 2002).

Many different factors associated with agricultural intensification (e.g., mechanization, and habitat destruction) and disturbances caused by activities on the adjoining arable field (e.g., close plowing) are made responsible for the decline in plant diversity in field margins (Marshall 1987; Freemark & Boutin 1995; Kleijn & Verbeek 2000). However, the increased use of agrochemicals, such as pesticides and fertilizers, and their misplacements in off-field habitats may have also contributed to a loss of biodiversity in these habitats (Marrs et al. 1989; Jobin et al. 1997; Firbank et al. 2008).

In general, synthetic fertilizers were introduced in the 19th century and became popular after the Second World War, when the synthetic fertilizer industry expanded (Bührer 2001). Since that time, fertilizer use has steadily increased, and currently, approximately 160 million tons of nitrogen fertilizers are applied worldwide each year. In Germany, the average annual use of nitrogen fertilizers is 1.6 million tons, which is over 3 times higher than in 1949 (Bührer 2001; Fuchs 2012).
Synthetic pesticides were introduced following the Second World War, and their use has also increased substantially to approximately 2.5 million tons per year worldwide (Sanchez-Bayo 2011). In Germany, the average pesticide use is 45 000 tons per year (2.3 times higher than in 1970), with herbicides comprising the largest percentage (44 %) (BVL 2013a; Riester & Huber 2013).

Herbicides used in agriculture are designed to kill or to suppress undesirable plants (often called weeds) in arable fields, which compete with crop plants for resources. These unwanted plants (e.g., wild herbs and grasses) in arable fields are, by definition, target species of herbicide applications, whereas wild plant species outside the field, growing, for example, in field margins, are non-target species. Generally, these non-target plant species should not be affected by herbicide applications on the adjoining arable field. However, the drift of herbicides to field margins can also cause negative effects on sensitive wild plant species in these habitats (Kleijn & Snoeijing 1997; Marrs et al. 1997; de Snoo et al. 2005; Kjaer et al. 2006a, 2006b; Damgaard et al. 2008; Strandberg et al. 2012).

Fertilizers are applied on cropped fields to increase the amount of plant nutrients in the soil, which are necessary to raise the overall productivity of crop plants and, thus, to enhance the crop yield. Fertilizer additions to grasslands, however, reduce the plant species richness of these habitats by encouraging a few plant species with high nutrient uptake. Numerous studies have documented such effects of nutrient supplies on grasslands (e.g., Willems et al. 1993; Hautier et al. 2009; Kleijn et al. 2009; Socher et al. 2013), whereas the effect of fertilizer inputs on the plant diversity of field margins has insufficiently studied thus far (Boatman et al. 1994; Wilson 1999; Kleijn & Snoejing 1997; Tsiouris & Marshall 1998).

Fertilizers and herbicides are both designed to influence vegetation, and therefore, their applications to field margins may also involve interactions with each other and will potentially cause combined effects on plants. Although the use of herbicides and fertilizers in agriculture is widespread and common practice since many decades, and although, it is assumed (as noted above) that such agrochemicals are responsible for biodiversity reductions in field margins, only three published studies have investigated such combined/interaction effects on non-target plant species (Kleijn & Snoeijing 1997; Gove et al. 2007; Strandberg et al. 2012).

In addition, plants in field margins are not only exposed to fertilizers and herbicides but also to other agrochemicals, such as insecticides, which could cause further stress to plants. Insecticides are generally used to control insect pests in arable fields and are not targeted against plant species. Nevertheless, their applications may indirectly affect plant populations by reducing the density of pollinators (Potts et al. 2010; Blair 1991; Gist & Pless 1985) (= possibly negative effects on plants) or by reducing the density of herbivores (Egan et al. 2014) (= possibly positive effects on plants). However, until now, such indirect effects on plants have received little attention.

In conventional agriculture, farmers apply fertilizers and pesticides multiple times every year. The yearly repeated exposures of plants in field margins might intensify the effects and/or cause cumulative effects of fertilizers and pesticides on the plant community composition. At present, little is known concerning the possible cumulative and long-term effects of repeated agrochemical inputs on the plant community composition and plant species diversity of field margins. In existing field margin plant communities, it is generally difficult to distinguish the effects of pesticides and fertilizers because the vegetation has been simultaneously exposed to these agrochemicals for the last 5-6 decades.

Increasing our knowledge on the individual and combined effects of fertilizers and pesticides on plants in field margins is crucial for protecting, conserving and restoring biodiversity in agroecosystems. The preservation of biodiversity in agricultural landscapes is also one of the six major targets of the new EU Biodiversity Strategy for 2020 (adopted in May 2011 by the European Commission) ("Target 3: More sustainable agriculture," European Commission 2011). Understanding and characterizing the effects of agrochemicals on plant communities in field margins are necessary for raising public and political awareness of such effects, which in turn is required to ensure the protection of wild plant species in field margins in agricultural landscapes.

1.4 Entry routes of agrochemicals into field margins

1.4.1 Pesticides

Pesticides can reach non-target plant species in habitats adjacent to fields via different entry routes during crop applications. Some of the most frequently mentioned ways of exposure are spray drift, run-off and/or volatilization. Of these entry routes, the registration authorities for pesticides indicated that **spray drift** (particulates that become air-borne during application) is the major exposure pathway that can affect non-target organisms in off-crop habitats (European Commission 2002; EPPO 2003). The quantity of sprayed pesticide that is deposited in field margins depends on meteorological conditions (e.g., wind speed, and direction, temperature, and humidity) and on technical features, such as droplet spectrum and travel speed during application. Ganzelmeier et al. (1997) and Rautmann et al. (2001) studied spray drift in a series of field trials. The basic drift values of these studies have been published and are currently used in pesticide registration in the European Union (Rautmann et al. 2001). The initial assessment of spray drift for arable fields is conducted at a distance of 1 m from the field edge (European Commission 2002).

However, because of this policy, the effects of agrochemical inputs on the first meter (0 -1 m) of a field margin directly adjacent to the field are currently not considered. This is presumably based on the wording used in the document “Environmental risk assessment scheme for plant protection products” published by the European and Mediterranean Plant Protection Organization (EPPO 2003). Accordingly, non-target areas do not border directly on treated areas because policymakers assume that a narrow vegetation strip is usually present between the treated and the non-treated area (EPPO 2003). However, in Germany, there is no transition area between the cropped field (in-field = treated area) and the field margin (off-field = non target area). Studies from the Netherlands (Kleijn & Verbeek 2000) and from Finland (Tarmi 2002) also reported that the permanent vegetation of field margins borders directly on arable fields. Hence, such field margins receive not only pesticide inputs via **spray drift** but also via **overspray**:

An overspray of field margins can occur because nozzles on boom sprayers are mounted in such a manner that their spray cones overlap. This overlapping is required to assure a full 100 % application rate in the field. In conventional agriculture, the application of arable land is conducted directly up to the field edge and thus, the last nozzle of the spray arm is placed above the field border. Due to the spray cone of this nozzle, parts of the adjacent field margin are oversprayed with 50 % of the field rate (Fig.1-1). The area of a field margin receiving an overspray during application depends on the field cultivation and on the corresponding height of the spray arm. For example, field margins adjacent to cereal fields are usually exposed to overspray in the first 75 cm, which is followed by spray drift with 15 % of the field rate at a distance of 76 cm and a 2.77 % drift rate at a distance of 1 m from the crop edge (personal communication D. Rautmann, Julius Kühn Institute, Braunschweig, Germany with C. Brühl, University Koblenz-Landau, Germany) (Fig.1-1).

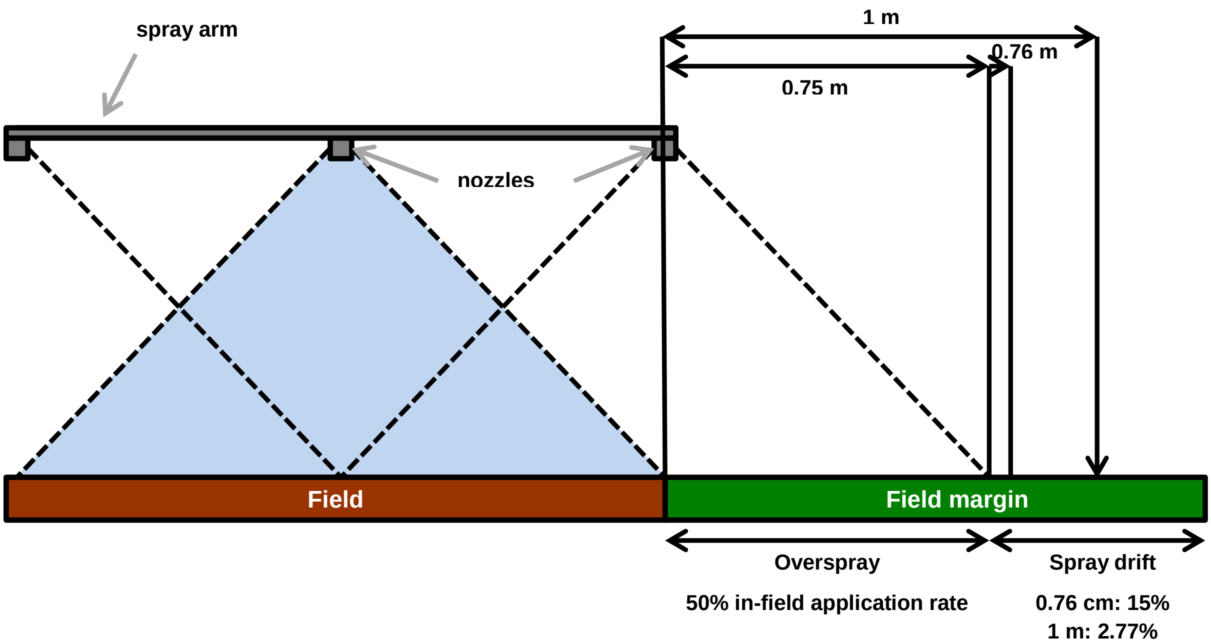

Fig. 1-1: Sketch of pesticide inputs via overspray and spray drift in cereal field margins. The blue colored area illustrates the spray cone of one nozzle. See text for explanation.

Pesticides are often labeled with product-specific risk-mitigation measures, such as in-field buffer zone distances between 5 and 20 m to terrestrial non-target areas to reduce pesticide inputs in these habitats. However, these regulations are often softened by exceptions in Germany (BVL 2013b). For example, field margins less than 3 m wide are exempt from such regulations and thus, farmers do not have to maintain a distance to field margins during applications and can legally spray in these margins, as previously mentioned. The problem is increased because the majority of field margins are only 1 to 2 m wide in Germany (Hahn et al. 2014). Consequently, most field margins are not protected by risk-mitigation measures.

Based on the circumstances described above, overspraying of field margins and the following spray drift are two major entry routes of pesticides in the frequently encountered narrow field margins in German agricultural landscapes.

1.4.2 Fertilizer

On cropped fields, fertilizers are usually applied in a dry granular form, which acts as a time-release capsule that allows nutrients to flow out over time. The most common type of fertilizer applicators used on farms is spreaders, which distribute the fertilizer via spinning disks from the back and sides of the spreader. The popularity of such spinning disc spreaders lies in their relatively low costs, easy maintenance, and, in particular, in their high working width (approximately 6 to 36 m) (Van Liedekerke et al. 2008). However, with this application method, fertilizer misplacements in field margins are likely to occur (Rew et al. 1995; Tsiouris & Marshall 1998; Wilson 1999). According to

Rew et al. (1992) and Tsiouris & Marshall (1998), who studied the patterns of granular fertilizer deposition in field margins, fertilizer misplacement can range from 25 % to 50 % of the field rate in the first meter of a field margin. Such relatively high fertilizer misplacements in field margins can occur because no distance requirements for the application of fertilizers near field margins exist.

1.5 Terrestrial non-target plants in the risk assessment of herbicides

The legal basis for authorizing pesticides in the EU was set with the Council Directive 91/414/EEC concerning the placing of plant protection products on the market, (Füll et al. 2000). This Directive was implemented in 1991 and was updated in 2009 by Regulation (EC) No 1107/2009. This new regulation aims to provide greater uniformity in the risk assessment of pesticides across Europe. In addition, issues that were considered likely to have been missed in the first Directive are now included (e.g., endocrine disruption, the negative effects of co-formulants, and the effects of combinations of chemicals). Moreover, the new Regulation (EC) No 1107/2009 explicitly lists biodiversity as a protection goal.

The Plant Protection Product Directive requires that pesticides are only used for their intended function and that such a use does not cause unreasonable effects either on human health or on the environment (EU Directive 1107/2009). Therefore, a comprehensive risk assessment procedure must be undertaken for each active substance before a pesticide can be authorized for marketing and use. Regarding herbicides, this procedure also includes a risk assessment for non-target terrestrial higher plants. Non-target plants are generally defined as non-crop plants located outside the treatment area in so-called off-crop habitats, such as field margins (European Commission 2002; EPPO 2003).

For regulatory purposes, Guidance Documents for risk assessment procedures of pesticides for terrestrial non-target organisms (including non-target plants) (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC) and plant testing guidelines (OECD 2006) were developed. According to these guidelines, the risk assessment of herbicides for non-target plants is currently based on emergence or vegetative vigor tests of single plant species in pots at young development stages (2-6 leaf stage). These tests must be performed in greenhouses under standardized conditions and last 21 to 28 days, and the most commonly used end-points are mortality and effects on plant biomass (OECD 2006). A few annual crop plants are used as test species, although non-crop species (annuals and perennials) should be protected in field margins. Crops are often chosen because they require no special treatment before sowing, usually have consistent and reliably high rates of germination and grow fast (White et al. 2009; Carpenter & Boutin 2010). However, recent studies have demonstrated that wild plant species can also be utilized in greenhouse phytotoxicity testing (Olszyk et al. 2008; White et al. 2009; Carpenter & Boutin 2010; Boutin et al. 2010). Moreover, phytotoxicity testing with crop plants alone as representative species may not be sufficiently protective for the entire non-target plant community in the field (Boutin & Rogers 2000; Olszyk et al. 2008). Only limited effects can be evaluated by the single species tests under greenhouse conditions. For example, no effects on competitive interactions between species can be assessed (Dalton & Boutin 2010). In addition, due to the short test duration of 21-28 days, only short-term effects (acute effects) can be determined. Long-term effects, and reproduction effects, cannot be detected, even though herbicides are often applied in the field at a time when plants are close to flowering and then, negative effects on the reproductive capacity (e.g., flowering, seed production) of wild plant species could be observed (Strandberg et al. 2012; Boutin et al. 2014).

However, the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, is currently under revision, and therefore, also the data requirements and testing methods for assessing herbicide effects on non-target plants. Therefore, there is an urgent need to investigate to what extent non-target plants are generally protected by the current risk assessment and which improvements can be made to refine it.

2 Thesis

2.1 Objectives

The objective of this dissertation was to examine the effects of agrochemical inputs (fertilizer, herbicide, and insecticide) on the plant community of a field margin. Therefore, a field experiment was conducted over three successive growing seasons (2010 – 2012). The study was specifically designed to separate the effects of the three stressors from each other and to investigate their combined effects because a field margin of a conventionally arable field is exposed to all of these stressors.

Before the experiment could be conducted, finding a suitable study site (see 2.2.) and obtaining a solid knowledge base to conduct such a large-scale field experiment were necessary. All preparations to carry out the project were made in 2009. These preparations included e.g., a literature search, personal communications with farmers and agrochemical suppliers concerning pesticides and fertilizers applied on fields in and around the study area, the development of an appropriate test design and its statistical analysis. In addition, establishing methods for e.g., the performance of the plot applications and for the vegetation assessments were necessary. Furthermore, I organized the financial support for my PhD position and for the project. This preliminary work was followed by three experimental years (2010-2012) on the study site.

2.2 Study site

The study site was an extensively managed hay meadow. Before the field experiment began, the meadow was mowed twice per year, without any fertilizer or pesticide additions. Existing field margins could not be used for this study because the vegetation of existing field margins had most likely already changed as a result of agrochemical inputs from adjacent field management practices in recent decades. This meadow was selected because it could be regarded as an original habitat that was not contaminated with pesticides or fertilizers and, therefore, represented the plant community of a surrogate field margin without this influence.

The meadow was approximately 1 ha in size, located near Landau (South Rhineland Palatinate, Germany), and consisted of a semi-natural, species-rich plant community (belonging to the Molinio-Arrhenatheretea meadows, Arrhenatherion community, Ellenberg et al. 1992) containing 54 species (40 herbs, and 14 grasses based on vegetation assessments conducted in May and June). The overall natural distribution of plant species was homogenous across the meadow. Some of the species were naturally more abundant than others, and consequently, these species were found more frequently (26 species). For example, herbs like the hedge bedstraw *Galium mollugo*, the ground ivy *Glechoma hederacea*, the common buttercup *Ranunculus acris*, the meadow vetchling *Lathyrus pratensis* and grasses such as the tall oatgrass *Arrhenatherum elatius* and the cock`s foot *Dactylis glomerata* were among the most common plant species on the study site. All species, their plant frequencies and supplementary information (life span, type of reproduction, Ellenberg`s indicator value for nitrogen, and German Red List status) are presented in Paper I, page 36.

The field experiment was set up as a randomized block design with seven treatments and one control. The treatments consisted of three single applications, i.e., one fertilizer (F), one herbicide (H), and one insecticide (I); all possible combinations of these treatments (F+I, H+I, F+H, and F+H+I); and one control (C). All treatments and the control were replicated eight times in plots, resulting in 64 plots. Each plot measured 8 m x 8 m, and a 2 m distance separated adjacent plots (Fig. 2-1). The vegetation of the 2 m paths between the plots was mowed with a lawn mower every 2 weeks from April to July. The lawn mower was equipped with a collection container to remove the freshly cut vegetation. The vegetation of the entire meadow was mowed and removed by the farmer with a rotary cutter mounted on a tractor once per year at the end of July.

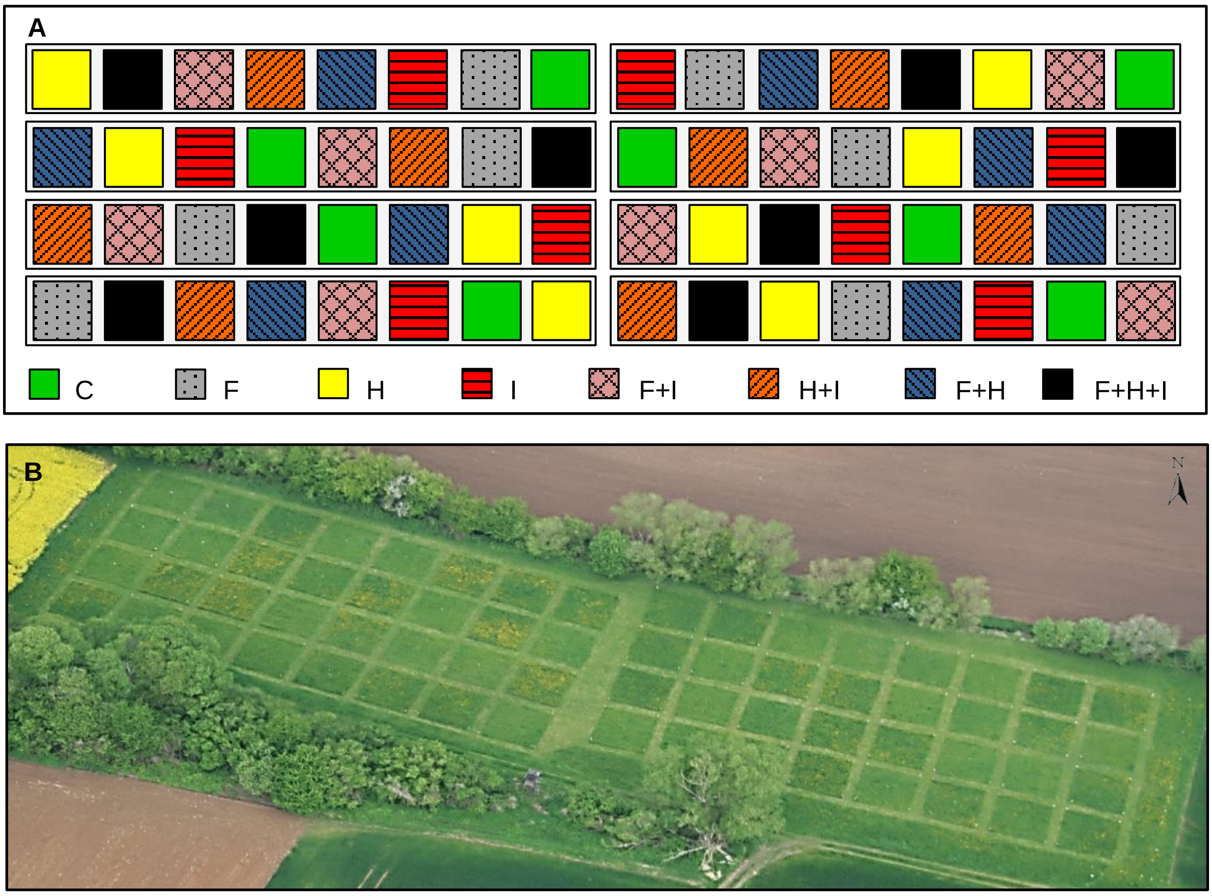

Fig. 2-1: Study design (randomized block design) (A) and aerial photograph of the study site (B). This photo was taken in May 2010, approximately two weeks after the first herbicide application on the study site[1]. Plot size: 8 m x 8 m; distance between plots: 2 m; distance to neighboring fields: at least 8 m. C = control; F = fertilizer; H = herbicide; I = insecticide.

[1] This photo shows first treatment effects on the flowering intensity of the common buttercup *Ranunculus acris*. See Paper II for details.

2.3 Agrochemical applications

The majority of farmed fields in Germany are winter wheat fields, and the selected meadow served as a surrogate for field margins adjacent to such fields. Therefore, the field management of winter wheat fields, with their agrochemical applications and application sequences, was imitated. The fertilizer rates (25 % of the field rate) and pesticide rates (30 % of the field rate) used for the plot applications were equal to their average input rates (overspray + drift) in the first meter of a field margin directly adjacent to a winter wheat field (see Paper II, page 41 for details). These fertilizer and pesticide input rates were simulated because these rates are highly relevant factors that can affect the plant community composition in the frequently encountered narrow field margins in German agricultural landscapes.

Fertilizer:

The recommended field rate of fertilizer in winter wheat fields is 200 kg nitrogen (N)/ha per year, which is usually applied in two equal applications (100 kg N/ha each), one at the beginning of the vegetation period in spring (when the wheat begins to grow) and the second a few weeks later (personal communications with farmers and agrochemical suppliers). In keeping with personal recommendations given by farmers and agricultural stores, a granular NPK (nitrate, phosphorus, potassium) fertilizer (14% N, Floral Düngemittel) was applied at the beginning of April, and a calcium carbonate and ammonium nitrate fertilizer (KAS, 27% N; Raiffeisen Markt) was applied approximately three weeks later in 2010, 2011 and 2012 (Table 2-1). Each time, 25 kg N/ha (= 25% of the field rate) was used. The exact fertilizer amount per plot was weighed before application. Then, the fertilizer was applied using a battery-driven hand spreader (Power Spreader by Wolf Garten; MTD Products Aktiengesellschaft). Similar to a spinning disc spreader used by farmers, the hand-operated fertilizer spreader distributes the fertilizer via a spinning disk in front and on the sides of the spreader. The fertilizer spreader had a spread range of 4 m, and thus, the plots could be treated from the 4 plot boundaries (Fig. 2-2). To ensure a homogenous distribution of fertilizer granules over the entire plot area, the spreader was calibrated before application.

Herbicide and insecticide:

For the pesticide applications, the herbicide Atlantis WG and the insecticide Karate Zeon were used. These pesticides were chosen because they were among the five most commonly used pesticides in winter wheat fields in Germany at the beginning of the study (Freier et al. 2008). In addition, farmers in the proximity of the study site used these pesticides for weed and pest control on their fields (personal communications with farmers).

The herbicide Atlantis WG (Bayer CropScience; active ingredients [a.i.]: 30 g/kg mesosulfuron-methyl, 6 g/kg iodosulfuron-methyl-natrium, 90 g/kg mefenpyr-diethyl [Safener]) was applied once per year in April 2010, 2011, and 2012 (Table 2-1). Atlantis WG is a selective sulfonylurea herbicide for the post-emergent control of black-grass, wild oats, rye-grasses, meadow-grasses, common chickweed and mayweeds in winter wheat fields. It is predominantly a foliar herbicide with less activity via the soil and does not reliably control weeds that emerge after spraying. The best results are obtained under good growing conditions (Atlantis WG product information, Bayer CropScience). Sulfonylurea herbicides are extremely effective inhibitors of plant cell division; these herbicides inhibit acetolactate synthase (ALS), which is a key enzyme in the pathway of branched-chain amino acids (leucine, isoleucine and valine) in plants (Russel et al. 2002). After spraying, Atlantis WG is readily translocated within the plant, inhibiting plant and root growth within hours of application. The first visible effects are noticeable after approximately 7 days; however, the full effect may not be apparent for up to 4 weeks, depending on the plant species, the treatment timing and the weather conditions. The recommended field rate for spraying Atlantis WG in spring (April) is 400 g/ha (Atlantis WG, product information, Bayer CropScience).

The insecticide Karate Zeon (Syngenta; a.i.: lambda-cyhalothrin 7.5 mL a.i./ha) was applied once per year at the end of May or at the beginning of June 2010, 2011, and 2012 in parallel with the peak of wheat flowering, when pest control applications were conducted in the surrounding agricultural area (Table 2-1). Karate Zeon is a pyrethroid, which is a non-systemic insecticide with contact and stomach action and repellent properties. It is effective on a broad range of insects at all stages of development. The insecticide rapidly penetrates the insect cuticle, disrupting nerve conduction within minutes and leading to feeding cessation, muscular control loss, paralysis and eventual death. The recommended field rate for spraying Karate Zeon is 75 mL/ha (Karate Zeon, product information, Syngenta).

The application rates used for the plot applications on the study site were 30 % of the recommended field rate for both, the herbicide (120 g Atlantis WG/ha) and the insecticide (22.5 mL Karate Zeon/ha). Each time, the plots were treated under good agricultural practice (wind speed < 5 m/s, temperature < 25°C, no rain 1 day before and after application). The products were applied using a purpose-built and air-assisted experimental field sprayer on wheels (Schachtner Gerätetechnik). The field sprayer was equipped with an 8 m spray boom with 15 flat-fan TeeJet nozzles (XR 11002-VS; Schachtner Gerätetechnik). The boom height above the vegetation canopy and the distance between the nozzles were 50 cm each. Following label recommendations for field applications, a spray volume of 400 L/ha was used. To ensure a homogenous distribution and a constant delivery rate, the sprayer was calibrated before applying the pesticides. Additionally, a flow measurement on the field sprayer documented the exact application volume during applications and assured that an application volume of ± 10 % was achieved. During plot applications, neighboring plots were protected against drift with plastic sheets (Fig. 2-3).

Time schedule of treatments:

The fertilizer and pesticide application times on the experimental study site are shown in Table 2-1. The agrochemicals were applied when the farmer applied agrochemicals to his cereal field in the proximity of the study site. The exact application times varied slightly among the years due to weather conditions. The following figures show the fertilizer (Fig. 2-2) and herbicide application (Fig. 2-3) at the experimental study site in April 2011.

Table 2-1: Application times of the agrochemicals applied in 2010, 2011 and 2012

	2010	**2011**	**2012**
NPK-Fertilizer (14% N)	15 April	4 April	3 April
KAS-Fertilizer (27% N)	6 May	19 April	23 April
Herbicide Atlantis WG	21 April	11 April	13 April
Insecticide Karate Zeon	4 June	24 May	30 May

Fig. 2-2: Fertilizer application at the experimental study site. The hand-operated fertilizer spreader (Power Spreader by Wolf Garten) with fertilizer granules is shown. Neighboring plots were protected against drift with plastic sheets. Photo taken on 4 April 2011.

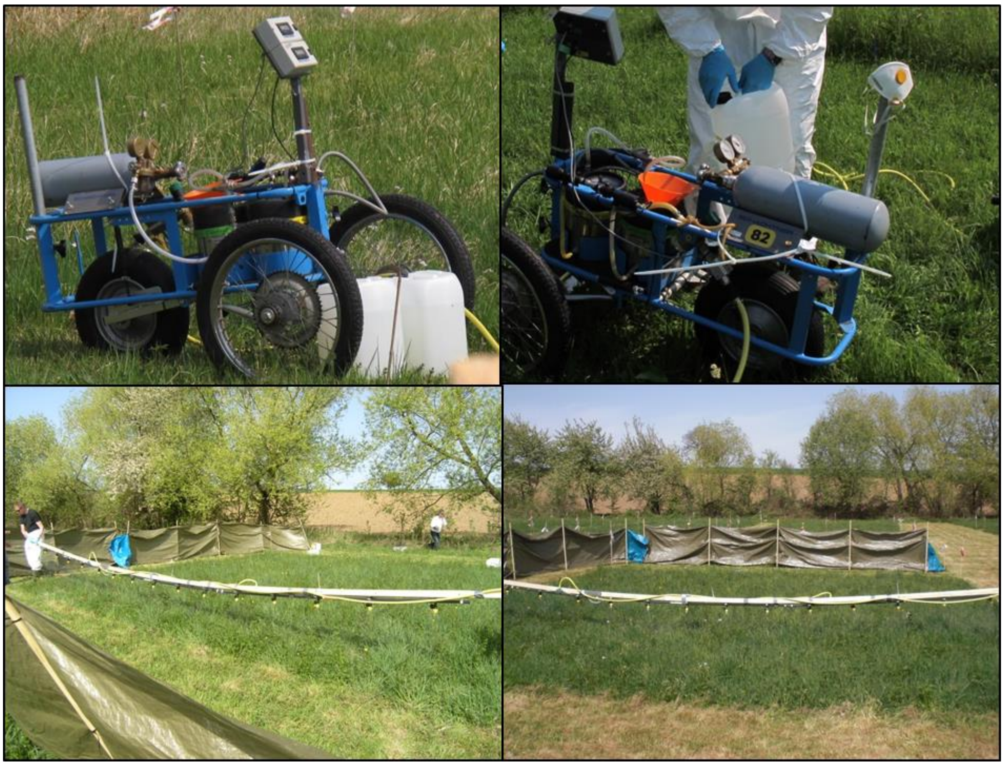

Fig. 2-3: Herbicide application at the experimental study site. The field sprayer and the 8 m spray boom with 15 flat-fan TeeJet nozzles (XR 11002-VS; Schachtner Gerätetechnik) are shown. Neighboring plots were protected against drift with plastic sheets. Photo taken on 11 April 2011.

2.4 Thesis structure

Field experiment:

The effects of the agrochemical applications on the plant community were assessed each year (2010-2012) to achieve three primary objectives:

- To assess the effects of pesticides and fertilizers on the **plant frequencies of individual species, the plant species composition** and **species diversity after three years of application**
  → Paper I: Schmitz, J., Hahn, M., Brühl, C.A. (2014): Agrochemicals in field margins – An experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community. *Agriculture, Ecosystems & Environment*, 193: 60–69.

- To assess the effects of pesticides and fertilizers on the **flowering intensity** of the common buttercup *Ranunculus acris*
  → Paper II: Schmitz, J., Schäfer, K., Brühl, C.A. (2013): Agrochemicals in field margins – Assessing the impacts of herbicides, insecticides and fertilizer on the common buttercup (*Ranunculus acris*). *Environmental Toxicology and Chemistry*, 32 (5): 1124-1131.

- To assess the effects of pesticides and fertilizers on the **reproductive capacity** of four selected species of the study site
  → Paper III: Schmitz, J., Schäfer, K., Brühl, C.A. (2014): Agrochemicals in field margins – Field evaluation of plant reproduction effects. *Agriculture, Ecosystems & Environment*, 189: 82-91.

Risk assessment:

In addition to the field experiment, a literature search was performed to review the current **published literature regarding higher-tier approaches (microcosms, mesocosms, and field experiments) for terrestrial non-target plants** and to provide an overview of these studies. The test designs of the investigated studies were evaluated concerning their realism and applicability for higher-tier testing in risk assessment procedures

→ Paper IV: Schmitz, J., Stahlschmidt, P., Brühl, C.A.: Assessing the risk of herbicides to terrestrial non-target plants using higher-tier studies. Manuscript.

2.5 Data sampling

Field experiment:

- **Plant community assessments** of all 64 plots of the study site were conducted once in mid-May and once in mid-June of each year (2010, 2011, and 2012). For this purpose, the frequency method with a mapping frame was used because this method is highly sensitive to detecting changes in plant communities over time (Elzinga et al. 1998). An additional advantage of this method is that a uniform plant community assessment can be obtained because the only decision required by the observer is whether the species is present within the sub-square (Elzinga et al. 1998). Visual estimates of plant species cover as usually conducted by other methods such as the Braun-Blanquet Method were not used because such methods are very subjective; thus, the level of variability among observers and years can greatly differ for different species. Therefore, the frequency method was selected to be able to compare the data from each assessment (Papers I, II, and III).

  The mapping frame had a size of 1 m^2 and was divided into 25 sub-squares of 20 cm x 20 cm. The frame was placed on top of the vegetation, and the presence of each plant species was recorded in each sub-square. The plant community assessments were conducted six times per plot along the diagonal of the plots (Fig. 5-1 in Paper III, page 60), resulting in 384 assessments (= 384 m^2) in May and 384 assessments (= 384 m^2) in June of each year (= 768 m^2 per year). Thus, 2304 vegetation assessments (= 2304 m^2) were conducted in all three years.

  All vegetation assessment data were stored in a Microsoft Office database. I specifically created the database to organize and manage the large amount of collected data points for each plant species (in total 54 plant species) and to be able to summarize the data for analysis. Thus, it was possible, for example, to calculate the frequency of each plant species per 1m^2, per plot and/or per treatment, depending on the target analysis.

- The average **vegetation heights of all herbs and grasses** were also measured in all square meters during the plant community assessments in May and June each year (Paper I).

- Furthermore, **plant biomass samples** were collected at the end of June in 2010, 2011, and 2012 by cutting the above-ground plant biomass in one quadrant measuring 1 m x 1 m in the middle of each plot (= 64 samples per year). The fresh weights of the samples were recorded in the field immediately after cutting and the data of plant biomasses and vegetation heights were also entered into the Microsoft Office database (Paper I).

- **Photo-documentation of the flowering intensity of the common buttercup *Ranunculus acris*** was performed in May 2010, 2011 and 2012 (shortly before the yearly vegetation assessment in May). *R. acris* was one of the most common plant species on the experimental study site, and during the photo-documentation, this species was the first and only yellow flowering plant species on the meadow. In each of the 64 plots on the study site, the 6 m^2 of the plant community assessments were photographed vertically from above using a 1 m^2 frame and an Olympus digital camera (Olympus C5060 wide-zoom digital camera). Thus, 384 photos were taken each year (6 photos per plot x 64 plots). For analyzing the photo-documentation and to determine the area covered with flowers per 1 m^2 and/or treatment, an image-analysis program (free software, GNU Image Manipulation Program [GIMP]) and an object-based image analysis program (Definiens, Professional 5; Trimble Navigation) were used (Paper II).

- In the third experimental season, **mature seeds (fruits)** of four selected species (*R. acris*, *Vicia sepium*, *Lathyrus pratensis*, and *Rumex acetosa*) were harvested in June to July 2012. For each species, the fruit collection was conducted six times per plot. Thus, the target was to collect 48 fruits per species and treatment (6 fruits per species and plot × 8 replicates (plots) per treatment), resulting in 384 fruits per species for all study plots (overall target = 384 fruits x 4 species = 1536 fruits). Afterwards, germination tests with a defined number of seeds collected in the field were performed in the climate chamber at the University Koblenz-Landau, Campus Landau (Paper III).
- The field experiment was accompanied by **monitoring *R. acris* in field margins** in the proximity of the study site in May 2011. The presence or absence of *R. acris* in field margins at 10 m intervals along a stretch of 11 km was recorded. At each monitoring point, the crop type adjoining the field margin was also noted (Paper I).

For the statistical analysis, various univariate and multivariate methods were used. Statistical analyses and further information regarding the sampling methods are provided in detail in the publications (the publications are listed in parentheses behind the above-described methods). The exact times of each sampling at the study site are shown in Table 2-2.

Table 2-2: Times of data sampling at the study site in 2010, 2011, and 2012

	2010	2011	2012
Photo-documentation of the flowering intensity of *R. acris*	5 May	2 May	10 May
Plant community assessments, measuring of vegetation heights	10 - 15 May	9 - 13 May	7 - 11 May
	14 - 18 June	14 - 18 June	11 - 15 June
Monitoring of *R. acris* in field margins	-	16 May	-
Seed collection of *R. acris*, *V. sepium*, *L. pratensis*, and *R. acetosa*	-	-	May - June
Plant biomass samples	28 June	27 June	27 June

Risk Assessment:

- The literature search for the evaluation of higher-tier approaches for terrestrial non-target plants (Paper IV) was performed using ISI Web of Knowledge, OvidSP and Google Scholar. Multiple search terms were used, e.g., “non-target plant”, “field margin”, “herbicide drift”, “phytotoxicity test”, “greenhouse experiment”, “microcosm”, “field study” and/or e.g., “plant community”, “margin”, “pesticide”, “herbicide”, and “agriculture”. The resulting hits were screened, and the cited sources and the articles in which this literature had been cited were analyzed. Relevance was based on papers describing methodologies for higher-tier tests including non-standard laboratory tests, mono-species field and multispecies greenhouse or field tests, as well as, field experiments.

Remark: Because this doctoral thesis is a cumulative dissertation, note that the following chapters are published in (chapters 3, 4 and 5), or submitted to (chapter 6), scientific journals; therefore, some redundancy in portions of the Introduction and Materials and Methods with the description above could not be avoided.

3 Agrochemicals in field margins – An experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community

Paper I

This chapter presents the author`s final version of the article:

Schmitz, J., Hahn, M., Brühl, C.A (2014): Agrochemicals in field margins – An experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community. *Agriculture, Ecosystems & Environment*, 2014: Vol. 193, pp. 60-69.

The published version of this article is available at Elsevier ScienceDirect via http://dx.doi.org/10.1016/j.agee.2014.04.025

Abstract - In agricultural areas, field margins are often the only remaining habitat for wild plant species. However, due to their proximity to agricultural fields, the vegetation of field margins may be affected by agrochemicals applied to the crop field. To investigate individual and combined effects of fertilizer, herbicide, and insecticide inputs on the plant community of field margins, a three-year field study with a randomized block design was performed. The applied fertilizer rates (25 % of the field rate) and pesticide rates (30 % of the field rate) were consistent with their average input rates (drift + overspray) in the first meter of a field margin directly adjacent to the field. Fertilizer and herbicide applications resulted in significantly reduced frequencies of several plant species. The fertilizer promoted plants with a high nutrient uptake and decreased the frequencies of small and subordinate species. In addition to the disappearance of a few species, the herbicide caused predominantly sublethal effects, which gradually reduced the frequencies of certain species. Significant herbicide-fertilizer interaction effects were also observed and could not be extrapolated from individual effects. The impacts of both agrochemicals became stronger over time, led to shifts in plant community compositions, and caused significantly lower species diversities than in the control plots. The insecticide application significantly affected the frequencies of two plant species. The results suggest that a continuous annual application of agrochemicals would cause further plant community shifts. Hence, to preserve biodiversity of agricultural landscapes, it is recommended to protect the vegetation in field margins from agrochemical inputs.

Keywords – Agro-ecosystem, Off-field habitats, Non-target species, Plant frequency, Interaction effects, Plant diversity

3.1 Introduction

A decrease in biological diversity in farmlands across Europe has been observed over the last several decades, and agricultural intensification was identified as a major driving force of this decrease (Berendse et al. 2004; Tscharntke et al. 2005). Agricultural intensification caused land-use changes, such as increases in farm size, specialization, and management intensity (Tscharntke et al. 2005; Firbank et al. 2008). Consequently, complex natural ecosystems and semi-natural habitats have decreased in number and size (Benton et al. 2003). Today, the majority of semi-natural habitats in agricultural landscapes are field margins (Marshall & Moonen 2002). These landscape structures are usually linear, permanent vegetation strips of grassy and herbaceous off-field habitats adjacent to agricultural fields (Kühne & Freier 2001; Kleijn & Verbeek, 2000; Hahn et al. 2014). Generally, these habitats are only a few meters wide. A study using digital orthophotos and geographical information systems indicated that field margins with a width of 1 to 2 meters are the typical margins remaining in intensively used agricultural landscapes in Germany (Hahn et al. 2014).

Field margins are beneficial for the conservation of biodiversity because they are often the only remaining habitat of a variety of wild plant species and farmland animals in agro-ecosystems (Nentwig 2000; Asteraki et al. 2004 and references therein).

However, the biodiversity of field margins can be affected by agrochemicals due to the proximity of these habitats to agricultural fields (Firbank et al. 2008). For example, the vegetation of field margins may be exposed to herbicides. Studies have shown that herbicide applications in field margins can negatively affect the plant community composition (Kleijn & Snoeijing 1997; Marrs & Frost 1997, de Snoo & van der Poll 1999). In Germany in particular, narrow field margins appear to be strongly affected. This is because field margins that are less than 3 meters wide are not considered as terrestrial non-target areas and therefore, are not protected from herbicide inputs by risk mitigation measures (e.g., in-field buffer zone distances to terrestrial non-target areas) (Kühne et al. 2000; BVL 2013b). Consequently, farmers in Germany and in other European countries do not have to maintain distances from field margins during pesticide applications and thus, these field margins receive pesticide inputs via overspray and spray drift (Schmitz et al. 2013).

Fertilizer misplacements in field margins are also supposed to affect the vegetation of field margins. Fertilizers are usually applied on the field using spreaders, which distribute the fertilizer via spinning disks that eject fertilizer backwards and sideways from the spreader. Thus, fertilizer misplacements in field margins are likely to occur (Rew et al. 1995; Tsiouris & Marshall 1998, Wilson 1999). Furthermore, because fertilizers and herbicides are both designed to affect vegetation, their application to field margins will most likely involve interactions with each other. However, to date, only a few studies have been concerned with such combined effects on natural plant communities (Perry et al. 1996; Kleijn & Snoeijing 1997; Gove et al. 2007; Strandberg et al. 2012). These studies demonstrated that herbicide and fertilizer inputs below recommended crop application rates can significantly affect the plant community.

Another concern involves the annual application sequences of agrochemicals on a field. A conventionally managed winter wheat field is treated annually with fertilizer, at least once with an herbicide, and also with an insecticide. Insecticides could probably cause indirect effects on plants by reducing herbivorous or flower-visiting insects, which have been less investigated until now. Furthermore, the annual repeated exposure of the vegetation to agrochemicals might intensify these effects and/or cause cumulative effects.

The aim of this study was to investigate effects of agrochemical misplacements (fertilizer, herbicide, and insecticide) on the plant community of a field margin during three successive growing seasons (2010 – 2012). The study was specifically designed to separate the effects of these three stressors from each other and to investigate their combined effects because a field margin of a conventionally arable field is exposed to all of these stressors.

3.2 Materials and methods

3.2.1 Study site

The study site was an extensively managed hay meadow that was mowed twice per year without any fertilizer or pesticide applications. The meadow was 1 ha in size, located near Landau (South Rhineland Palatinate, Germany), and consisted of a semi-natural species-rich plant community (belonging to the Molinio-Arrhenatheretea meadows, Ellenberg et al., 1992) containing 54 species (40 herbs, 14 grasses, based on vegetation assessments conducted in May and June). All species and supplementary information (life span, type of reproduction, Ellenberg`s indicator value for nitrogen, German Red List status) are listed in Appendix A. The overall natural distribution of plant species was homogenous across the meadow. A few of the species were naturally more abundant than others, and consequently these species were found more frequently (26 species; a species was classified as common when at least two individuals per plot were documented; see chapter 3.2.3 for details of vegetation assessments).

The field experiment was established in spring 2010 and was designed to study individual and combined effects of repeated agrochemical applications on a surrogate field margin in successive growing seasons (Schmitz et al. 2013). We used a randomized block design with seven treatments and one control. The treatments consisted of three single applications, i.e., – one fertilizer (F), one herbicide (H), and one insecticide (I); and all possible combinations of these treatments (F+I, H+I, F+H, F+H+I); and one control (C). Hence, the experiment was planned as a fully factorial design where the three factors (fertilizer, herbicide, and insecticide) had 2 levels (applied versus not applied) resulting in a 2x2x2 factorial design. All of the treatments (including the control) were replicated eight times in plots, resulting in a total of 64 plots. Each plot measured 8 m x 8 m, and 2 m of distance separated adjacent plots (Schmitz et al. 2013).

3.2.2 Agrochemical applications

The majority of farmed fields in Germany are winter wheat fields, and the selected meadow served as a surrogate for field margins adjacent to such fields. Therefore, the field management of winter wheat fields, with their agrochemical applications and application sequences, was imitated. Fertilizer and pesticide rates used for the plot applications were equal to their average input rates in the first meter of a field margin directly adjacent to such a field.

During fertilizer application, there is usually an input rate of 25% of the field rate in the first meter of a field margin (Tsiouris & Marshall 1998), and during pesticide application, the average input rate in the first meter of a field margin is approximately 30 % of the field rate (direct overspray and spray drift) (see Schmitz et al. 2013 for details). The recommended field rate of fertilizer is 200 kg nitrogen (N)/ha per year, which should be applied in two equal rates (100 kg N/ha each), one at the beginning of the vegetation period in spring (when the wheat starts to grow) and the second a few weeks later (personal communications with farmers and agrochemical suppliers). We applied a granular NPK (nitrate, phosphorus, potassium) fertilizer (14% N, Floral Düngemittel) and a calcium carbonate and ammonium nitrate fertilizer (27% N; Raiffeisen Markt) at the beginning of April (NPK fertilizer) and approximately three weeks later (calcium carbonate and ammonium nitrate fertilizer) in 2010, 2011, and 2012. Each time, 25 kg N/ha (= 25 % of the field rate) was applied. The fertilizer was applied using a hand-operated fertilizer spreader (Power Spreader by Wolf Garten; MTD Products Aktiengesellschaft).

We used the herbicide Atlantis WG (sulfonylurea; recommended field rate 400 g/ha, active ingredients [a.i.] 30 g/kg mesosulfuron-methyl, 6 g/kg iodosulfuron-methyl-natrium, 90 g/kg mefenpyr-diethyl [Safener], mode of action: inhibitor of plant cell division [e.g., acetolactate synthase], Bayer CropScience). We applied the product once per year in April 2010, 2011, and 2012. The insecticide Karate Zeon (pyrethroid; recommended field rate 75 ml/ha, a.i. lambda-cyhalothrin, 7.5 ml a.i./ha, mode of action: nonsystemic insecticide with contact and stomach action, repellent properties, Syngenta) was applied once per year at the end of May or at the beginning of June 2010, 2011, and 2012. These pesticides were chosen because they were among the five most commonly used pesticides in winter wheat fields in Germany at the beginning of the study (Freier et al. 2008). The application rates of the herbicide and the insecticide were 120 g Atlantis WG/ha and 22.5 ml Karate Zeon/ha, respectively, which represented 30 % of their corresponding recommended field application rates. Each time, the plots were treated under good agricultural practice (wind speed < 5 m/s, temperature < 25°C, no rain 1 day before and after application). The products were applied using a purpose-built and air-assisted experimental field sprayer on wheels (Schachtner Gerätetechnik). The field sprayer was equipped with an 8-m spray boom with 15 flat-fan TeeJet nozzles (XR 11002-VS; Schachtner Gerätetechnik). The boom height above the vegetation canopy and distance between the nozzles was 50 cm. Following label recommendations for field applications, a spray volume of 400 L/ha was used.

3.2.3. Plant community assessments

The plant community was assessed using the frequency method (Elzinga et al. 1998) with a mapping frame of 1 square meter, which was divided into 25 sub-squares of 20 cm x 20 cm. We placed the frame on top of the vegetation and recorded the presence of each plant species in each sub-square. This method allows for the identification of changes in plant communities over time (Elzinga et al. 1998). An additional advantage of this method is that a uniform plant community assessment can be obtained because the only decision required by the observer is whether the species is present within the sub-square (Elzinga et al. 1998). Study staff was comprehensively trained in species identifications. The plant community assessments were conducted six times per plot along the diagonal of the plots. All of the community assessments (6 vegetation assessments per plot x 64 plots = 384) were performed within a one-week period in mid-June of each year (2010, 2011, and 2012). We calculated the frequency of each species per plot and treatment. If a species was recorded 150 times per plot (25 sub-squares x 6 assessments per plot), then this species exhibited a plant frequency of 100 %.

3.2.4. Biomass samples

Plant biomass samples were collected at the end of June in 2010, 2011, and 2012 by cutting the above-ground plant biomass in one quadrant measuring 1 m x 1 m in the middle of each plot. The fresh weights of the samples were recorded in the field immediately after cutting.

3.2.5. Statistical analyses

Univariate analysis

The data from each year (2010, 2011, and 2012) were analyzed separately. The statistic program Primer (Version 6) with the Permanova+ add-on was used (Anderson et al. 2008). We tested the differences in the number of species, species diversities, and biomass samples between the seven treatments and the control with a one-way permutational univariate analysis of variance (PerAnova). Euclidean distance was used as a distance measurement to generate resemblance matrices. To test at a significance level of 0.001, 9999 permutations were generated (Anderson et al. 2008). Post-hoc PerAnova pairwise comparisons were adjusted using a Bonferroni correction.

To test for treatment effects on individual species, a nested PerAnova was performed. We used a mixed-effect model design with the block as a random factor and the treatment as a fixed factor. The six vegetation assessments per plot were nested within the factor treatment (settings: Euclidean distance, 9999 permutations, and Bonferroni corrections).

In addition, the factorial design of the experiment allowed us to test for main effects of individual factors (F, H, and I) and interaction effects between the three factors (FxH, FxI, HxI, FxHxI). The main effect is the overall (or average) effect of one factor averaged across the levels of other factors.

An interaction effect occurs when the effect of one factor depends on another factor. We performed a three-factorial PerAnova to analyze main and interaction effects on the number of species, biomass, and plant frequency of individual species. The analyses were performed with the Euclidean distance and 9999 permutations.

Multivariate analysis

Nonmetric multidimensional scaling (NMDS) was performed to examine similarities in plant community compositions among the seven treatments and the control. The data from each year (2010, 2011, and 2012) were analyzed separately. Statistical analyses were performed using the open-source software R (www.r-project.org, version 3.01) and the Biodiversity R package (Kindt and Coe 2005). We performed a two-dimensional NMDS based on the species data (obtained from plant community assessments in June) in each plot. Those species with a plant frequency of < 1% were excluded. The ordination method NMS standard (function NMSrandom), the Bray-Curtis dissimilarity index, and 100 NMS permutations were used.

In addition, we performed a two-dimensional NMDS with weighted-average species scores and a subsequent vector fitting of environmental variables (function metaMDS in the R package vegan (Oksanen 2013)) with the data from the plant community assessments in June 2012. This analysis were performed to present individual species, individual factors (F, H, I) and the control (C) on one ordination plot and to check whether certain species are associated with specific factors (or the control) after the third experimental season. We used the Bray-Curtis dissimilarity index, and the NMDS was started with a maximum of 50 random starts. Species scores and significant vectors (999 permutations) were added to the ordination plot (function envfit in the R package vegan (Oksanen 2013)).

Permutational multivariate analysis of variance (PerManova) was additionally performed to find significant differences in species compositions among the treatments and the control. The data from each year (2010, 2011, and 2012) were analyzed separately. We used the Bray-Curtis dissimilarity index, 9999 permutations and Bonferroni corrections. The analyses were performed using Primer with the Permanova+ add-on (Anderson et al. 2008).

3.3. Results

3.3.1. Number of species

The mean number of species per treatment changed only slightly over the years (2010-2012). We observed a significantly lower mean number of dicotyledons in the treatment combinations of fertilizer and herbicide (F+H, and F+H+I) than in the control plots during the third experimental season (Fig. 3-1). The mean number of monocotyledons (grasses) was not significantly affected by the treatments during the experiment.

Analysis of main effects (three factorial PerAnova) indicated a significant main effect of fertilizer on the mean number of dicotyledons in June 2011 ($p = 0.007$) and in June 2012 ($p = 0.002$). In addition, a significant main effect of the factor herbicide ($p = 0.03$) was found in June 2012. Interaction effects between the factors were not identified.

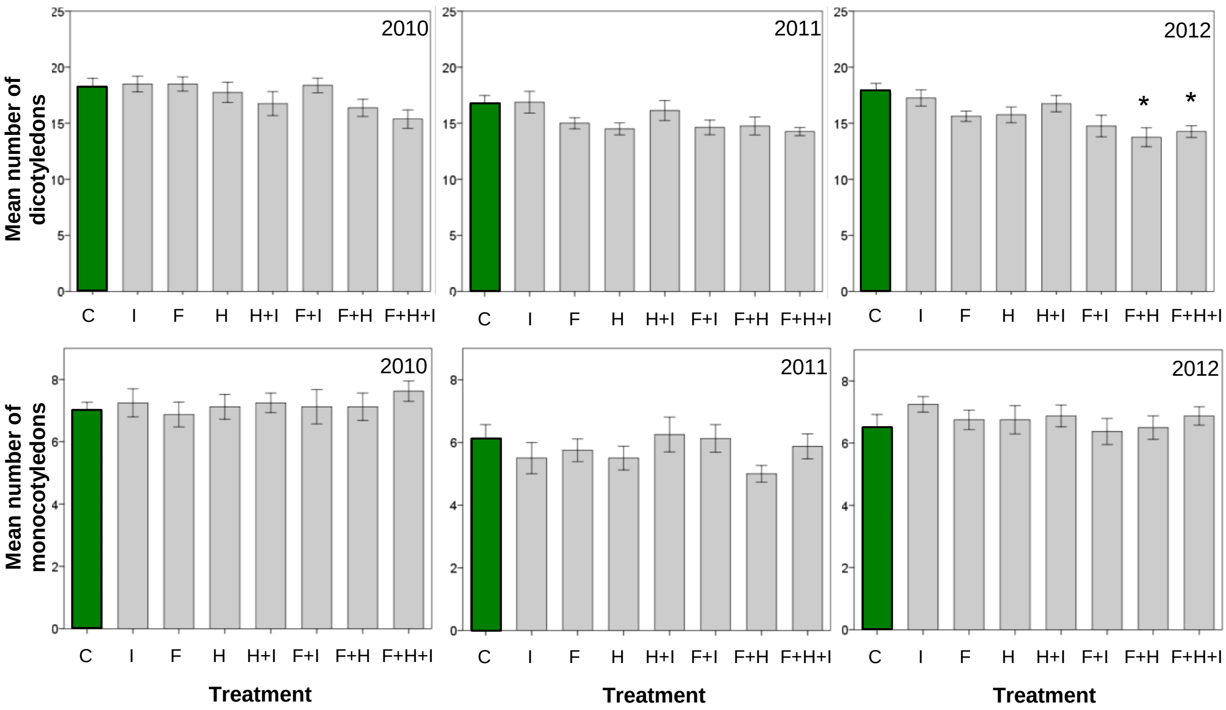

Fig. 3-1: Mean number (± standard error) of dicotyledons and monocotyledons in June 2010, 2011, and 2012; replicates per treatment = 8. * Significantly different from the control, $p < 0.05$ (one-way analysis of variance with permutations [PerAnova]); p values Bonferroni corrected. C = control (highlighted in green), I = insecticide, F = fertilizer, H = herbicide.

3.3.2. Mean plant frequency of individual species

The mean frequencies of most species gradually changed in the plots treated with fertilizer and herbicide during the experiment. For example, in 2010, the treatment combination of fertilizer, herbicide, and insecticide (F+H+I) significantly altered the frequencies of nine species (*Galium mollugo*, *Lathyrus pratensis*, *Vicia sepium*, *Rhinanthus alectorolophus*, *Glechoma hederacea*, *Stellaria graminea*, *Vicia hirsuta*, *Trifolium pratense*, and *Heracleum sphondylium* (Table 3-5 in supplementary data). Over time, significant treatment effects became more apparent. As a result, the number of treatment effects, e.g., in the F+H+I treatment, nearly doubled in third year compared with the first year (Table 3-1 and Table 3-2).

Table 3-1: Mean plant frequency [%] of the most common dicotyledons (plant frequency > 1%) per plot and treatment in June 2012. A frequency of 100% was possible, when the species was recorded in each sub-square of the vegetation assessments (in total 150 sub-squares per plot). Replicates per treatment = 8. Species are listed in descending order of frequency. The last two columns show the results of the three-factorial PerAnova (= main effects and interaction effects). C = control, I = insecticide, F = fertilizer, H = herbicide. SE = standard errors [%], P = perennial, A = annual.

			Control		Single treatments						Combined treatments									
	Species		C	(± SE)	I	(± SE)	F	(± SE)	H	(± SE)	H+I	(± SE)	F+I	(± SE)	F+H	(± SE)	F+H+I	(± SE)	Main effect	Inter-action
1	*Galium mollugo*	P	85.3	(3.4)	89.5	(2.8)	89.5	(2.2)	83.3	(6.4)	91.9*	(2.4)	92.6**	(3.1)	89.8	(2.3)	85.5	(4.5)	/	/
2	*Glechoma hederacea*	P	71.8	(3.9)	65.3	(7.4)	50.3***	(8.7)	59.6*	(4.9)	45.1***	(8.7)	65.0	(6.0)	34.3***	(5.4)	35.3***	(9.6)	F**, H***	/
3	*Ranunculus acris*	P	58.2	(4.1)	54.3	(3.6)	31.1***	(6.5)	45.4**	(8.0)	52.3	(7.6)	23.3***	(3.5)	27.7***	(6.2)	33.2***	(5.5)	F***	/
4	*Lathyrus pratensis*	P	53.2	(7.6)	54.3	(5.0)	16.2***	(4.8)	27.8***	(5.5)	31.3***	(5.0)	17.4***	(5.6)	15.3***	(4.5)	12.5***	(4.3)	F***, H***	FxH**
5	*Vicia sepium*	P	49.6	(3.1)	45.8	(5.2)	28.0***	(5.6)	25.7***	(5.5)	30.7***	(3.8)	33.8***	(4.8)	11.2***	(3.5)	13.7***	(2.3)	F***, H***	/
6	*Ajuga reptans*	P	39.8	(4.8)	43.8	(5.4)	9.5***	(1.8)	38.8	(6.5)	32.3	(4.0)	8.3***	(1.7)	22.0**	(6.3)	16.6***	(2.4)	F***	FxH*
7	*Rumex acetosa*	P	38.3	(4.5)	29.4	(4.4)	23.3***	(6.0)	21.5***	(4.0)	15.8***	(3.4)	23.4***	(5.1)	9.8***	(1.5)	10.8***	(1.5)	F**, H***	/
8	*Veronica chamaedrys*	P	33.3	(8.5)	38.0	(8.7)	11.3***	(3.6)	47.3	(7.1)	25.8	(6.0)	15.9**	(6.4)	33.8	(8.7)	24.1	(7.9)	F**	/
9	*Ranunculus repens*	P	25.6	(4.8)	20.5	(5.9)	15.8	(5.1)	6.0***	(3.3)	4.2***	(1.8)	12.1**	(4.2)	1.3***	(0.6)	1.5***	(1.0)	F*, H***	/
10	*Plantago lanceolata*	P	16.4	(4.3)	13.3	(4.4)	5.3***	(1.8)	21.4	(6.0)	15.9	(5.6)	2.9***	(1.5)	11.2	(5.1)	10.8	(7.5)	F**	/
11	*Vicia hirsuta*	A	14.8	(4.6)	8.4	(3.3)	1.3***	(0.5)	4.1***	(2.9)	4.0***	(2.9)	2.0***	(1.6)	0.4***	(0.2)	0.8***	(0.4)	F***, H*	/
12	*Lotus corniculatus*	P	13.6	(4.7)	10.7	(4.4)	1.4***	(0.7)	13.1	(8.0)	17.1	(7.8)	3.3**	(2.1)	3.9**	(1.8)	8.3	(4.5)	F**	/
13	*Rhinanthus alectorolophus*	A	11.0	(8.4)	6.3	(3.7)	0.2***	(0.1)	0.0***	(0.0)	0.1***	(0.1)	0.6***	(0.6)	0.0***	(0.0)	0.1***	(0.1)	F*, H*	FxH*
14	*Calystegia sepium*	P	7.7	(4.7)	6.5	(3.9)	9.2	(4.9)	9.7	(4.8)	9.7	(6.4)	4.8	(2.3)	10.6	(4.8)	11.8	(5.6)	/	/
15	*Stellaria graminea*	P	6.3	(3.6)	22.8***	(7.2)	17.3**	(6.0)	1.1***	(0.6)	3.8	(1.3)	20.5***	(7.4)	0.3***	(0.2)	0.0***	(0.0)	H***, I*	/
16	*Hypericum perforatum*	P	4.2	(2.4)	0.8	(0.7)	1.3	(1.3)	1.4	(1.4)	2.3	(1.5)	1.1	(0.7)	1.0	(0.8)	4.6	(2.6)	/	/
17	*Leucanthemum vulgare*	P	3.4	(2.8)	1.1	(0.7)	0.8	(0.4)	6.7	(5.7)	2.8	(1.5)	0.2**	(0.1)	0.1***	(0.1)	1.1	(1.0)	/	/
18	*Achillea millefolium*	P	3.1	(1.6)	3.0	(0.8)	3.8	(1.8)	3.8	(2.0)	3.8	(1.9)	5.3	(2.0)	2.3	(1.2)	3.8	(1.9)	/	/
19	*Heracleum sphondylium*	P	3.1	(1.2)	1.2	(0.6)	5.0	(2.8)	1.1	(0.4)	1.2	(0.4)	0.3**	(0.2)	1.1	(0.6)	0.7*	(0.3)	/	/

Grey background = plant frequency was significantly negatively affected by the treatment in comparison to the control, framed cells = plant frequency was significantly positively affected by the treatment in comparison to the control. * p < 0.05, ** p < 0.01, *** p < 0.001, p values Bonferroni corrected, nested PerAnova.

Table 3-2: Mean plant frequency [%] of the most common monocotyledons (plant frequency > 1%) per plot and treatment in June 2012. A frequency of 100% was possible, when the species was recorded in each sub-square of the vegetation assessments (in total 150 sub-squares per plot). Replicates per treatment = 8. Species are listed in descending order of frequency. The last two columns show the results of the three-factorial PerAnova (= main effects and interaction effects). C = control, I = insecticide, F = fertilizer, H = herbicide. SE = standard errors [%], P = perennial.

			Control		Single treatments						Combined treatments									
	Species		C	(± SE)	I	(± SE)	F	(± SE)	H	(± SE)	H+I	(± SE)	F+I	(± SE)	F+H	(± SE)	F+H+I	(± SE)	Main effect	Inter-action
20	*Arrhenatherum elatius*	P	82.4	(4.8)	73.4	(5.5)	83.1	(5.9)	30.1***	(4.0)	30.8***	(4.4)	86.1	(3.3)	35.0***	(4.6)	34.9***	(5.3)	H***	/
21	*Dactylis glomerata*	P	56.1	(4.4)	48.1	(6.4)	68.3*	(8.6)	53.9	(4.5)	60.3	(4.9)	59.8	(7.3)	72.3**	(4.3)	72.0**	(5.7)	F**	/
22	*Agrostis capillaris*	P	26.8	(6.9)	26.2	(6.3)	7.6***	(2.4)	31.3	(7.2)	33.4	(4.4)	11.7**	(4.9)	29.5	(5.5)	18.6	(4.7)	F**	/
23	*Holcus lanatus*	P	26.6	(7.7)	19.8	(5.1)	16.5*	(4.5)	26.3	(4.0)	19.3	(3.9)	13.2**	(3.5)	9.5***	(2.5)	15.4**	(4.0)	F**	/
24	*Alopecurus pratensis*	P	6.8	(2.1)	2.3**	(1.2)	10.8	(4.1)	1.3***	(1.0)	1.4***	(0.8)	3.3	(0.9)	0.5***	(0.2)	2.0**	(0.8)	H***, I*	/
25	*Festuca arundinacea*	P	5.4	(1.0)	2.7	(0.5)	1.9*	(0.7)	11.7**	(4.0)	12.3**	(2.6)	2.7	(0.8)	5.2	(1.6)	9.4	(3.0)	F*, H***	/
26	*Anthoxanthum odoratum*	P	3.6	(1.5)	3.9	(2.0)	0.2***	(0.1)	0.3***	(0.2)	0.2***	(0.2)	0.0***	(0.0)	0.1***	(0.1)	0.0***	(0.0)	F***, H***	FxH**

Grey background = plant frequency was significantly negatively affected by the treatment in comparison to the control, framed cells = plant frequency was significantly positively affected by the treatment in comparison to the control. * p < 0.05, ** p < 0.01, *** p < 0.001, p values Bonferroni corrected, nested PerAnova.

Based on the results listed in Tables 3-1 and 3-2, three primary groups of responses of individual species to the treatments were recognized after three years, which are described in the following sections and summarized in Table 3-3.

Response 1:

Eight species (*Glechoma hederacea*, *Ranunculus acris*, *Lathyrus pratensis*, *Vicia sepium*, *Rumex acetosa*, *Vicia hirsuta*, *Rhinanthus alectorolophus*, and *Anthoxanthum odoratum*) were significantly negatively affected by the separate herbicide and fertilizer treatment but showed no significant response to the insecticide treatment. In addition, we detected significant herbicide-fertilizer interaction effects on three of these species: *L. pratensis* (factorial PerAnova: FxH: p = 0.007), *R. alectorolophus* (factorial PerAnova: FxH: p = 0.03), and *A. odoratum* (factorial PerAnova: FxH: p = 0.001) (Table 3-1, last column).

Response 2:

Six species (*Ajuga reptans*, *Veronica chamaedrys*, *Plantago lanceolata*, *Lotus corniculatus*, *Agrostis capillaris*, and *Holcus lanatus*) were significantly negatively affected by the separate fertilizer treatment but not by the herbicide or insecticide treatment. However, it appears that the herbicide in the combined fertilizer and herbicide treatments (F+H and F+H+I) compensated for the negative fertilizer effect on four species of this group (*A. reptans*, *V. chamaedrys*, *P. lanceolata*, and *A. capillaris*); frequencies of these species were either not significantly affected by the treatment combinations (F+H and F+H+I) (*V. chamaedrys*, *P. lanceolata*, and *A. capillaris*) or were less affected than in the fertilized plots (F) *(A. reptans*). A significant herbicide-fertilizer interaction effect was detected for one of these species: *A. reptans* (factorial PerAnova: FxH: p = 0.01) (Table 3-1, last column).

Response 3:

Six species (*Galium mollugo*, *Calystegia sepium*, *Hypericum perforatum*, *Leucanthemum vulgare*, *Achillea millefolium*, and *Heracleum sphondylium*) were not significantly affected by the separate fertilizer, herbicide, or insecticide treatment. We found no herbicide-fertilizer interactions or main effects, despite the fact that the one-way PerAnova showed significant effects for *G. mollugo* (H+I and F+I), for *L. vulgare* (F+I and F+H) and for *H. sphondylium* (F+I and F+H+I) (Table 3-1).

The remaining six species in Tables 3-1 and 3-2 (*Ranunculus repens*, *Stellaria graminea*, *Arrhenatherum elatius*, *Dactylis glomerata*, *Alopecurus pratensis*, and *Festuca arundinacea*) were not assigned to response groups, as they showed different reactions to the treatments. *R. repens*, *S. graminea*, *A. elatius*, and *A. pratensis* were significantly negatively affected by the herbicide, whereas they differed in their responses to the insecticide and fertilizer treatment. For example, the separate fertilizer and insecticide treatment increased the mean frequency of *S. graminea* by factors of 2.75 to 3.62 (mean frequency F: 17.3%; I: 22.8%) compared to the control (mean frequency 6.3%).

F. arundinacea also exhibited a unique response to the treatments. The separate fertilizer treatment reduced its frequency, whereas the separate herbicide treatment significantly increased its frequency.

Moreover, it seems that the positive herbicide effect neutralized the negative fertilizer effect in the F+H and F+H+I treatments (Table 3-2).

D. glomerata was significantly positively affected by the separate fertilizer treatment, whereas the herbicide treatment caused no significant effect.

Table 3-3: Response groups[a] of individual species to the treatments in June 2012. Separate treatments (F = fertilizer, H = herbicide, I = insecticide) are listed. Symbols within brackets indicate whether the treatment caused a significant decrease (—) or no significant change (0) in the mean plant frequency in comparison to the control. Numbers next to the species (within parentheses) correspond to the number designations of the species in Tables 3-1 and 3-2.

	Response 1	**Response 2**	**Response 3**
	F(—) **H**(—) **I**(0)	**F**(—) **H**(0) **I**(0)	**F**(0) **H**(0) **I**(0)
Dicotyledons	*G. hederacea (2)* *R. acris (3)* *L. pratensis* (4) *V. sepium* (5) *R. acetosa* (7) *V. hirsuta* (11) *R. alectorolophus* (13)	*A. reptans* (6) *V. chamaedrys* (8) *P. lanceolata* (10) *L. corniculatus* (12)	*G. mollugo* (1) *C. sepium* (14) *H. perforatum* (16) *L. vulgare* (17) *A. millefolium (18)* *H. sphondylium* (19)
Monocotyledons	*A. odoratum* (26)	*A. capillaris* (22) *H. lanatus* (23)	

[a] A response group was created when more than five individual species showed the same response to a treatment. Six species (*R. repens* (9), *S. graminea* (15), *A. elatius* (20), *D. glomerata* (21), *A. pratensis* (24), and *F. arundinacea* (25)) could not be assigned to response groups.

3.3.3. Community diversity

Species diversity (expressed as the mean Shannon index, Fig. 3-2) was highest in the control and insecticide treated plots during all three years of the study. The separate fertilizer and herbicide treatments and their treatment combinations (F+H and F+H+I) reduced species diversities compared to the control. These reductions were significant in the F+H+I, F+H and H+I treatments during the first experimental season. In the third year, in all fertilizer and herbicide treatments a significantly lower species diversity than in the control was observed (Fig. 3-2).

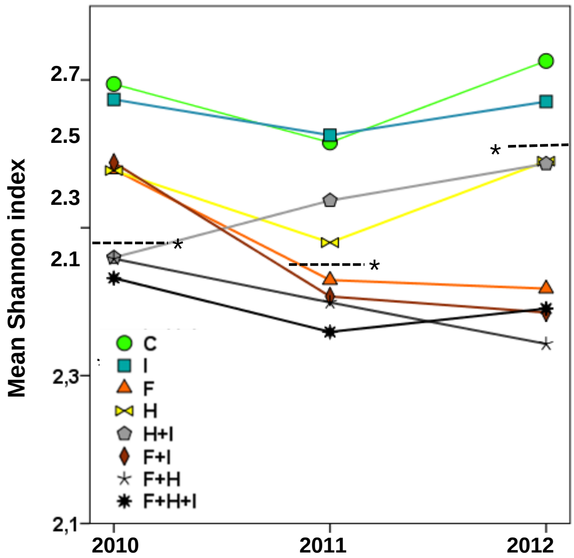

Fig. 3-2: Species diversity per treatment and year (2010-2012) expressed as the mean Shannon index [H' = -SUM(Pi*log(Pi))]. The indices below the dotted lines (-----) differ significantly from the control. Asterisks (*) indicate significance: $p < 0.05$ (one-way analysis of variance with permutations [PerAnova]; p values are Bonferroni corrected). C= control, F = fertilizer, H = herbicide, I = insecticide.

3.3.4. Community composition

Plant community compositions of the treated and control plots were similar during the first and second years (Fig. 3-3A, B). The NMDS diagram of the third year revealed three clearly distinct groups: one group (1) consisted of the control and insecticide treated plots (C and I), another group (2) consisted of the fertilizer treated plots (F and F+I), and the last group (3) consisted of the herbicide treated plots (H and H+I) and those receiving combinations of fertilizer and herbicide (F+H and F+H+I) (Fig. 3-3C). We observed significant differences between all three groups (one-way PerManova, $p < 0.01$ for all pairwise comparisons) in 2012, whereas treatments belonging to one group (with one exception) did not differ significantly from one another. The exception was the F+H and H treatment (group 3), for which significant differences were detected (one-way PerManova, $p = 0.008$, pairwise comparison).

The NMDS diagram with the weighted-average species scores of June 2012 indicated that the species composition was affected by the fertilizer and herbicide treatments (Fig. 3-3D). We found no significant correlation between the species composition and the insecticide treatment. Analysis of main effects (three factorial PerManova) confirmed this finding and indicated a significant main effect of fertilizer ($p = 0.0001$) and herbicide ($p = 0.0001$) on the plant community composition in 2012. No significant herbicide-fertilizer interactions were found at the community level.

NMDS revealed that those species that were negatively affected by the herbicide and fertilizer treatments (Response 1, Table 3-3) were plotted near the control (Fig. 3-3D). In contrast, species that were only negatively affected by the fertilizer treatment (Response 2, Table 3-3) were plotted in the opposite direction of fertilizer, i.e., between the herbicide and control, as their frequencies were similar in the herbicide and control plots. Those species that were not affected by the treatments (Response 3, Table 3-3) displayed no discernible correlation with any factor (Fig. 3-3D).

3.3.5. Biomass

The fresh weights of the biomass samples yielded similar results each year. The mean fresh weights were highest in the plots receiving the fertilizer treatments (F and F+I), followed by those of the control and insecticide treatments (Fig. 3-4). The lowest fresh weights were found in the plots treated with the herbicide (H and H+I). Analysis of main effects (three factorial PerAnova) showed a significant main effect of fertilizer and herbicide on the biomass every year ($p < 0.01$). In addition, slightly significant herbicide-fertilizer interactions were identified in June 2010 (factorial PerAnova, FxH, $p = 0.04$) and June 2012 (factorial PerAnova, FxH, $p = 0.02$).

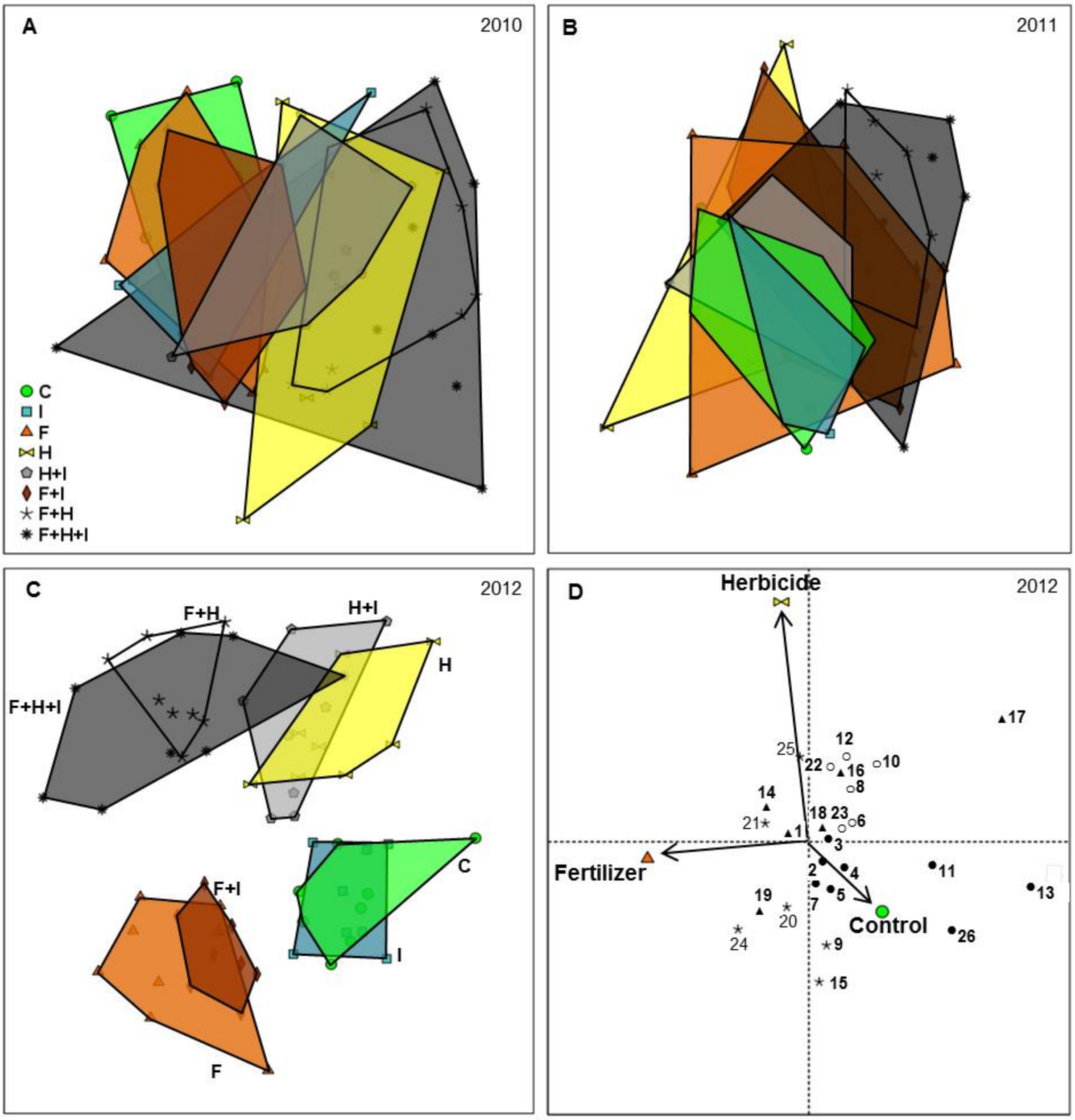

Fig. 3-3A-C: Two-dimensional NMDS graph of the 64 plots in June of 2010 (A), 2011 (B), and 2012 (C). Plots are labelled to their treatments. Different symbols represent different treatments and polygons enclose all plots of the same treatment. C = control, I = insecticide, F = fertilizer, H = herbicide (stress-values ranged from 0.20 to 0.25).

D. Two-dimensional NMDS graph of the weighted-average species scores (June 2012) (stress-value 0.21). Vectors significantly correlated with ordination (species composition) are shown (fertilizer, $r^2 = 0.61$, $p = 0.001$; herbicide, $r^2 = 0.79$, $p = 0.001$; control, $r^2 = 0.19$, $p = 0.002$). Numbers correspond to the designations assigned to the species in Tables 3-1 and 3-2. The symbols represent species response groups summarized in Table 3-3: Response 1(●), Response 2 (o), Response 3 (▲), and species not assigned to response groups (*).

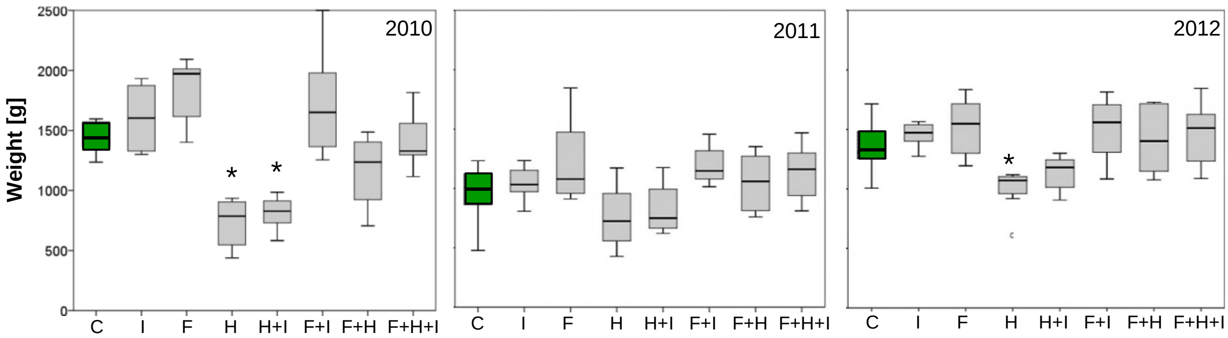

Fig. 3-4: Mean fresh weights [g] of the biomass samples (1 m^2) per plot and treatment in June 2010, 2011, and 2012. Replicates per treatment = 8. * Significantly different from the control (one-way analysis of variance with permutations [PerAnova]; p values Bonferroni corrected; $p < 0.05$.). C = control (highlighted in green), I = insecticide, F = fertilizer, H = herbicide.

3.4. Discussion

3.4.1. Effects of fertilizer, herbicide, and insecticide on individual plant species

Agrochemical effects on certain species were already observed in the first year, but became stronger over time. As a result, 20 of the 26 abundant species were significantly affected by the treatments in the third year. Tables 3-1 and 3-2 show that 17 species were significantly affected (15 negatively, 2 positively) by the separate fertilizer treatment and that 13 species were significantly affected (12 negatively, 1 positively) by the separate herbicide treatment. The insecticide treatment affected 2 species significantly (1 positively, 1 negatively).

The fertilizer effect on several species is not surprising because negative effects of fertilizers on plant species are well-documented in the literature (e.g., Clark & Tilman 2008; Kleijn et al. 2009; Socher et al. 2013). Effects of eutrophication with low application rates of fertilizer similar to the rates used in our study (25 % of the field rate) often develop slowly and reduce the population size of plant species over time. Species losses or a decrease in certain species are often caused by competitive advantages of a few nitrogen-tolerant species. Tall grasses such as *D. glomerata* (as in our study) benefit from fertilization because they can increase in abundance over smaller herbs (Socher et al. 2013). Small species are usually replaced by faster-growing species (Jumpponen et al. 2005) and by species with a high leaf canopy (Wilson 1999). This variation enhances effects of shading and, consequently, the competition for light, which can further suppress the frequencies of small and subordinate species (Hautier et al. 2009), such as *G. hederacea* and *A. reptans*. Certain species with low nitrogen-values (Ellenberg et al. 1992) were also negatively affected (e.g., *R. alectorolophus* (N-value = 3), *V. hirsuta* (N-value = 4)).

The herbicide affected the plant species in a different manner. The herbicide treatment caused nearly a complete disappearance of three species (*R. alectorolophus, S. graminea,* and *A. odoratum*) in 2012 (Tables 3-1 and 3-2). Similar reductions were also observed in the first and second years. The herbicide that was applied is designed to control grasses and annual herbs. *R. alectorolophus* is an annual, which may explain its high sensitivity, whereas *S. graminea* is a perennial; thus, a generalization is difficult. These findings, however, are of great concern because *R. alectorolophus* is on the Red List "V" in Germany (V = vulnerable = species likely to become endangered in the near future).

During the vegetation assessments, it was also observed that the herbicide visibly affected the vegetation. Several leaves in the herbicide treated plots were slightly yellow or brown. The affected species were still recorded after the herbicide applications but, presumably, they were more vulnerable and sensitive to natural stress. Their fitness and competitive ability were most likely reduced, and the repeated herbicide applications intensified the effects, which reduced the abundance of the most sensitive species over time. This trend indicates that certain species were more sensitive to the herbicide than others when growing in a natural plant community.

A few of the dicotyledons were also negatively affected by the herbicide due to flower suppression. In the same field experiment, significant reductions in the seed production of *R. acris*, *L. pratensis*, and *V. sepium* were observed in the herbicide-treated plots (Schmitz et al. 2013, and 2014a). Such reductions in fruit sets during one growing season, especially when combined with other stresses (e.g., herbivores, weather conditions) are likely to be sufficient to hamper recovery, and reproduction of most plants (Carpenter & Boutin 2010). These findings may explain why herbicide effects often became apparent the first year after application. With recurring exposure, the seed bank eventually will be depleted, which will reduce population size (Roberts &Neilson 1981; Ball 1992). Other studies have also demonstrated that herbicides may reduce seed sets when sprayed at the bud stage or shortly before the onset of reproduction (Fletcher et al. 1996; Boutin et al. 2000; Kjaer et al. 2006).

An additional herbicide effect was the inhibition of plant growth, as indicated by the biomass samples. The biomass correlated well with the vegetation height, which was also lowest in the herbicide treated plots (Fig. 3-4, see Fig. 3-5 in supplementary data for vegetation heights in June 2012). This inhibition increased the light intensity and most likely was an advantage for less herbicide-sensitive dicotyledons (*A. reptans*, *V. chamaedrys*, *P. lanceolata*, *L. corniculatus*) and monocotyledons (*D. glomerata*, *A. capillaris*, *H. lanatus*, *F. arundinacea*). Their frequencies were either not significantly negatively affected or increased slightly (not significant) (*V. chamaedrys*, *P. lanceolata*) or significantly (*F. arundinacea*) with the herbicide treatment (Table 3-1).

The insecticide treatment increased the frequency of *S. graminea* by a factor of 3.6 compared to the control. Similar increases were observed during all three years. The insecticide is a pyrethroid, which may have toxic or repellent effects for insects (Gist & Pless 1985; Blair 1991). For example, the micro-moth *Coleophora striatipennella* uses the flowers of *S. graminea* for oviposition, and the fruits/seeds are a food source for their larvae (Database of Insects and their Food Plants 2013). This treatment may have had negative effects on insects (direct effect) and, in consequence, positive effects on the plant (indirect effect) because certain herbivorous and seed eating insects decreased in number.

In contrast, the frequency of *A. pratensis* was significantly reduced by the insecticide. Perhaps *A. pratensis* has mutualistic relationships with specific arthropods. These possibilities, however, are only speculations.

The results also indicated that there were no main effects on the frequencies of six plant species (Table 3-1). Five of these species *(C. sepium*, *H. perforatum*, *L. vulgare*, *A. millefolium*, and *H. sphondylium*) were present in low frequencies (with high standard errors); thus, it is difficult to develop firm conclusions regarding their sensitivity to the agrochemicals. However, *G. mollugo* was the most abundant species in the study site and seems to be not sensitive to the fertilizer and herbicide products that were used in our study.

We used only one product per treatment (herbicide, and insecticide). In general, species sensitivity varies greatly with the pesticide used, and agrochemicals containing other active ingredients might cause other effects. Therefore, it is difficult to extend our findings to other agrochemicals. Moreover, we used formulated products in our field experiment, and the ingredients in other formulations may differ. These various ingredients may also influence species sensitivity, which is largely unknown until now. Thus, it may be valuable to investigate other agrochemicals and their effects on nontarget plants in future studies.

3.4.2. Interaction effects of fertilizer, herbicide, and insecticide on individual plant species

We observed significant herbicide-fertilizer interaction effects on four species (Table 3-1 and Table 3-2). However, it seems that interaction effects between fertilizer and herbicide also occurred for other species. For example, the herbicide neutralized the negative fertilizer effects in the treatment combinations F+H and F+H+I for V. *chamaedrys*, *P. lanceolata*, *A. capillaris*, and *F. arundinacea*. Conversely, it appears likely that there were additive effects on *G. hederacea*, *V. sepium*, and *R. acetosa*, as their frequencies were negatively affected by the single fertilizer and herbicide treatment, whereas their frequencies were more strongly reduced by the treatment combinations of fertilizer and herbicide (F+H and F+H+I). Thus, it is not possible to extrapolate from individual effects of herbicide and fertilizer to their combined effects on specific species. There are two possible explanations: On the one hand, agrochemicals can interact with one another by neutralizing effects or by causing synergistic or additive effects, as mentioned above. On the other hand, the application of agrochemicals to natural plant communities can (simultaneously) affect or influence the sensitivity of certain species; thus, their competitiveness within the community is altered. For example, Damgaard et al. (2011) investigated the combined effects of nitrogen and glyphosate on two grasses (*Festuca ovina* and *A. capillaris*) and observed significant positive interactions of glyphosate and nitrogen on the growth of *A. capillaris*. The authors suggested that positive herbicide-fertilizer interactions on this species were caused by altered plant competition (Damgaard et al. 2011).

Interaction effects in natural plant communities are complex and depend on interaction effects between agrochemicals on certain plant species (e.g., additive, synergistic effects) and on interaction effects between species (e.g., changed competition, shading effects). The relative intensity of species competition along environmental gradients (e.g., agrochemicals) may also depend on species density in the plant community (Damgaard & Fayolle 2010). Thus, it is most likely that fertilizer-herbicide interaction effects differ with the type of vegetation. Interaction effects of agrochemicals may also vary with the applied fertilizer and herbicide products due to different modes of action.

3.4.3. Effects of fertilizer, herbicide, and insecticide on the plant community composition

Before the experiment began, the study site was not contaminated with fertilizer and/or pesticides and thus, the plant community may be regarded as a habitat free of any influence of agrochemicals. Plant communities of unaffected habitats are relatively stable in their composition and change only slowly following low fertilizer or pesticide application rates. These effects take time to appear, as reflected by the results of the NMDS analysis. Although the plant frequencies of a few species were already affected during the first and second years, these effects were most likely too weak to cause clear separations of the plant communities in the different treatments (Fig. 3-3A, B). With each year of application, effects on the frequencies of individual species became stronger, and after three years the composition of the plant community was altered by the herbicide and fertilizer treatments (Fig. 3-3C, D). The NMDS diagram of the 64 plots based on the June 2012 data shows a separation of the fertilizer-treated plots (F and F+I) from all other treatments (Fig. 3-3C). This result illustrates the severe effect of fertilizer on the frequencies of several species. Herbicide treatments (H and H+I) and treatment combinations of fertilizer and herbicide (F+H and F+H+I) are plotted closer to each other in the NMDS diagram due to the above-mentioned herbicide-fertilizer interaction effects. It is likely that a stronger separation of the treatment combinations (F+H and F+H+I) and the herbicide-treated plots (H and H+I) would occur in the course of time, based on the fact that a significant difference between the F+H and H treatments was observed in June 2012.

The number of species was slightly reduced in the treatment combinations of fertilizer and herbicide in June 2012 (Fig. 3-1). Although the separate fertilizer and herbicide treatments did not significantly affect the mean number of species, we observed significantly lower species diversities in all fertilizer and herbicide treatments compared to the control in the third year (Fig. 3-2). It may be assumed that these effects would become more pronounced with each year of agrochemical applications until the most robust and least susceptible species dominate the plant community.

3.5. Conclusion

This study revealed that fertilizer and herbicide misplacements in field margins are major factors that affect field margin plant communities. Previous studies have often focused only on the effects of herbicide drift on the vegetation in off-field habitats, and the effects of fertilizer were not considered. Our results demonstrate that both agrochemicals - herbicide and fertilizer - can reduce mean frequencies of several species, although these agrochemicals affect different species in different ways. In our study, fertilizer directly promoted the growth and spread of species with a high nutrient uptake, whereas it indirectly reduced the growth of small species. In contrast, the herbicide caused predominantly sublethal effects (phytotoxic and reproduction effects) and nearly a complete disappearance of a few species.

Reproduction effects require time to be measurable in plant frequency assessments; nonetheless, these effects are expected to reduce population size of plant species in the long term. Thus, investigations that only focus on short-term herbicide effects on mean plant frequencies may underestimate the full herbicide effect. In our field study, negative effects on plant frequencies and plant reproduction, contributed together to the alterations of the plant communities. Moreover, the application of the insecticide significantly affected the frequencies of two species, which may have resulted from effects on plant-insect interactions, although this assumption requires more specific investigations.

An additional key finding was that treatment combinations of fertilizer and herbicide caused interaction effects (e.g., additive effects) and that these effects could not be extrapolated from the individual fertilizer or individual herbicide effect.

Treatment effects (individual and combined) on most species gradually became apparent. Thus, low fertilizer and herbicide rates may not significantly affect plant community compositions in field margins in the short-term (within one or two years), but long-term misplacements of agrochemicals may cause significant changes in species composition and reduce species diversity significantly, as was observed in our study. We believe that continuous annual applications of agrochemicals on the study site would cause further plant community shifts and would likely lead to the disappearance of certain affected plants. Small and subordinate species with high herbicide and fertilizer sensitivity will most likely become less abundant or completely disappear. The most robust and least susceptible species (predominantly tall grasses and a few species of dicotyledons) may dominate the plant community after several years.

Narrow field margins in Germany are most likely similarly affected, as simulated in our study. As a result, the vegetation in these margins has already been degraded due to agrochemical inputs over the last five to six decades. Although herbicide risk assessment (RA) aims to protect nontarget plants in off-field habitats (field margins) from adverse effects, reproduction effects are not considered so far. The RA of pesticides is also carried out for only one specific compound, and therefore, influences of combined effects of pesticides are not taken into account. Therefore, it seems that the RA currently performed provides insufficient protection for nontarget plants and their habitats. Furthermore, there are no distance requirements for fertilizer applications next to field margins, which could prevent fertilizer misplacements.

Adaptations of the current RA and development of general risk mitigation measures (e.g., in-field buffers) for the application of herbicides and fertilizers are urgently required to conserve natural plant communities in field margins.

Supplementary data[2]: Agrochemicals in field margins – An experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community

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Table 3-4: Species identified during vegetation assessments on the experimental study site in May and June of 2010, 2011 and 2012. Frequent species in the meadow are highlighted with a grey background (A species was classified as frequent, when its average plant frequency was > 1% per plot (i.e., more than 2 individuals per plot were recorded). Species are arranged alphabetically.

Life span: P = perennial, A = annual, B = biennial; reproduction: S = seed, SV = seed and vegetatively, SSV = mostly by seed, rarely vegetatively, VVS = mostly vegetatively, rarely by seed; Ellenberg`s indicator value for nitrogen: 1 = extremely infertile sites, 2 = between 1 and 3, 3 = more or less infertile sites, 4 = between 3 and 5, 5 = sites of intermediate fertility, 6 = between 5 and 7, 7 = plant often found in highly fertile places, 8 = between 7 and 9, 9 = extremely rich conditions; Red List status in Germany: V = vulnerable.

(all Information on species from Klotz, S., Kühn, I., Durka, W., (2002): BIOLFLOR - Eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland. Schriftenreihe für Vegetationskunde 38. Bundesamt für Naturschutz (BfN), Bonn.)

	Dicotyledons	**Common name**	**Familiy**	**Life Span**	**Repro-duction**	**Ellenberg`s indicator value for nitrogen**	**Red List status (Germany)**
1	*Achillea millefolium*	Yarrow	Asteraceae	P	SV	5	not endangered
2	*Agrimonia eupatoria*	Common agrimony	Rosaceae	P	S	4	not endangered
3	*Ajuga reptans*	Common bugle	Lamiaceae	P	SV	6	not endangered
4	*Alchemilla vulgaris*	Common Lady's Mantle	Rosaceae	P	SV	6	V
5	*Bellis perennis*	Common daisy	Asteraceae	P	SV	6	not endangered
6	*Calystegia sepium*	Hedge bindweed	Convolvulaceae	P	SV	9	not endangered
7	*Cardamine pratensis*	Cuckoo Flower	Brassicaceae	P	VVS	indifferent	not endangered
8	*Cerastium fontanum*	Common mouse-ear chickweed	Caryophyllaceae	P	SV	5	not endangered
9	*Cirsium arvense*	Cursed Thistle	Asteraceae	P	SV	7	not endangered
10	*Galium mollugo*	Hedge Bedstraw	Rubiaceae	P	S	unknown	not endangered
11	*Glechoma hederacea*	Ground ivy	Lamiaceae	P	SV	7	not endangered
12	*Heracleum sphondylium*	Hogweed	Apiaceae	B, P	SSV	8	not endangered
13	*Hypericum perforatum*	Common St. Johnswort	Hypericaceae	P	SV	4	not endangered
14	*Hypochaeris radicata*	Catsear	Asteraceae	P	SSV	3	not endangered
15	*Lathyrus pratensis*	Meadow vetchling	Fabaceae	P	SSV	6	not endangered
16	*Leucanthemum vulgare*	Oxeye daisy	Asteraceae	P	SV	3	not endangered
17	*Linaria vulgaris*	Common Toadflax	Scrophulariaceae	P	SV	5	not endangered
18	*Lotus corniculatus*	Bird's-foot Trefoil	Fabaceae	P	SSV	3	not endangered
19	*Lychnis flos-cuculi*	Ragged Robin	Caryophyllaceae	P	SV	indifferent	V
20	*Lythrum salicaria*	Purple loosestrife	Lythraceae	P	SSV	indifferent	not endangered

[2] Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2014.04.025

Table 3-4 continued:

21	*Plantago lanceolata*	Narrowleaf plantain	Plantaginaceae	P	SSV	indifferent	not endangered
22	*Potentilla anserina*	Common Silverweed	Rosaceae	P	SV	7	not endangered
23	*Prunella vulgaris*	Common self-heal	Lamiaceae	P	SV	indifferent	not endangered
24	*Ranunculus acris*	Common buttercup	Ranunculaceae	P	S	indifferent	not endangered
25	*Ranunculus repens*	Creeping buttercup	Ranunculaceae	P	SV	7	not endangered
26	*Rhinanthus alectorolophus*	European yellow rattle	Scrophulariaceae	A	S	3	V
27	*Rumex acetosa*	Common sorrel	Polygonaceae	P	SV	6	not endangered
28	*Rumex crispus*	Curly dock	Polygonaceae	P	SV	6	not endangered
29	*Sanguisorba officinalis*	Great burnet	Rosaceae	P	SV	5	V
30	*Saxifraga granulata*	Meadow Saxifrage	Saxifragaceae	P	VVS	3	V
31	*Stellaria graminea*	Grass-like starwort	Caryophyllaceae	P	SV	3	not endangered
32	*Symphytum officinale*	Common comfrey	Boraginaceae	P	SSV	8	not endangered
33	*Tragopogon pratensis*	Jack-go-to-bed-at-noon	Asteraceae	B	S	6	not endangered
34	*Trifolium pratense*	Red clover	Fabaceae	P	S	indifferent	not endangered
35	*Trifolium repens*	White clover	Fabaceae	P	SV	6	not endangered
36	*Urtica dioica*	Stinging nettle	Urticaceae	P	SV	9	not endangered
37	*Valeriana officinalis*	Valerian	Valerianaceae	P	SV	5	not endangered
38	*Veronica chamaedrys*	Germander Speedwell	Scrophulariaceae	P	SV	6	not endangered
39	*Vicia hirsuta*	Tiny vetch	Fabaceae	A	S	4	not endangered
40	*Vicia sepium*	Bush vetch	Fabaceae	P	SSV	5	not endangered

	Monocotyledons	**Common name**	**Familiy**	**Life Span**	**Repro-duction**	**Ellenberg`s indicator value for Nitrogen**	**Red List status (Germany)**
1	*Elytrigia repens*	Quackgrass	Poaceae	P	VVS	7	not endangered
2	*Poa trivialis*	Rough bluegrass	Poaceae	P	SV	7	not endangered
3	*Anthoxanthum odoratum*	Sweet vernal grass	Poaceae	P	SV	indifferent	not endangered
4	*Arrhenatherum elatius*	Tall oatgrass	Poaceae	P	SSV	7	not endangered
5	*Calamagrostis epigejos*	Wood Small-reed	Poaceae	P	SV	6	not endangered
6	*Festuca arundinacea*	Tall fescue	Poaceae	P	S	4	not endangered
7	*Agrostis capillaris*	Colonial bentgrass	Poaceae	P	SV	4	not endangered
8	*Bromus hordeaceus*	Soft brome	Poaceae	A	S	3	not endangered
9	*Alopecurus pratensis*	Meadow foxtail	Poaceae	P	SV	7	not endangered
10	*Trisetum flavescens*	Yellow oatgrass	Poaceae	P	SSV	5	not endangered
11	*Cynosurus cristatus*	Crested dogstail grass	Poaceae	P	S	4	not endangered
12	*Dactylis glomerata*	Cock's-foot	Poaceae	P	SSV	6	not endangered
13	*Holcus lanatus*	Common velvet grass	Poaceae	P	SV	5	not endangered
14	*Poa pratensis*	Kentucky Bluegrass	Poaceae	P	SV	6	not endangered

Table 3-5: Treatment effects on plant species (plant frequency > 1%) in June 2010. In the control column the mean plant frequencies [%] per plot are shown. Significant treatment effects compared to the control are marked with asterisks. The last column shows the results from the three-factorial PerAnova (main effects). C = control, I = insecticide, F = fertilizer, H = herbicide. ns = not significant.

2010

Dicotyledons	Control	Single treatments			Combined treatments				Main effect
	C	I	F	H	H+I	F+I	F+H	F+H+I	
Galium mollugo	85.0	ns	ns	***	ns	ns	**	***	H**
Ranunculus acris	60.6	ns	ns	ns	ns	ns	ns	ns	F*
Lathyrus pratensis	60.5	ns	ns	***	***	**	***	***	F*, H***
Vicia sepium	42.6	ns	ns	ns	ns	ns	***	***	H***
Rhinanthus alectorolophus	28.9	ns	ns	***	***	ns	***	***	H***
Lotus corniculatus	23.0	ns	ns	ns	ns	ns	ns	ns	/
Glechoma hederacea	22.8	ns	ns	ns	ns	ns	ns	**	/
Plantago lanceolata	17.2	ns	ns	ns	ns	**	ns	ns	/
Veronica chamaedrys	16.7	ns	ns	ns	ns	ns	ns	ns	/
Rumex acetosa	16.5	ns	ns	ns	ns	ns	ns	ns	/
Stellaria graminea	15.7	ns	ns	***	***	*	***	***	H***
Vicia hirsuta	11.9	ns	**	***	***	ns	***	***	H***
Trifolium pratense	8.3	ns	**	ns	**	**	***	***	F**, H*
Achillea millefolium	7.3	ns	ns	ns	ns	ns	ns	ns	/
Calystegia sepium	7.3	ns	ns	ns	ns	ns	ns	ns	/
Heracleum sphondylium	5.9	ns	ns	ns	*	**	ns	**	/
Ajuga reptans	4.0	ns	ns	ns	ns	***	ns	ns	/
Leucanthemum vulgare	3.7	ns	ns	ns	ns	ns	ns	ns	/
Lychnis flos-cuculi	1.8	ns	ns	ns	ns	ns	ns	ns	/
Hypericum perforatum	1.5	ns	ns	ns	ns	ns	ns	ns	/
Ranunculus repens	1.0	*	ns	ns	ns	*	*	ns	F*

Monocotyledons	Control	Single treatments			Combined treatments				Main effect
	C	I	F	H	H+I	F+I	F+H	F+H+I	
Holcus lanatus	44.2	ns	**	ns	ns	**	ns	ns	/
Dactylis glomerata	22.2	ns	ns	ns	ns	ns	ns	ns	/
Arrhenatherum elatius	20.9	ns	ns	ns	ns	ns	ns	ns	/
Agrostis capillaris	18.1	ns	ns	*	ns	ns	**	ns	H**
Festuca arundinacea	10.2	ns	ns	ns	ns	ns	*	ns	/
Anthoxanthum odoratum	8.4	ns	ns	**	ns	ns	ns	ns	H*
Alopecurus pratensis	3.2	ns	ns	ns	*	ns	**	ns	H*

Grey background = plant frequency was significantly negatively affected by the treatment in comparison to the control, framed cells = plant frequency was significantly positively affected by the treatment in comparison to the control. * p < 0.05, ** p < 0.01, *** p < 0.001, p values Bonferroni corrected, nested PerAnova.

Table 3-6: Treatment effects on plant species (plant frequency > 1%) in June 2011. In the control column the mean plant frequencies [%] per plot are shown. Significant treatment effects compared to the control are marked with asterisks. The last column shows the results from the three-factorial PerAnova (main effects). C = control, I = insecticide, F = fertilizer, H = herbicide. ns = not significant.

2011

Dicotyledons	Control	Single treatments			Combined treatments				Main effect
	C	I	F	H	H+I	F+I	F+H	F+H+I	
Galium mollugo	79.0	ns	ns	***	**	ns	ns	ns	/
Glechoma hederacea	40.1	**	ns	ns	ns	ns	ns	***	F*
Ranunculus acris	38.2	ns	***	ns	ns	***	ns	***	F**
Vicia sepium	31.4	ns	ns	ns	ns	ns	***	ns	/
Lathyrus pratensis	30.0	ns	***	ns	**	***	**	***	F***
Ajuga reptans	25.8	*	***	ns	ns	***	ns	ns	F***
Rumex acetosa	23.1	ns	ns	*	***	ns	***	***	H**
Plantago lanceolata	21.9	ns	ns	ns	ns	ns	ns	ns	/
Veronica chamaedrys	20.1	ns	ns	ns	ns	ns	ns	ns	/
Ranunculus repens	18.1	ns	ns	*	ns	ns	ns	*	H*
Stellaria graminea	11.9	***	***	***	ns	***	***	***	H***, I*
Lotus corniculatus	8.7	ns	ns	*	*	*	ns	ns	F*
Rhinanthus alectorolophus	8.5	ns	***	***	***	***	***	***	H**
Calystegia sepium	6.3	ns	ns	ns	ns	*	ns	ns	/
Hypericum perforatum	3.2	ns	ns	ns	ns	ns	ns	ns	/
Achillea millefolium	3.0	ns	ns	ns	ns	ns	ns	ns	/
Trifolium pratense	2.4	ns	ns	ns	ns	ns	ns	ns	/
Heracleum sphondylium	1.3	ns	ns	ns	ns	ns	ns	ns	/
Leucanthemum vulgare [a]	0.9	ns	ns	*	ns	ns	ns	*	/
Trifolium repens [a]	0.8	ns	ns	ns	ns	ns	ns	ns	/
Vicia hirsuta [a]	0.5	ns	ns	ns	ns	ns	ns	ns	/

Monocotyloedons	Control	Single treatments			Combined treatments				Main effect
	C	I	F	H	H+I	F+I	F+H	F+H+I	
Arrhenatherum elatius	63.7	ns	ns	***	*	ns	*	ns	H*
Dactylis glomerata	51.2	ns	ns	ns	ns	ns	***	***	F***
Holcus lanatus	46.6	*	**	ns	ns	ns	***	***	F*
Anthoxanthum odoratum	9.2	ns	***	ns	ns	***	***	***	F**
Agrostis capillaris	8.8	ns	**	ns	**	ns	ns	*	F*, H*
Festuca arundinacea	3.6	ns	ns	ns	ns	ns	**	ns	/
Alopecurus pratensis	3.1	*	ns	*	ns	ns	***	ns	/

Grey background = plant frequency was significantly negatively affected by the treatment in comparison to the control, framed cells = plant frequency was significantly positively affected by the treatment in comparison to the control. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, p values Bonferroni corrected, nested PerAnova.

[a]frequencies < 1%, these species are listed for a comparison of their frequencies in 2010 and 2012.

Mean vegetation height of monocotyledons and dicotyledons in June 2012

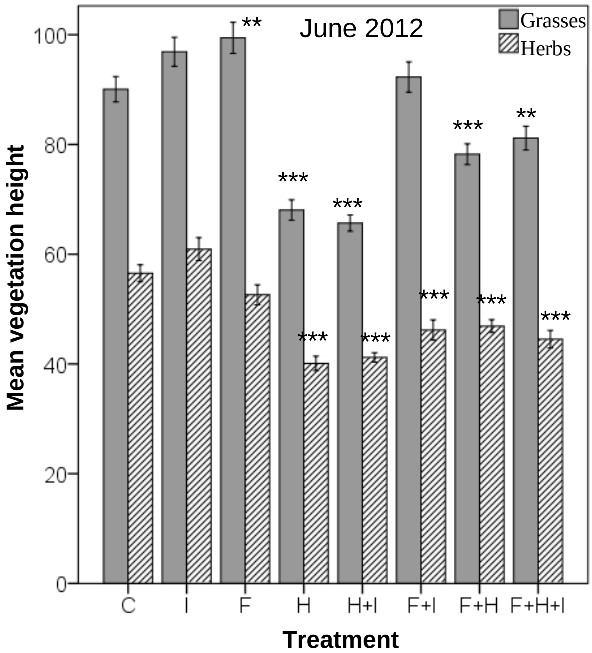

Fig. 3-5: Mean vegetation height [cm] of monocotyledons and dicotyledons per plot and treatment (measured in June 2012). Replicates (plots) per treatment = 8 (6 separate measurements per plot). C = control, I = insecticide, F = fertilizer, H = herbicide. * Significantly different from the control (nested analysis of variance with permutations [nested PerAnova]; p values Bonferroni corrected; *p < 0.05, ** p < 0.01, ***p < 0.001).

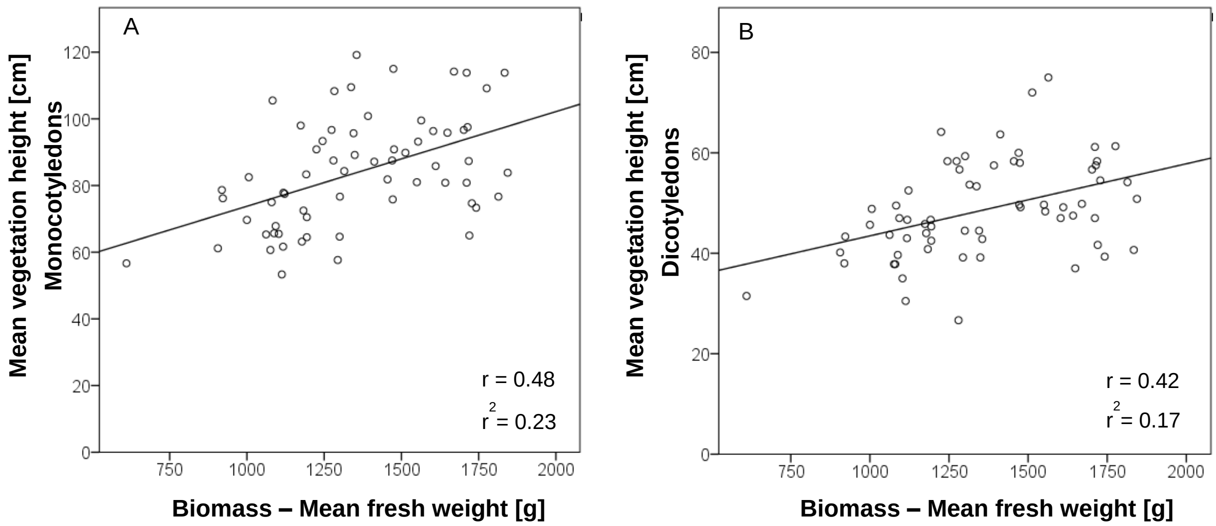

Fig. 3-6: Correlation between the mean vegetation height of monocotyledons (A) and dicotyledons (B) and biomass per plot and treatment in June 2012. The vegetation height was measured during plant community assessments in mid-June. Biomass samples were taken approximately 2 weeks later. Pearson correlation r = 0.48, p = 0.001 for vegetation high of monocotyledons and biomass (A); Pearson correlation r = 0.42, p = 0.001 for vegetation high of dicotyledons and biomass (B).

4 Agrochemicals in field margins – Assessing the impacts of herbicides, insecticides and fertilizers on the common buttercup (*Ranunculus acris*)

Paper II

This chapter presents the author`s final version of the article:

Schmitz, J., Schäfer, K., Brühl, C.A. (2013): Agrochemicals in field margins – Assessing the impacts of herbicides, insecticides and fertilizers on the common buttercup (*Ranunculus acris*). *Environmental Toxicology and Chemistry*, Vol. 32, No. 5, pp. 1124–1131.

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Abstract - The effects of herbicide, insecticide, and fertilizer inputs on the common buttercup *Ranunculus acris* in field margins were studied in an experimental field study. The test design allowed us to investigate the single and combined effects of repeated herbicide, insecticide, and fertilizer applications in successive growing seasons. To assess the effects of the agrochemical applications on *R. acris*, plant community assessments were carried out and a photo-documentation of the flowering intensity was performed over two years. In addition, the authors conducted a monitoring survey of *R. acris* in field margins in the proximity of the study site. In the field experiment, *R. acris* plant density decreased significantly with treatments including fertilizer. The herbicide caused a sublethal effect by reducing flower intensity by 85%. In the long run, both effects will result in a decline of *R. acris* and lead to shifts in plant communities in field margins. This was confirmed by the monitoring survey, where *R. acris* could hardly be observed in field margins directly adjacent to cereal fields, whereas in margins next to meadows the species was recorded frequently. Besides the implications for the plants, the sublethal effects may also affect many flower-visiting insects. The results indicate that the current risk assessment for non-target plants is insufficiently protective for wild plant species in field margins and that consideration of sublethal effects is crucial to preserve biodiversity in agricultural landscapes.

Keywords - Risk assessment, Field margin, Non-target plant, Pesticide, Fertilizer

4.1 Introduction

Herbicides are the most widely used type of pesticide in the agricultural landscape. In Europe the use of herbicides has increased considerably in the last decades, and to date herbicides represent more than 50 % of all pesticides used throughout the world (Cooper & Dobson 2007). Therefore, studying the negative effects of herbicides on the biodiversity of agricultural landscapes is becoming ever more important. Generally, herbicides are used to control certain plant species (target plants) on the agricultural area, which compete with crop plants for resources. However, the vegetation of seminatural habitats directly adjacent to agricultural fields consisting of so-called non-target plants is also affected by pesticide misplacements (Kleijn & Snoeijing 1997; Marrs & Frost 1997).

Field margins represent the majority of seminatural habitats in the intensively farmed agricultural landscapes in Germany as well as in other parts of Europe, Canada, and the United States (Marshall & Moonen 2002). The exact definition of field margins can slightly vary between different countries and authors (Marshall & Moonen 2002; Kühne & Freier 2001). Here, field margin is defined as a linear, permanent vegetation strip of mostly grassy and herbaceous off-crop habitats adjacent to agricultural fields. These habitats are usually just a few meters wide and are mown periodically (Kühne & Freier 2001). Field margins are useful for the conservation of biodiversity in agroecosystems since they enhance plant diversity within farmland and may serve as corridors for the movement of fauna and flora (Marshall & Moonen 2002). They can also act as valuable habitats for many insects including pollinators and beneficial arthropods, which move into adjoining arable fields and provide ecosystem services (Power 2010; Pywell et al. 2004).

The risk assessment of herbicides aims to protect non-target plants in off-crop habitats such as field margins from adverse effects of pesticides (European Commission 2002). For this purpose, tests with single and annual plant species are performed in young development stages (two- to four-leaf stage) in greenhouse experiments. Although testing guidelines (OECD 2006; US EPA 1996) allow using non-crop species, the standard risk assessment uses crop plants for phytotoxicity testing even though non-crop species (annual and perennial species) are to be protected in field margins. However, Boutin and Rogers (2000) mentioned that phytotoxicity testing with crop plants alone as representative species is not necessarily protective for wild species and could underestimate their sensitivity. Furthermore, single-species tests under greenhouse conditions cannot provide sufficient safety for the entire plant community in field margins because competitive interactions between species are not assessed with these testing methods (Dalton & Boutin 2010).

In the risk assessment, spray drift is considered to be the key exposure route for non-target plants in field margins (European Commission 2002). In accordance with the proposals of the guidance document on terrestrial ecotoxicology, the initial assessment of spray drift should be conducted for a distance of 1 m from the field edge for crops (European Commission 2002; EPPO 2003). However, the first meter of a field margin directly adjacent to the field, which is affected most by pesticide inputs, is not considered. This is probably due to a statement in the "Environmental Risk Assessment Scheme for Plant Protection Products” by the European and Mediterranean Plant Protection Organization (EPPO 2003), which mentions that nontarget areas generally do not border on a treated area directly. According to this document, there is usually a narrow vegetation strip between the treated area and the nontarget area (EPPO 2003). However, in Germany, for instance, there is no border between the treated area and the nontarget area.

Although spray drift mitigation strategies and some regulations for the application of pesticides to reduce pesticide drift (e.g., product-specific sanctions including buffer zone distances to terrestrial off-crop habitats) are in place, they are often softened by exceptions. In Germany, a farmer does not have to follow these mitigation regulations when the field margin is less than 3 m wide since then it is not considered as a terrestrial nontarget habitat (Kühne et al. 2000). Thus, these narrow field margins are not protected. Consequently, if the farmer does not keep a distance to the field margin during field application, overspraying of the field margin will take place. Overspraying can occur since the spray nozzles are mounted on a spray arm in such a way that the spray cones of two nozzles overlap, which is necessary to assure a full 100 % application rate in the field. The last nozzle of the spray arm is then placed above the field edge, and as a result, parts of the adjacent field margin (depending on the field cultivation and the corresponding height of the spray arm) are oversprayed. This difficulty is compounded by the fact that field margins in Germany are often only a few meters (1–2 m) wide. For this reason, the precise impact of overspraying and the following drift in the first meter of field margins are highly relevant factors, which can affect the plant composition in frequently encountered narrow field margins in the agricultural landscapes in Germany.

In addition, field margins are exposed to fertilizer misplacements, which can range from 25 to 50 % of the field rate in the first meter of a field margin (Tsiouris & Marshall 1998). Fertilizer inputs encourage plant species with a high nutrient uptake and lead to changes in community composition and a lower plant diversity (De Cauwer et al. 2006).

These effects can also interact with or be influenced by herbicide inputs in field margins (e.g., cumulative, synergetic effects) (Kleijn & Snoeijing 1997; Gove et al. 2007) because of the annual application sequences of herbicides and fertilizer on one field.

Furthermore, the vegetation of field margins is exposed to agrochemical inputs over several growing seasons, which probably intensify the effects. So far, these possible cumulative effects or the repeated exposures of plants to fertilizers and herbicides on plant species and communities in field margins have not been well investigated. In addition, the annual application of insecticides can probably have indirect effects on plants by decreasing the density of herbivorous insects.

The aim of the present study was to detect short- and medium- term effects of fertilizer and pesticide (herbicide and insecticide) inputs in narrow field margins on the plant community. In the following, we present the effects of fertilizer and pesticides on the common buttercup *Ranunculus acris* L. over two successive seasons (2010 and 2011). This species was chosen because it is widely distributed throughout Europe and is considered a common plant species of the agricultural landscape. Furthermore, *R. acris* was one of the most common species in the experimental study site. The yellow buttercup flowers, which can be seen between May and September, form a prominent part of the flowering aspect of many grasslands or ruderal habitats during spring and summer (Steinbach & Gottsberger 1994). In addition, monitoring of *R. acris* in field margins in the study area was undertaken to document its presence in field margins of the agricultural landscape.

4.2 Materials and Methods

4.2.1 Experimental study site

The field study was carried out on a meadow (1 hectare) near Landau (South Rhineland Palatinate, Germany), which had been extensively managed for feed for horses by mowing (twice a year) without any fertilizer additions for the previous 10 years. The meadow is surrounded by a ditch with a dense hedge and tree row (north), a cart track and small woodland (south), and neighboring fields (west and east; Fig. 2-1, page 10). Existing field margins were not used for this experiment because it could not be excluded that the fauna and flora of field margins had already changed as a result of the agrochemical inputs from the adjacent field management. The meadow was selected since it can be regarded as an original habitat that was not contaminated with agrochemicals and, therefore, as representing the plant community of a surrogate field margin without this influence. The vegetation of the meadow was homogeneous and consisted of tall grasses, for example, *Holcus lanatus* and *Arrhenatherum elatius*, and herbaceous plants like *Galium mollugo*, *R. acris*, and *Lathyrus pratensis* (in total approx. 40 herbaceous plants, 13 grasses).

4.2.2 Test design

The treatment of the meadow represented that of a surrogate field margin adjacent to winter wheat fields since this crop constitutes the majority of farmed fields in Germany. We simulated the field management of winter wheat fields in the study area with their recommended agrochemical products and application rates. Furthermore, the pesticides are among the five most commonly used pesticides in winter wheat fields in Germany (Freier et al. 2008).

The fertilizer and pesticide rates applied on the study site were consistent with the average input rates in the first meter of a field margin directly adjacent to a field under good agricultural practices. The test design consisted of three single applications: one fertilizer, one herbicide, and one insecticide. The combination of these treatments was used to investigate the effects of interaction (in total, seven treatments and one control; Fig. 2-1, page 10) A randomized block design was chosen to take into account potential underlying environmental gradients. Each treatment was replicated eight times in plots of 8 m x 8 m with a 2-m distance to each plot (in total 64 plots). The local management system for field margins with cutting and removing the vegetation once a year in July was maintained during the experiment.

4.2.3 Agrochemical applications

For the plot applications with fertilizer, the lower input rate (25 % of the field rate) occurring in field margins (Tsiouris & Marshall 1998) was chosen. The recommended application rate for fertilizer in winter wheat fields is approximately 200 kg nitrogen (N)/ha per year, which is normally applied in two equal rates at the beginning of the vegetation period and two to four weeks later (personal communications with farmers and agricultural stores). Accordingly, fertilizer was applied at the beginning of April and approximately three weeks later (each time 25 kg N/ha = 25 % field rate) in 2010 and 2011. In keeping with personal recommendations given by farmers and agricultural stores, a granular N, phosphorus (P), and potassium (K) fertilizer (14 % N; Floral Düngemittel) was used for the first fertilizer application, and for the second application a fertilizer made of calcium carbonate and ammonium nitrate (27 % N; Raiffeisen Markt) was applied with a hand-operated fertilizer distributor (Power Spreader by Wolf Garten; MTD Products Aktiengesellschaft). The distributor had a spread range of 4 m, and the plots were treated from outside the plot boundaries. Before application, the distributor was calibrated to ensure a homogenous distribution of fertilizer granules over the plot area.

The pesticide input in field margins consist of two entryways: direct overspray and spray drift. In cereal fields, the first 0.75 m of field margins are exposed to overspray (= 50 % of the field rate), followed by spray drift with an amount of 15 % of the field rate at a distance of 0.76 m (D. Rautmann, Julius Kühn Institute, Braunschweig, Germany, personal communication) and a 2.77% drift rate at a distance of 1 m to cereal crops (Fig. 4-1A) (Ganzelmeier et al. 1995, Rautmann et al. 2001). Based on these known rates, we calculated the average input over the first meter of a field margin (see equations in Fig. 4-1B). This resulted in an application rate of 39.5 % of the field rate. In order not to overestimate the pesticide input, we decided to treat the plots with 30 % of the field rate.

As an herbicide, Atlantis WG (Bayer CropScience, sulfonylurea; recommended field rate 400 g/ha, active ingredient [a.i.] 30 g/kg mesosulfuron-methyl, 6 g/kg iodosulfuron-methyl-natrium, 90 g/kg mefenpyr-diethyl [Safener], mode of action: inhibitors of plant cell division [e.g., acetolactate synthase]) was used and applied once a year in April 2010 and 2011. At this time the vegetation was approximately 20 to 30 cm high. *Ranunculus acris* started to sprout in early spring, and therefore, its phenological stages during herbicide application were approximately one to two weeks before onset of flowering. For the insecticide application, the insecticide Karate Zeon (Syngenta, pyrethroid; field rate = 75 ml/ha, a.i. lambda-cyhalothrin 7.5 ml a.i./ha, mode of action: nonsystemic insecticide with contact and stomach action, repellent properties, gives rapid knockdown and long residual activity) was applied once a year at the end of May or at the beginning of June 2010 and 2011.

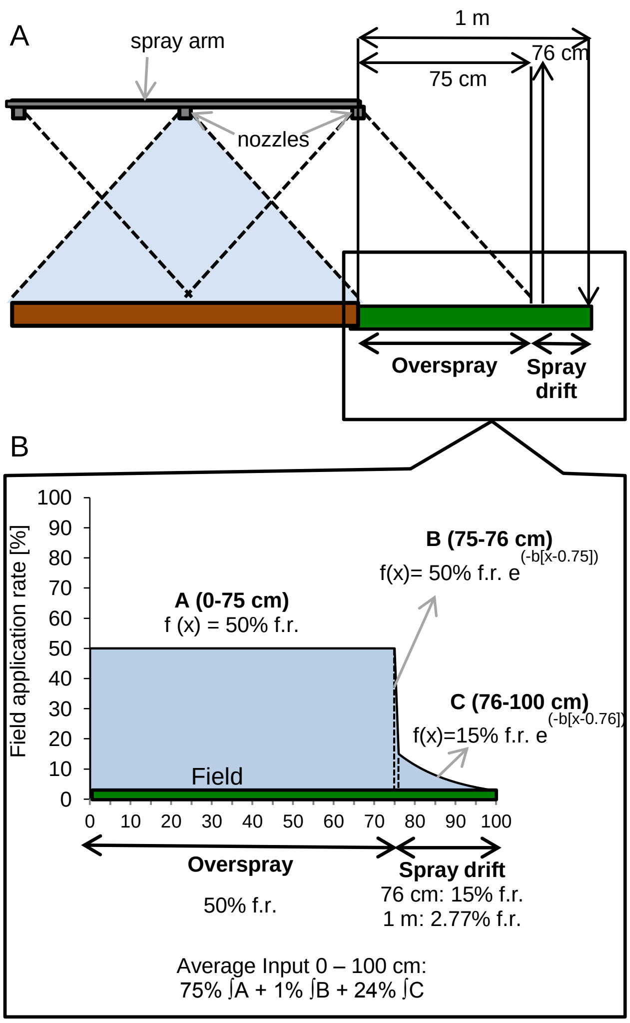

Fig. 4-1A: Schematic of pesticide inputs via overspray and spray drift in cereal field margins. The blue area illustrates the spray cone of one nozzle. **B:** Enlarged part of Figure 4-1A; detailed sketch of pesticide inputs via overspray and spray drift in a cereal field margin with equations to describe the input mathematically in different parts of the first meter of a field margin. f.r. = field rate.

Both applications were made using a purpose-built and air-assisted experimental field sprayer on wheels, which can be handled like a wheelbarrow (Schachtner Gerätetechnik). An 8-m spray boom equipped with 15 110° flat-fan TeeJet nozzels (XR 11002-VS; Schachtner Gerätetechnik) was mounted on the field sprayer. Nozzle spacing and boom height above the vegetation canopy were 50 cm. A spray volume of 400 L/ha was used in accordance with label recommendations, with an operating pressure of 4 bar. Before application, the field sprayer was calibrated to ensure a homogenous distribution and a constant delivery rate. In addition, a flow measurement on the field sprayer documented the exact application volume during the plot applications and assured that an application volume of ± 10 % was achieved. All applications were performed under good agricultural practices (wind speed < 5 m/s, temperature < 25°C, no rain 1 d before and after application). During applications, neighboring plots were covered with plastic sheets to prevent contamination due to drift.

4.2.4 Assessment of *R. acris*

To detect the effects of the agrochemical application on *R. acris*, plant community assessments were performed in mid-May and mid-June in 2010 and 2011 (four assessments in total). For this purpose, a mapping frame of 1 m^2 was placed on top of the vegetation. The frame was subdivided into 25 subsquares (20 cm x 20 cm) to record the occurrence of plant species in each subsquare. A plant species could reach a plant density (frequency) of 100 % when the species was recorded in each of the 25 subsquares. This method is very appropriate to document vegetation changes in the plots over time. However, the key advantage of the method is that several technicians can usually measure frequency with minimal training on methodology; furthermore, a uniform plant community assessment independent of the technicians is obtained (Elzinga et al. 1998). All vegetation assessments were replicated six times per plot within a defined vegetation assessment scheme along the diagonal of the plots. The results of the plant community assessments were stored in a database to calculate the plant density of each plant taxa per square meter, plot, and/or treatment.

4.2.5 Photo-documentation

In May 2010 and 2011, a photo-documentation of the flowering intensity of *R. acris* was performed. For this purpose, the 6 m^2 of the plant community assessments in each plot were photographed from the same height and angle using a frame and an Olympus digital camera (Olympus C5060 wide-zoom digital camera). For analysis of the photo-documentation, an image- analyzing program (free software, GNU Image Manipulation Program [GIMP]) was used together with object-based image analysis software (Definiens, Professional 5; Trimble Navigation). In the GIMP program, the gradation curve was modified to increase the contrast of the colors. In a second step, the photographs were analyzed with the Definiens program. At first, the segmentation of the picture and then the classification of the yellow flowers were performed to obtain the area covered with flowers per square meter. During the time of the photo-documentation, *R. acris* was the first and only yellow flowering plant species on the meadow.

4.2.6 Field monitoring

The field experiment was accompanied by monitoring of *R. acris* in field margins around the study area in May 2011. We recorded the presence or absence of *R. acris* in field margins at 10-m intervals along a stretch of 11 km. At each monitoring point, we additionally recorded the type of crop adjoining the field margin.

4.2.7 Statistical analyses

Statistical analyses were performed using Primer (Version 6) with the PERMANOVA+ add-on (Anderson et al. 2008). Analysis of variance with permutations (PerANOVA) was used since the data of the field experiment were not normally distributed. To analyze the assessments of *R. acris* and the photodocumentation, a nested PerANOVA was used to detect differences between treatments. In addition, we used the block as a random factor (mixed-effect model design). The data from the field monitoring (presence or absence data) were analyzed using a one-way PerANOVA to test the differences of *R. acris* in field margins between different crop cultures (unbalanced design).

Significant differences between treatments as well as post hoc PerANOVA pairwise comparisons were evaluated with 1,000 permutations in accordance with the recommendations for tests at an a level of 0.05. The multiple comparisons were adjusted with a Bonferroni correction. Prior to analysis, the data were transformed (log [x + 1]) to remove heteroscedasticity. Resemblance matrices were generated choosing Euclidean distance as a distance measurement in all analyses (Anderson et al. 2008). Interaction effects between the factors fertilizer, herbicide, and insecticide were assessed by a three-way PerANOVA with the above-mentioned settings.

4.3 Results

4.3.1 Assessment of *R. acris*

The plant density of *R. acris* was significantly affected by the fertilizer applications over the course of time (Fig. 4-2). In May 2010, two to three weeks after the first herbicide and fertilizer application, the plant density of *R. acris* was comparable in all treatments (approx. 80 % per plot and treatment), which points to the homogenous distribution of *R. acris* in the field study at the beginning of the experiment (Fig. 4-2A). Four weeks later, in June 2010, the density of *R. acris* was approximately 60 % in the control plots due to natural variations. However, in the fertilizer treatments (single as well as in combination with the insecticide and herbicide) the plant density was slightly affected, although these effects were not significant (Fig. 4-2B).

In 2011, after the second application season on the study site, these effects became stronger. A significant fertilizer effect (three-way PerANOVA, p = 0.04 in May and p = 0.002 in June) could be detected (Fig. 4-2C, D). All plots which had been treated with fertilizer showed a reduced density of *R. acris* in 2011. The average decrease in the density of *R. acris* was almost 20 % in May 2011, increasing to even 40 % in June 2011 in plots in which fertilizer had been applied (fertilizer [F], F+ insecticide [I], F+ herbicide [H], F+H+I; mean plant density in May = 68 % ± 2 standard errors [SE], mean plant density in June = 23 % ± 2 SE) in comparison to control plots (mean plant density in May = 82 % ± 2 SE, mean plant density in June = 38 % ± 3 SE) (Fig. 4-2C, D).

By contrast, herbicide applications had no significant effect on the density of *R. acris* plants. In May and June 2010 as well as in May and June 2011, the density of *R. acris* plants was similar in the herbicide and control plots (Fig 4-2). A significant interaction effect between the treatments was not detected. The insecticide did not show any effects on *R. acris*.

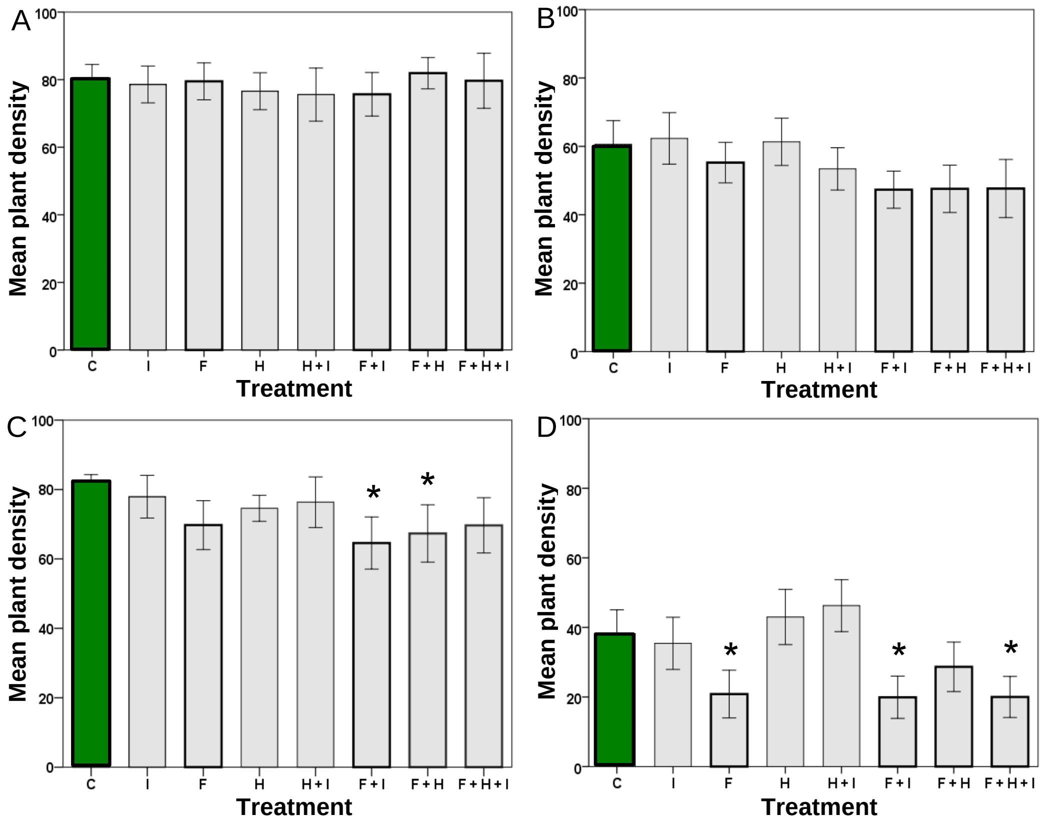

Fig. 4-2: Mean (± standard error) plant density of *R. acris* in May 2010 (A), June 2010 (B), May 2011 (C), and June 2011 (D) per plot and treatment; n per treatment = 48. Plots treated with fertilizer are highlighted with a frame, and control plots are marked in green. * Significantly different from the control, $p < 0.05$ (Nested analysis of variance with permutations); p values Bonferroni-corrected. C = control; I = insecticide; F = fertilizer; H = herbicide.

4.3.2 Photo-documentation

Ranunculus acris showed sublethal effects after the herbicide applications. Flower intensity was significantly reduced in all herbicide-treated plots two weeks after the first herbicide application in 2010 (Fig. 4-3). In 2011, the effects were similar (Fig. 4-3B). The reduction in flower intensity by the herbicide application was 85% in plots that had been treated with herbicide (H, H+I, F+H, F+H+I; mean flower intensity 3 ‰ ± 0.2 SE) in comparison to control plots (mean flower intensity 20 ‰ ± 2 SE). Flower intensity in the treatment combination of F+H and F+H+I in 2011 (mean flower intensity 2.5 ‰ ± 0.2 SE) was also lower than in the H and H+I treatment (mean flower intensity 4 ‰ ± 0.3 SE; Fig. 4-3B).

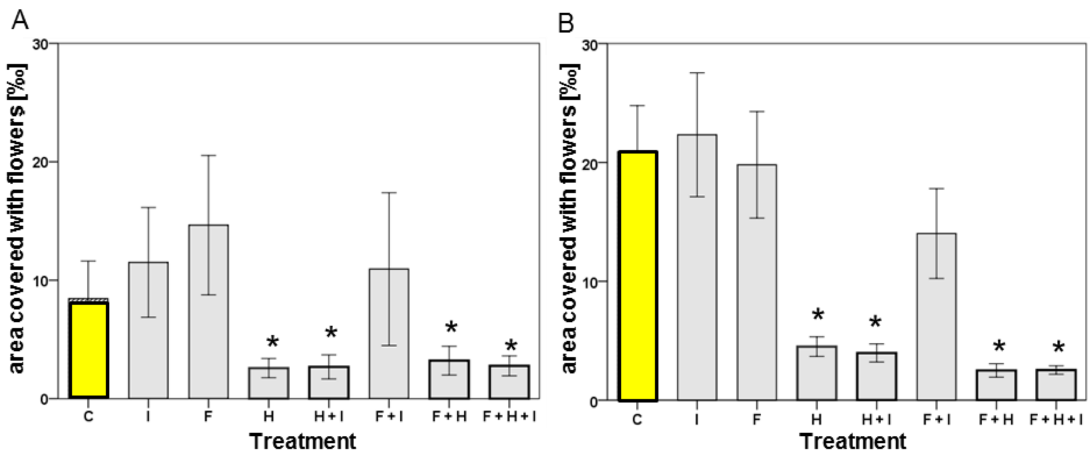

Fig. 4-3: Mean (± standard error) area covered with flowers of *R. acris* in May 2010 (A) and May 2011 (B) per plot and treatment; n per treatment = 48. Plots treated with herbicides are highlighted with a frame, and control plots are marked in yellow. * Significantly different from the control, $p < 0.05$ (Nested analysis of variance with permutations), p values Bonferroni-corrected. C= control; I = insecticide; F = fertilizer; H = herbicide.

4.3.3 Field monitoring

In total, 1,130 monitoring points were recorded in field margins; 844 data points (75 %) were located in field margins next to cereal fields, whereas the other data points were recorded adjacent to vineyards, hedges, orchards, or extensively managed meadows (Table 4-1). In total, *R. acris* was recorded 76 times, though in negligibly small proportions in field margins adjoining cereal crops (2 %) and vineyards (7 %). Adjacent to meadows, *R. acris* was found frequently (85 %), followed by field margins next to orchards (30 %) and hedges (29 %) (Table 4-1). The presence of *R. acris* in field margins adjacent to cereal crops differed significantly from all other field margins. Only field margins located next to hedges and orchards did not differ significantly from each other.

Table 4-1: Monitoring points (m.p.) and the occurrence of *Ranunculus acris* in field margins adjacent to different cropped areas or hedges.

neighboring crop /structure	**m.p.**	**m. p. with *R. acris***		**Significance**
		n	**[%]**	
cereal	844	16	2	A
vine	172	12	7	B
Orchard	46	14	30	C
Hedge	42	12	29	C
Meadow	26	22	85	D
Overall	**1130**	**76**		

[a] Different letters indicate significant differences (analysis of variance with permutations, $p < 0.05$) between the occurrences of *R. acris* in different field margins.

4.4 Discussion

It is often supposed that the application of pesticides and the use of fertilizer are two major drivers of biodiversity loss in the agricultural landscape (Firbank et al. 2008). The main objective of the present study was to investigate the impacts of pesticide and fertilizer inputs in field margins on the common buttercup *R. acris* and to separate the two stressors. Therefore, the input (overspray and drift) of agrochemicals in field margins was simulated with an experimental field sprayer. With this method the plots (plants) were directly sprayed. Drift differs from direct spray (overspray): drift consists of smaller droplets with possibly higher concentrations of the compound but has less power to penetrate the vegetation than direct spraying (De Snoo et al. 2005). Former studies have also shown that the responses of plants directly sprayed with low herbicide rates may be different from those of plants exposed to spray drift under natural conditions (Koch et al. 2004). However, since 0.75 m of the first meter of a field margin receive overspray in an arable application scenario; the application method described in the present study seems to be appropriate for assessing realistic effects of agrochemical inputs on plant species in the first meter of a field margin.

Generally, *R. acris* starts to sprout in early spring. Therefore, in May 2010 (the first experimental season), the plant density of *R. acris* reached approximately 80 % per plot and treatment (Fig. 4-2A). At this particular time *R. acris* represents one of the tallest flowering plant species on the meadow. In June, the vegetation is generally higher than in May due to weather conditions (higher temperatures) and the associated increase in growth of the whole plant community. Hence, there is a higher proportion of sprouted plants/total biomass on the meadow and, thus, the density of *R. acris* was reduced to 60 % in June 2010. However, it is striking that the density of *R. acris* showed a larger reduction from May to June (in the control plots) in the second year of the experiment (June 2011) in comparison to the first year (June 2010; Fig. 4-2B and D). This might be explained by the dry weather conditions between May and June 2011 (average precipitation, 102 mm in May 2010 and 25 mm in May 2011) since *R. acris* prefers humid habitats.

Besides these natural variations in the plant density of *R. acris* in May and June, the results of the present study demonstrated negative effects of fertilizer on *R. acris* in the second experimental season (Fig. 4-2C, D). The fertilizer treatment (single as well as in combination with the herbicide and the insecticide) caused an average decrease in the density of *R. acris* in May and June 2011. Fertilizer application increases the availability of N, P, and other plant nutrients and, thus, leads to increased overall productivity and favoring of some plant species (Hautier et al. 2009). As a result, plant diversity is usually reduced due to the increase of only a few plant species with a high nutrient uptake and rapid growth, typically grasses (e.g., *Dactylis glomerata*), which have the advantage of fast spreading due to the new habitat conditions. Plants with a relatively small stature like *R. acris* respond negatively because they can be overtopped by the taller and faster-growing plants (Jumpponen et al. 2005). A further and major mechanism of decreasing plant diversity is the increasing competition for light after eutrophication (Hautier et al. 2009), which supresses the density of *R. acris* even further.

The effects of fertilization were not significant in the first experimental season but became obvious in the second year. This is hardly surprising since eutrophication with low fertilizer concentrations as used in the present study is a slow process (Hejcman et al. 2007) and only long-term studies can correctly assess fertilizer effects on plant communities. Nevertheless, the results of the first two experimental seasons indicate that *R. acris* will probably decrease in field margins with recurrent fertilizer inputs.

Regarding plant density after two years of experimental input, the herbicide treatment seems to have no effect on *R. acris*. Since *R. acris* was one of the tallest species on the meadow during herbicide application, a shielding effect from herbicide exposure was not given. However, Atlantis WG is an herbicide that is used to control mainly grasses and a few annual herbs; *R. acris* is a perennial plant species and, therefore, not one of the target species of this herbicide. Nevertheless, sublethal effects caused by the herbicide were noticeable. In comparison to control plots, the flower intensity of *R. acris* was reduced by 85 % in plots treated with the herbicide (Fig. 4-3B). As a consequence, it is likely that the seed production of *R. acris* was affected. Herbicides, especially sulfonylureas, are known to be very effective at reducing seed set when sprayed at the onset of reproduction, for example, at flower bud (Boutin et al. 2000; Fletcher et al. 1996). This suggests that *R. acris* might decline over time in the herbicide-treated plots due to a reduction of the seed bank. And this, in turn, leads to shifts in the plant community. The loss of seeds in soil varies between plant species, but in general, the population of buried seeds decreases exponentially at a rate of 20 to 40 % per year, resulting in a very small seed population after 10 years (Sarukhan 1974). It is sometimes also mentioned that *R. acris* has a vegetative form of reproduction; however, this is limited to the occasional branching of its short rhizomes (Sarukhan 1974) and thus, is not sufficient for the existence of *R. acris* in plant communities.

Therefore, both agrochemicals (herbicide and fertilizer) contribute to the decrease of *R. acris* in the agricultural landscape, although in different ways. The fertilizer results in a relatively immediate (within two years) measurable decrease of plant density, and the herbicide caused sublethal effects, which will probably need more time to be reflected in plant density since seed production may be reduced.

A significant interaction effect of the herbicide and fertilizer treatment on *R. acris* could not be detected during the two years of the field study. However, the treatment combination of F+H as well as the F+H+I treatment in 2011 showed reduced flower intensity (reduction of 37 %) in comparison with the H and H+I treatment (Fig. 4-3B). Therefore, it seems likely that the sublethal effects caused by the herbicide and the reduced density of *R. acris* caused by shifts in the plant community due to fertilizer applications are additive in the long run.

The results of the field experiment are supported by the monitoring survey. *Ranunculus acris* was rarely observed in field margins directly adjacent to cereal fields where fertilizer and herbicides were used. In vineyards and orchards, herbicides and fertilizer are also used. However, in these crops herbicides and fertilizer are applied directly at the stem base, and therefore misplacements in field margins are rather limited. This management difference might explain why in field margins next to orchards *R. acris* was recorded more frequently (30 %, Table 4-1). However, in field margins next to vineyards, *R. acris* was observed in only 7 % of the monitoring points. This might have been caused by the high mechanical disturbances in this crop such as mowing of margins and driving across with tractors several times a year. These management practices have a negative effect on *R. acris*, too. Frequent vegetation cuts reduce the flowering of *R. acris* and, thus, its occurrence (Lamoureaux & Bourdot 2007). Field margins next to hedges are normally not exposed to agrochemicals, and as a result, *R. acris* can occur in these field margins at a similar percentage (29 %, Table 4-1) as in field margins next to orchards (30 %, Table 4-1). In field margins next to meadows, the occurrence of *R. acris* was relatively high (*R. acris* was detected in 85 % of the monitoring points). This can be explained by the fact that *R. acris* is a typical and frequently found species in extensively managed meadows and, therefore, *R. acris* can also spread to the field margins. Herbicides are generally not applied on such meadows, though it cannot be excluded that they are fertilized. However, meadows are fertilized much less frequently than cultivated fields and with lower application rates of approximately 60 to 90 kg N/ha per year (BMLFUW 2006). That means that fertilizer input in field margins next to meadows is less than half of the fertilizer input in, for example, cereal field margins. Hence, it seems that *R. acris* can occur in field margins with small amounts of fertilizer input, if the input is not frequent and under the prerequisite that there is no herbicide input causing sublethal effects (e.g., reduced flowering intensity).

Regarding our results it seems problematic that there are no regulations for fertilizer applications next to field margins. Some sanctions, including buffer zone distances to field margins, would be necessary to protect the vegetation of field margins from fertilizer inputs since fertilizer misplacements in field margins affect plant composition and might interact with herbicide effects.

Another area of concern lies in the current testing scheme of herbicides for non-target plants. In this testing scheme, the sublethal effects of herbicides are not considered at all. In standard tests, plant species are exposed as seedlings based on the assumption that this stage is the most sensitive (Breeze et al. 1992). However, the results of our study showed that *R. acris* is very sensitive, particularly in the budding stages just before flowering. This results in negative impacts on reproduction and potentially the population development of *R. acris*. For most herbicides, the impact on the reproductive stage of wild plants is not known, although the vegetation of field margins usually consists of annual and perennial plant species in different developmental stages at the time of field application.

Furthermore, with the standard test methods (OECD 2006) interaction effects (e.g., competition) between plant species that can also be altered by exposure to herbicides are not taken into account. Field or microcosm studies are expensive and time-consuming. However, without considering and predicting the interaction effects, it is not possible to understand the effects of herbicides on plant communities.

Plants in field margins are also exposed to repeated pesticide applications with alternating pesticides during the growing season every year. This might lead to additive or synergistic effects, which are difficult to study because long-term studies are generally not conducted.

In addition to these implications for the plant community, the sublethal effects of herbicides such as suppressed flower intensity (*R. acris*, 85% reduction) may also affect flower-visiting insects due to a reduced density of pollen plants. This food source decrease might be especially severe for specialist pollinators such as the solitary bee *Chelostoma florisomnis*, which depends entirely on *Ranunculus* pollen. However, the pollen of *R. acris* is consumed by many insects, and Weiner et al. (2011) recorded recently a total of 117 flower-visiting insects on this plant species alone. Hence, if the current risk-assessment scheme is to be tailored to preserve biodiversity (European Commission 2009), it is crucial to take account of sublethal effects in plants and their consequences for pollinators and herbivores.

4.5 Conclusion

The present study revealed that the misplacement of herbicides and fertilizer in field margins causes negative effects on *R. acris*. While fertilizer input in field margins increases the availability of nutrients and promotes plants with a high nutrient uptake, and thus decreases the density of *R. acris*, the herbicide input in field margins produces sublethal effects (reduced flowering intensity). So far, these sublethal effects are not taken into account in risk-assessment procedures for pesticides. Over time, sublethal effects are expected to cause the disappearance of *R. acris* in agricultural field margins. In addition, plants in field margins are exposed to repeated agrochemical inputs during a growing season over several years, and these application sequences can be additive or synergistic. This is also the reason why the fertilizer effects in the present study were stronger in the second experimental season. Moreover, the sublethal effects of the herbicide applications can also cause follow-up effects, for example, on flower-visiting insects and, thus, affect biodiversity in agricultural landscapes.

To improve the current risk-assessment scheme of agrochemical inputs in field margins, we recommend investigating sublethal effects and long-term effects in future research targeted at wild plant species. Here, it is particularly important to study effects on plants at other phenological stages than the seedling stage.

5 Agrochemicals in field margins – Field evaluation of plant reproduction effects

Paper III

This chapter presents the author`s final version of the article:

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Abstract - Field margins are important habitats for various plant species in agro-ecosystems but they can also be exposed to agrochemicals. In this experimental field study, effects of herbicide, insecticide, and fertilizer misplacements in field margins on the plant frequency and reproductive capacity of four wild plant species (*Ranunculus acris*, *Lathyrus pratensis*, *Vicia sepium*, *Rumex acetosa*) were investigated from 2010 to 2012. Individual and combined effects of the agrochemicals were studied in a randomized block design and plant community assessments were performed every year. Additionally, seeds of the four species were harvested in 2012 to detect effects on plant reproduction. Plant frequencies of the four species were significantly reduced in all herbicide and fertilizer treatments in the third year. The plant frequency of *R. acris* and *L. pratensis* was more affected in the fertilizer treatments than in the herbicide treatments, whereas the plant frequency of *V. sepium* and *R. acetosa* was similarly affected by fertilizer and herbicide treatments. However, the treatment combinations of fertilizer and herbicide resulted in additive effects on the plant frequency of *V. sepium* and *R. acetosa*. Furthermore, herbicide treatments suppressed the formation of flowers and, hence, led to a significantly reduced seed production of *R. acris*, *L. pratensis*, and *V. sepium*. Because field margins are exposed to repeated agrochemical applications over several years, the observed effects will possibly lead to shifts in plant community compositions and will cause the disappearance of the affected plants in the long run. In the current risk assessment of herbicides for nontarget plants no reproduction effects are considered, and therefore, it seems that herbicide effects on wild plants species are potentially underestimated.

Keywords - Non-target plants; Off-crop habitats; Herbicide; Fertilizer; Reproduction; Seed production

5.1 Introduction

In recent years, the interest in seminatural habitats in intensively farmed agricultural landscapes has increased considerably since these structures are the last remaining habitats for wildlife in farmlands (Marshall & Moonen 2002; Boutin et al. 2012). In Europe, the majority of seminatural habitats in agricultural landscapes are field margins (Marshall & Moonen 2002). In the present paper, the term field margin is defined as linear, permanent vegetation strips of mostly grassy and herbaceous off-crop habitats that are directly adjacent to agricultural fields (Kühne & Freier 2001). Generally, these habitats are only a few meters wide and are mown periodically. Field margins are ecologically important because they enhance plant diversity within farmlands (Kleijn & Verbeek 2000; Nentwig, 2000; Asteraki et al. 2004). Plants are the primary producers and form the basis of any food web in an ecosystem, and thus, high plant diversity in field margins is also essential to many farmland animals (Wilson et al. 1999; Kleijn & Verbeek 2000). For example, a multitude of herbivorous insects (e.g. grasshoppers, caterpillars, cicadas, etc.) consume various parts of plants and these insects represent the food of other predatory arthropods such as spiders, parasitoid flies, and wasps. Furthermore, not only the green leaves are eaten by herbivorous insects, also wildflowers in field margins offer important sources of nectar and pollen for butterflies, wasps, bumblebees, and solitary bees (Bäckman & Tiainen 2002; Carreck & Williams 2002; Holzschuh et al. 2009), which support and provide ecosystem services in agricultural landscapes (Pywell et al. 2004; Power 2010). These arthropods are essential food for other animals such as insectivorous birds (Wilson et al. 1999). In general, it is rather difficult to estimate how many arthropods are associated with one plant species. For instance, an extrapolation indicated that per plant species 100 – 300 arthropod species can be expected (Nentwig 2000 and references therein). Additionally, diverse vegetation structure in field margins provides important nesting habitats for arthropods (Roß-Nickoll et al. 2004) and other species, including small mammals and birds (Tew 1994; Vickery et al. 2009).

Large-scale monitoring studies detected reductions in plant diversity in field margins during the last decades (Bunce et al. 1994; Kleijn & Verbeek 2000; Roß-Nickoll et al. 2004). Factors contributing to reductions in plant diversity include mechanization, close ploughing, and fertilizer misplacements. Herbicides are also causing declines in plant diversity in field margins (Andreasen & Streibig 2011; Kleijn & Snoeijing 1997; Marrs & Frost 1997; De Snoo 1999). In particular, the vegetation of narrow field margins can strongly be affected by herbicides because these elements receive herbicide inputs via overspray and spray drift. This is due to the fact that the application of an arable land is conducted right up to the border of the field and consequently, parts of the adjacent field margin are directly oversprayed and exposed to spray drift (see Schmitz et al. (2013) for details of overspraying and spray drift in field margins).

Herbicides are often labeled with product-specific risk mitigation measures (e.g. in-field buffer zone distances to terrestrial non-target areas) to reduce herbicide inputs in terrestrial non-target areas (BVL, 2013b). These regulations, however, are often softened by exceptions. For example, field margins less than 3 m wide are not considered as terrestrial non-target areas in Germany and thus, these field margins are exempt from such regulations (Kühne et al. 2000; BVL 2013b). The problem is increased because the typical field margin is only 1–2 m wide and consequently, a large extent of field margins (0–3 m wide margins) are not protected from pesticide inputs by risk mitigation measures in Germany.

Before a pesticide can be used on the market, it undergoes a risk assessment scheme mandatory for registration. Risk assessment of herbicides was implemented to protect non-target plants in off-crop habitats, such as field margins (E.U. Directive 1107/2009; European Commission 2002). Currently, this risk assessment is based on phytotoxicity tests with single and annual plant species (mainly crop plants) in young development stages (two- to four- leaf stage). According to the OECD guideline, test durations are usually 21–28 days, and the most commonly used effect end-points are mortality and effects on plant biomass (OECD 2006). Because only young plants are used, reproduction effects cannot be detected. However, herbicides can also affect the reproductive capacity of wild plant species (Riemens et al. 2008; Carpenter & Boutin 2010; Strandberg et al. 2012). Reproductive endpoints, such as flowering and seed production, can be highly sensitive (Kjaer et al. 2006a, 2006b; Strandberg et al. 2012). To date, effects on flowering and seed production are rarely investigated, although the latter, as well as the germination rate of seeds, can be crucial for the persistence of many species. Some plants have also the possibility to reproduce asexually (e.g., individuals produced from stolons or rhizomes). However, this form of reproduction is associated with problems because it yields only little new genetic variation in the next generation (Travers et al. 2011). In contrast, sexual reproduction increases genetic variation in offspring, which is beneficial for new trait diversity, adaptability, and resilience of populations (Travers et al. 2011). Therefore, the ability of plants to reproduce by seeds can be highly relevant for the long-term survival of a plant species (Travers et al. 2011) and, thus, an investigation of herbicide effects on the reproductive capacity seems to be crucial.

Plants in field margins are not only exposed to herbicides, but also to fertilizers and insecticides, which can cause further stress to plants. In conventional agriculture, farmers apply these agrochemicals every year and thus, the vegetation of field margins is exposed to agrochemical inputs over several growing seasons. These repeated exposures might cause cumulative effects, as well as interaction effects between e.g., herbicides and fertilizers.

To investigate individual and combined effects of pesticide (herbicide and insecticide) and fertilizer inputs on the plant community of field margins, a field experiment was established in 2010 (Schmitz et al. 2013). This field experiment was a 3-year project and first results after two years of the experiment (2010 and 2011) on the flower intensity of one selected plant species (*Ranunculus acris*) already have been published (Schmitz et al. 2013).

The present paper describes the successional changes of four plant species (*Ranunculus acris*, *Lathyrus pratensis*, *Vicia sepium*, and *Rumex acetosa*) during the experiment. Furthermore, seed production and germination rates of the four species were assessed after the third year to detect effects of agrochemical applications on their reproductive capacity.

5.2 Materials and methods

5.2.1 Experimental design

A field experiment on a low productive meadow (1 hectare) was established in spring 2010 (Schmitz et al. 2013). The experiment was located near Landau (South Rhineland Palatinate, Germany) and was designed to study individual and combined effects of repeated agrochemical applications on a surrogate field margin in successive growing seasons (2010, 2011 and 2012). We used a randomized block design with seven treatments and one control. Each treatment and control was replicated eight times in 8 m × 8 m (64 m^2) plots with 2 m distance between each plot (in total 64 plots). Treatments included three single applications (one fertilizer (F), one herbicide (H) and one insecticide (I)), as well as all combinations of these treatments (F+I, H+I, F+H, F+H+I). Detailed information on the experimental study site, and test design, can be found in Schmitz et al. (2013).

5.2.2 Agrochemical applications

Applications of the agrochemicals and their application sequences imitated the field management of winter wheat fields in the study area. Fertilizer and pesticide rates used for the plot applications were equal to the average input rates of pesticides and fertilizers in the first meter of a field margin directly adjacent to a winter wheat field (Schmitz et al. 2013).

During fertilizer applications on a cereal field, there is generally an input rate of 25% of the field rate in the first meter of a field margin (Tsiouris & Marshall 1998). The recommended application rate for fertilizer in cereal fields is 200 kg nitrogen (N)/ha (field rate per year), which is usually applied in two equal rates (100 kg N/ha) at the beginning of the vegetation period and 2–4 weeks later (personal communications with farmers and agrochemical suppliers). We applied a granular nitrogen (N), phosphorus (P), and potassium (K) fertilizer (14% N; Floral Düngemittel) at the beginning of April in 2010, 2011, and 2012. Approximately three weeks later (each year), a calcium carbonate and ammonium nitrate fertilizer (27% N; Raiffeisen Markt) was applied. Each time 25 kg N/ha (=25% of the field rate) was used. Fertilizer was applied with a hand-operated fertilizer distributor (Power Spreader by Wolf Garten; MTD Products Aktiengesellschaft).

During pesticide applications, the input rate in the first meter of a field margin is 30% of the field rate (direct overspray and spray drift) (see Fig. 2 in Schmitz et al. (2013) for detailed information). We used the herbicide Atlantis WG (sulfonylurea; recommended field rate 400 g/ha, active ingredients [a.i.] 30 g/kg mesosulfuron-methyl, 6 g/kg iodosulfuron-methylnatrium, 90 g/kg mefenpyr-diethyl [Safener], mode of action: inhibitors of plant cell division, Bayer CropScience) and the insecticide Karate Zeon (pyrethroid; field rate 75 ml/ha, a.i. lambda-cyhalothrin 7.5 mla.i./ha, mode of action: nonsystemic insecticide with contact and stomach action, repellent properties, Syngenta). Each was applied once a year in April (herbicide) and at the end of May or the beginning of June (insecticide). Application rates were 30 % of the field rate for both, the herbicide (120 g Atlantis WG/ha) and the insecticide (22.5 ml Karate Zeon/ha). Applications were performed with a purpose-built and air-assisted experimental field sprayer on wheels, which can be handled like a wheelbarrow (Schachtner Gerätetechnik). The field sprayer was equipped with an 8-m spray boom with 15 flat-fan TeeJet nozzles (XR 11002-VS; Schachtner Gerätetechnik). The distance between the nozzles and the boom height above the vegetation canopy was 50 cm. Following label recommendations for field applications, a spray volume of 400 L/ha was used.

5.2.3 Selected species

Four plant species commonly found in the experimental study site were selected for the present study: *R. acris*, *L. pratensis*, *V. sepium*, and *R. acetosa* (Table 5-1). These species are widely distributed throughout Europe and are common plant species in agricultural landscapes. All species are perennials with flowering times between May and September (Table 5-1).

Table 5-1: Characteristics of the four study species (Information from Klotz et al. 2002).

Species	Common name	Family	Main habitats	Flowering time	Reproduction
Ranunculus acris	Common buttercup	Ranunculaceae	Meadows, pastures, wayside strips	May - September	Seeds
Lathyrus pratensis	Meadow vetchling	Fabaceae	Meadows, pastures, wayside strips, edge of forests	June - August	Mostly by seeds, rarely vegetatively
Vicia sepium	Bush vetch	Fabaceae	Meadows, pastures, wayside strips, forests	May - July	Mostly by seeds, rarely vegetatively
Rumex acetosa	Common sorrel	Polygonaceae	Meadows, pastures, shores	May - July	Seeds and vegetatively

5.2.4 Assessment of *R. acris*, *L. pratensis*, *V. Sepium*, and *R. acetosa*

Plant community assessments were performed using the frequency method with a mapping frame. This method is sensitive to detect changes in plant communities over time (Elzinga et al. 1998). The mapping frame was 1 m^2 and was subdivided into 25 subsquares (each 20 cm × 20 cm) (Fig. 5-1A).We placed the frame on top of the vegetation, and recorded the occurrence (presence) of each plant species in each subsquare. Plant community assessments were conducted six times per plot (Fig. 5-1B). All community assessments (6 vegetation assessments per plot × 64 plots) were completed within one week in mid-June every year (2010, 2011, and 2012). The plant frequency was calculated for each species per square meter, plot, and treatment. A plant frequency of 100% could be reached by a species, if the species was recorded in each of the 25 subsquares per mapping frame (1 m^2) or rather 150 times per plot (25 subsquares × 6 assessments per plot).

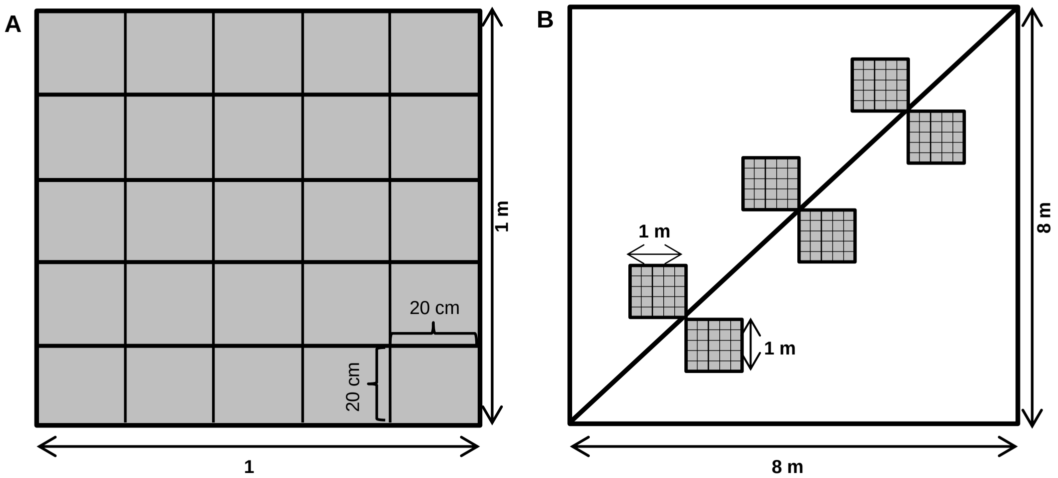

Fig. 5-1: (A) Schematic of the 1m x1m mapping frame. The frame was subdivided into 25 subsquares (each 20 cm x 20 cm). (B) Schematic of the plant community assessment per plot. Plot size 8 m x 8 m (= 64 m^2). Vegetation assessments were conducted six times per plot along the diagonal of the plots with the mapping frame described in (A).

5.2.5 Assessment of reproduction effects

5.2.5.1 Seed collection

We assessed the effects of the agrochemical applications on the reproductive capacity of the four selected species in June and July 2012. For this purpose, fruits of the species were harvested at maturity. For *R. acris*, *L. pratensis*, and *V. sepium*, one fruit (or pod) per plant was collected (Fig. 5-2). For *R. acetosa*, one fruit stalk of 10 cm per plant was collected because one fruit (= nut) of this species is small and comprises only one seed. For each species, the fruit collection was conducted six times per plot (= 1 fruit from 6 different plants per species = 6 fruits per species and plot). Thus, our target was to collect 48 fruits per species and treatment (6 fruits per species and plot × 8 replicates (plots) per treatment).

The collected fruits were stored in a dry place over several weeks, and then the seeds were counted and weighted. We assessed three different reproduction parameters per treatment: the number of fruits per species, the mean number of seeds per fruit, and the mean weight of one seed. To determine the weight of one seed, all seeds of one fruit were weighted together and afterwards the mean 1-seed weight was calculated.

Fig. 5-2: Fruits of *Ranunculus acris*, L*athyrus pratensis*, *Vicia sepium*, and *Rumex acetosa*.

5.2.5.2 Germination tests

Germination tests were performed with the seeds collected from the field. We used 20 seeds per plot (160 seeds per treatment) for *R. acris*, *L. pratensis*, and *R. acetosa*. Only five seeds per plot (40 seeds per treatment) could be used for *V. sepium* because fruits (pods) of this species comprised a lower number of seeds (approximately 3 or 4 seeds per fruit) than the other species. Additionally, during the drying process some seeds of *V. sepium* were lost due to larval herbivores, which hatched in the fruits and fed on the seeds.

Seeds of some wild plant species need a pretreatment (e.g. stratification) before they can germinate (Finch-Savage & Leubner-Metzger 2006). Therefore, we used gibberellic acid (GA3). This plant hormone is used in laboratory or greenhouse tests to trigger germination in seeds, which would otherwise remain dormant (personal communication with a laboratory assistant of Appels Wilde Samen GmbH, Darmstadt, Germany). Therefore, the seeds of *R. acris*, *L. pratensis*, and *V. sepium* were submerged in a 0.1 % GA3 solution and were stored in a refrigerator (4°C) for 24 h. Afterwards, the seeds were rinsed carefully with tap water. Seeds of *R. acetosa* were not pretreated because seeds are non-dormant (Van Assche et al. 2002).

Our germination test system was a container with a diameter of 11.5 cm and a height of 3 cm (one per replicate (= plot)). The bottoms of the containers were covered with cotton wool followed by a layer of filter paper. Both layers were moistened with water and then the seeds were surface sown. The containers were covered with a plastic wrap to prevent evaporation. They were top watered as required to ensure that neither the seeds nor the layers dried out, and monitored until the germination stagnated. Germination was recorded every week. The germination test was performed in a climatic chamber with 20°C and a day/night rhythm of 12 h. Upon completion of the germination tests, the average germination rate for each species in each of the treatments was determined.

5.2.6 Statistical analyses

Data from the field experiment were not normally distributed, and therefore, an analysis of variance with permutations was performed. For all statistical analyses, the statistic program Primer (Version 6) with the Permanova+ add-on was used (Anderson et al. 2008). We used a nested permutational univariate analysis of variance (nested PerAnova) to detect differences in the plant frequency of each species between treatments. Euclidean distance was used to generate resemblance matrices. As a PerAnova design, a mixed effect model with the block as the random factor and the treatment as the fixed factor was chosen. The six vegetation assessments per plot were nested within the factor treatment. The tests were followed by post-hoc PerAnova pairwise comparisons, which were adjusted with a Bonferroni correction. Significant differences were evaluated with 9999 permutations as recommended for tests at an α-level of 0.001 (Anderson et al. 2008).

The seed data were analyzed with a nested PerAnova (balanced design: number of fruits per treatment; unbalanced design: seeds per fruit and 1-seed weight per treatment) and germination was tested using a PerAnova (unbalanced design). Both analyses were performed as above (Euclidean distance, 9999 permutations).

5.3 Results

5.3.1 Assessment of *R. acris*, *L. pratensis*, *V. sepium*, and *R. acetosa*

Mean plant frequency is shown respectively for the four different species and treatments in June 2010, 2011, and 2012 in Fig. 5-3. The reactions of the species to the treatments were slightly different in the first experimental season (Fig. 5-3A, D, G, J). *L. pratensis* and *V. sepium* already showed some significant treatment effects in the first season, but *R. acris* und *R. acetosa* were first affected by the treatments in the second year. However, common to all species was that effects of fertilizer and herbicide applications became stronger over time, and the insecticide caused no effects on the plant frequencies. In the third experimental season, the fertilizer and herbicide applications reduced the plant frequencies of all four species significantly (Fig. 5-3C, F, I, L).
Two types of responses (1 and 2) to the treatments could be recognized after three years:

1. The plant frequencies of *R. acris* and *L. pratensis* were more strongly affected by the fertilizer treatment than by the herbicide treatment, although both treatments reduced the plant frequencies significantly. In June 2012, the average decrease in the frequency of *R. acris* was 22 % in the herbicide treated plots (H: mean plant frequency 45 % ± 8 standard error [SE]) and 47 % in the fertilizer treated plots (F: mean plant frequency 31 % ± 7 SE) compared with the control plots (C: mean plant frequency = 58 % ± 4 SE) (Fig. 5-3C). The effects for *L. pratensis* were similar, but with a much stronger reduction (nearly twice as large) of the plant frequency in all treatments. In June 2012, the fertilizer caused an average decrease of 70 % (F: mean plant frequency 16 % ± 5 SE) in the frequency of *L. pratensis*, and the herbicide caused an average decrease in the frequency of almost 50 % (H: mean plant frequency 28 % ± 6 SE) compared with the control plots (C: mean plant frequency = 53 % ± 8 SE (June 2012) (Fig. 5-3F).

2. The plant frequencies of *V. sepium* and *R. acetosa* were similarly affected by the herbicide and fertilizer treatment. In June 2012, the fertilizer or herbicide treatment caused an average decrease in the frequency of *V. sepium* of approximately 45 % (F: mean plant frequency 28 % ± 6 SE; H: mean plant frequency 26 % ± 6 SE) compared with the control plots (C: mean plant frequency = 50 % ± 3 SE) (Fig. 5-3I), and the frequency of *R. acetosa* was reduced by approximately 40 % in the fertilizer or herbicide treated plots (F: mean plant frequency 23 % ± 6 SE; H: mean plant frequency 22 % ± 4 SE) compared with the control plots (C: mean plant frequency = 38 % ± 5 SE) (Fig. 5-3L). However, effects of the herbicide and fertilizer treatments appeared to be additive for these two species because the plant frequencies were further reduced by approximately 50 % (and more) in the plots treated with fertilizer and herbicide in combination (F+H and F+H+I) compared with the individual treatments (F and H).

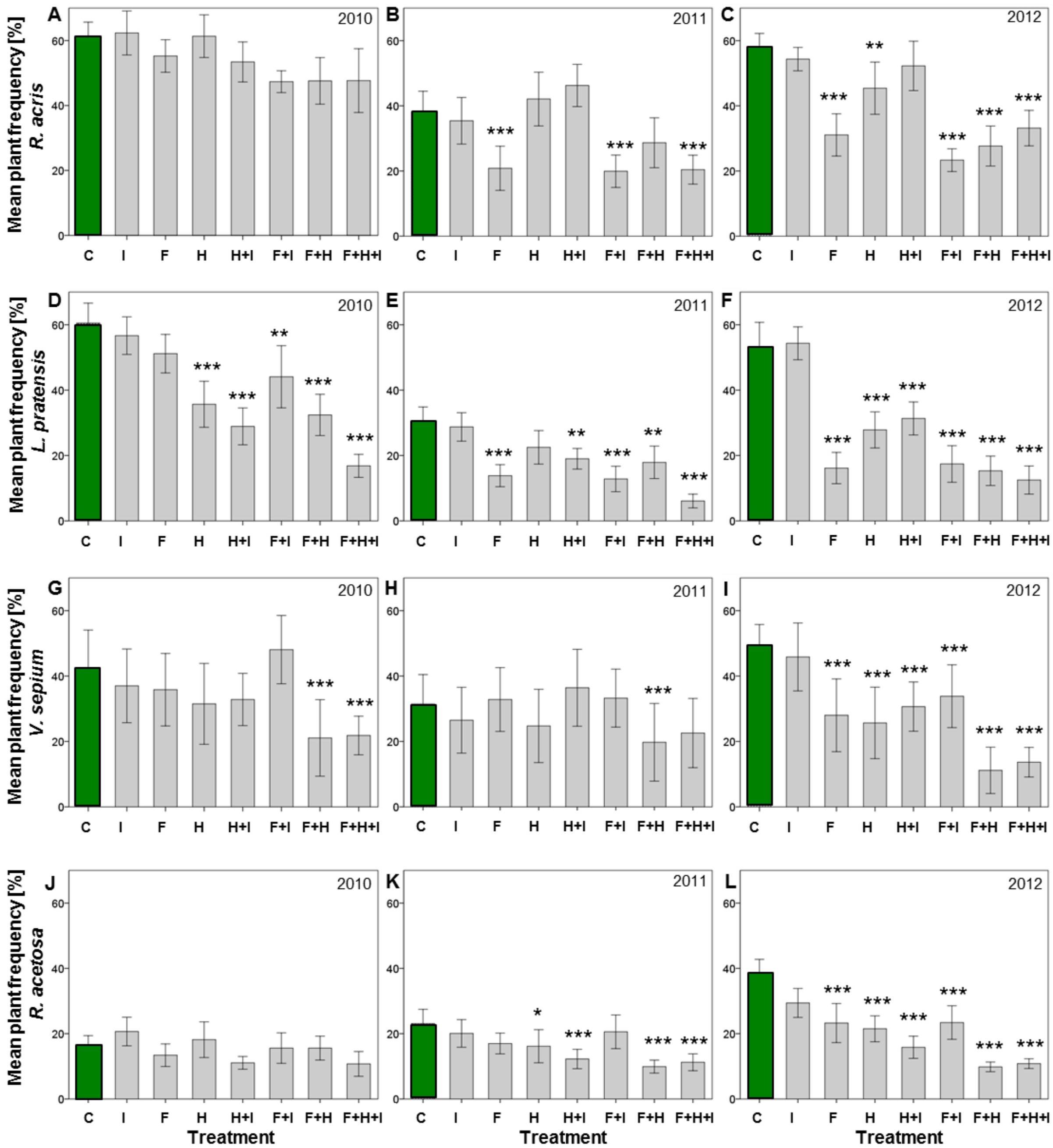

Fig. 5-3: Mean (±standard error) plant frequency of *Ranunculus acris* (A–C), *Lathyrus pratensis* (D–F), *Vicia sepium* (G–I), and *Rumex acetosa* (J–L) per plot and treatment in June 2010, 2011, and 2012; replicates per treatment = 8. Asterisks indicate significant differences from the control, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Nested analysis of variance with permutations [nested PerAnova]); p values Bonferroni corrected. C = control (highlighted in green), I = insecticide, F = fertilizer, H = herbicide.

5.3.2 Assessment of reproduction effects

5.3.2.1 Seed collection

The herbicide suppressed the formation of flowers in *R. acris*, *L. pratensis*, and *V. sepium* and thus, the total seed production of these three species in the herbicide treated plots was significantly reduced

(Table 5-2). Therefore, it was not always possible to find 48 fruits in the herbicide treated plots. We were able to find enough fruits for harvesting in the control, insecticide, and fertilizer treatments.

The mean number of seeds per fruit was not affected by the herbicide treatment. We detected only one slightly significant increase in the mean number of seeds per fruit in one fertilizer treatment (F+I) for *R. acris* (Table 5-2). The herbicide treatment (H) significantly reduced the mean seed weight of *R. acris*. Moreover, in all herbicide treated plots (H, H+I, and F+H+I) the mean seed weight of *R. acris* (H = 0.6 mg, H + I = 0.7 mg, F + H + I = 0.5 mg) was only about one third of that in the control plots (C = 1.6 mg).

Table 5-2: Number of collected fruits per treatment (target 48 fruits per treatment, but flower suppression in herbicide treatments resulted in lower numbers), mean number of seeds per fruit and treatment, and mean 1-seed weight of *Ranunculus acris*, *Lathyrus pratensis*, *Vicia sepium*, and *Rumex acetosa* per treatment. Treatments significantly different from the control were highlighted with a gray background and asterisks, * p < 0.05, ** p < 0.01, *** p < 0.001 (nested analysis of variance with permutations [nested PerAnova]), SE = standard error. C = control (highlighted in green), I = insecticide, F = fertilizer, H = herbicide. n.d. = not determined because no fruits could be collected.

		C	I	F	H	H+I	F+I	F+H	F+H+I
R. acris	no. of fruits (sum)	48	48	48	8***	2***	48	0***	1***
	seeds/fruit (± SE)	28 (± 1.1)	29 (± 1.2)	31 (± 1.2)	24 (± 2.7)	32 (± 1.0)	33 (± 1.2)*	n.d.	30
	1-seed weight [mg] (± SE)	1.6 (± 0.1)	1.5 (± 0.1)	1.3 (± 0.1)	0.6 (± 0.2)*	0.7 (± 0.5)	1.4 (± 0.1)	n.d.	0.5[a]
L. pratensis	no. of fruits (sum)	48	48	48	0***	5***	48	3***	0***
	seeds/fruit (± SE)	5 (± 0.3)	6 (± 0.3)	5 (± 0.3)	n.d.	6 (± 1.1)	5 (± 0.3)	3 (± 0.3)	n.d.
	1-seed weight [mg] (± SE)	9.5 (± 0.5)	11.6 (± 0.6)	11.5 ± (0.6)	n.d.	8.8 (± 1.9)	9.1 (± 0.5)	14.8(±3.5)	n.d.
V. sepium	no. of fruits (sum)	48	48	48	25**	33**	48	12***	14***
	seeds/fruit (± SE)	4 (± 0.2)	4 (± 0.2)	4 (± 0.3)	4 (± 0.4)	4 (± 0.4)	4 (± 0.3)	4 (± 0.5)	4 (± 0.5)
	1-seed weight [mg] (± SE)	19.4 (± 0.9)	17.5 (± 1.1)	18.3 (± 0.9)	16.2 (± 0.9)	18.2 (± 1.4)	17.7 (± 0.8)	19.2(±2.4)	18.2 (± 2.0)
R. acetosa	no. of fruit stalks (sum)	48	48	48	48	48	48	48	48
	seeds/stalk (± SE)	30 (± 1.7)	26 (± 1.6)	32 (±2.6)	30 (± 1.8)	29 (± 1.7)	25 (± 2.1)	35 (± 2.1)	29 (± 1.6)
	1-seed weight [mg] (± SE)	0.8 (± 0.1)	0.8 (± 0.1)	0.8 (± 0.1)	0.8 (± 0.1)	0.8 (± 0.1)	0.9 (± 0.1)	0.8 (± 0.1)	0.8 (± 0.1)

[a] no standard error and no statistical analysis could be calculated/conducted since only one value was available (1 fruit)

5.3.2.2 Germination tests

The germination test duration differed among the four species because it depended on the species-specific germination rate. *R. acetosa* achieved the highest germination rate. Ten days after sowing, 80 % of the seeds had already germinated and after 17 days the germination test could be stopped for this species due to its high germination rate in all treatments. All other species showed a lower germination rate. *R. acris* started to germinate eight to ten days after sowing and achieved a germination rate of around 30 % in the control after 30 days. For this species, the germination test was stopped 53 days after sowing because only a few more seeds germinated per week. The germination test for *L. pratensis* also ran for 53 days, whereby the seeds showed a low germination rate of < 10 % in the control plots. The germination of this species stagnated approximately 30 days after sowing. The germination of *V. sepium* was observed for 30 days. However, a stagnation of the germination rate was already reached 13 days after sowing in all treatments.

Table 5-3: Mean germination rate (g. r. [%]) of *Ranunculus acris*, *Lathyrus pratensis*, *Vicia sepium*, and *Rumex acetosa* per plot and treatment. Number of plots, in which seeds could be collected and used for germination tests (plots/treatment), sown seeds per plot, and total sown seeds, are listed. Germination test duration for *R. acris* = 53 days, *L. pratensis* = 53 days, *V. sepium* = 30 days and *R. acetosa* = 17 days. C = control (highlighted in green), I = insecticide, F = fertilizer, H = herbicide. SE = standard error. n.d. = not determined since no seeds or not enough seeds could be collected.

	R. acris					***L. pratensis***				
	mean g.r. [%]	SE [%]	plots/ treatment	sown seeds/plot	seeds in total	mean g.r. [%]	SE [%]	plots/ treatment	sown seeds/plot	seeds in total
C	36.3	6.2	8	20	160	7.5	1.4	8	20	160
I	44.4	7.4	8	20	160	5.7	1.2	8	20	160
F	35.7	5.3	8	20	160	6.9	2.7	8	20	160
H	22.5	7.5	2	20	40	n.d.	n.d.	0	0	0
H+I	0	0	2	20	40	20	n.d.	1	20	20
F+I	43.8	4.1	8	20	160	13.8	2.7	8	20	160
F+H	n.d.	n.d.	0	0	0	n.d.	n.d.	0	0	0
F+H+I	0	0	1	20	20	n.d.	n.d.	0	0	0
	V. sepium					***R. acetosa***				
	mean g.r. [%]	SE [%]	plots/ treatment	sown seeds/plot	seeds in total	mean g.r. [%]	SE [%]	plots/ treatment	sown seeds/plot	seeds in total
C	14.2	7.2	7 [a]	5	35	83.2	5.5	8	20	160
I	8.6	4.0	7 [a]	5	35	84.4	2.8	8	20	160
F	15.0	7.4	8	5	40	68.8	5.8	8	20	160
H	8.0	8.0	5	5	25	70.0	4.7	8	20	160
H+I	20.0	10.4	6	5	30	79.4	4.9	8	20	160
F+I	12.6	3.6	8	5	40	79.4	5.5	8	20	160
F+H	10.0	10.0	2	5	10	81.9	5.5	8	20	160
F+H+I	8.0	5.0	5	5	25	67.5	8.2	8	20	160

[a] In one plot, seeds were lost due to herbivores and consequently, not enough seeds remained for germination tests.

In some herbicide treated plots, no fruits (mature seeds) could be collected because flowering was suppressed (Table 5-2). This was the case for *R. acris*, *L. pratensis*, and *V. sepium* and consequently, the number of replicates (plots/treatment) for the germination test was reduced in these treatments (Table 5-3). We detected no significant differences in the mean germination rate among the treatments, maybe caused by the relative high standard errors, but particularly also caused by the low number of replicates (e.g. only one or two replicates) in the herbicide treated plots. This is especially true for *R. acris* for which little or no germination in some herbicide treated plots (H, H+I, and F+H+I) was recorded. For the F+H+I treatment (*R. acris*), no significant difference could be detected because in this treatment only one replicate was available, in which seeds of *R. acris* could be harvested.

5.4 Discussion

Herbicide and fertilizer treatments caused negative effects on the plant frequencies of the four study species. However, these effects became gradually apparent. Treatments reduced plant frequencies in the first experimental season for only two species, *L. pratensis* and *V. sepium*. In contrast, the plant frequencies of the two other species, *R. acris* and *R. acetosa*, were significantly affected from the second year of application (Fig. 5-3). Moreover, the results of the third experimental season revealed that the herbicide and fertilizer effects became stronger over time. We observed significant reductions in plant frequencies in all herbicide and fertilizer treatments (and treatment combinations) in the third year. Thus, long-term field studies are necessary to assess the entire herbicide and fertilizer effects on the plant frequencies. This is especially evident in plant communities of unaffected meadows (not contaminated with agrochemicals before the study began). These communities are relatively stable in their composition or change only slowly following low fertilizer or herbicide application rates, such as those rates used in our study (Hejcman et al. 2007). Fertilizer additions increase the availability of plant nutrients in soil. It directly promoted the growth and spread of tall grasses such as *Dactylis glomerata*, whereas it indirectly reduced the growth and spread of smaller plants (e.g. *R. acris, L. pratensis, V. sepium*), which were overtopped and replaced by the taller and faster-growing plants (Schmitz et al. 2014b). In contrast, the herbicide used in this study caused sublethal effects (phytotoxicity effects) to all four species, and these effects required time to be measurable in the plant frequency assessments. The herbicide treatment may have reduced plant fitness and competitiveness. Furthermore, the repeated agrochemical applications over several growing seasons intensified herbicide and fertilizer effects on the plant frequency.

Interaction effects of fertilizer and herbicide treatments were also detected, occurring first in the third year. It appears that the separate fertilizer and herbicide treatment caused similar decreases in the plant frequency in *R. acetosa* and *V. sepium*, but the treatment combinations of fertilizer and herbicide (F+H, and F+H+I) resulted in much stronger reductions. Thus, an additive effect seems likely. In general, interaction effects between fertilizer and herbicide are less investigated. We found only two other field studies that investigated fertilizer and herbicide interaction effects on natural plant communities (Kleijn & Snoeijing 1997; Strandberg et al. 2012). Both studies found combined effects that increased sensitivity of certain plant species.

We studied only perennials, but annuals and biennials, which have a relatively short life-span, may be more vulnerable to herbicides and, thus, effects might be visible faster. Annuals and biennials need to produce viable seeds in their life cycle. In contrast, perennial herbs can persist for many growing seasons because generally only the above ground parts of the plants die back each winter and regrow the following spring. However, even perennial species need to reproduce by seeds on occasion to avoid their decline in the plant community. Therefore, it is also important to detect effects of herbicide applications on reproductive capacity in addition to effects on the plant frequency.

Three of the four investigated species (*R. acris*, *L. pratensis*, and *V. sepium*) were affected in their reproductive capacity by the herbicide treatment. Flowering patterns of these three species were significantly suppressed, and in some herbicide treated plots, no fruits were formed. During the presented field experiment, the flowering intensity of *R. acris* was already investigated in the first and second experimental season (2010 and 2011) and it was detected that the herbicide reduced the flowering intensity by 85 % compared with the control plots in 2011 (Schmitz et al. 2013). The results of the present study (third experimental season) showed that also the mean 1-seed weight of *R. acris* was reduced in the herbicide treated plots, which could reduce germination rates of these seeds. However, statistical analysis did not find a significant reduction in the germination rate of *R. acris*, which may be caused by the reduced number of replicates (collected fruits) in the herbicide treated plots. Without flowering and seed production, however, there will be no new seeds to germinate. Such reproduction effects will probably require more than one or two years to be reflected in the plant frequency assessments, particularly when species are perennials and can also reproduce vegetatively. Nevertheless, sexual reproduction is essential to maintaining genetic variations in natural plant communities and thus, this form of reproduction is beneficial for the competitive ability of a population over time (Travers et al. 2011). Repeated herbicide applications every year, ultimately reduces the soil seed bank. Longevity of buried seeds varies between plant species and determines if species can form transient (short-term) or long-term persistent seed banks (Thompson et al. 1993; Bekker et al. 1998). Longevity, in turn, depends on different seed morphology and the vertical distribution of seeds in the soil. Small seeds (1- seed weight < 3 mg) can persist for at least five years in soil (e.g. *R. acris*, *R. acetosa*, Table 5-2), whereas significantly heavier seeds with a higher variance in seed shape (e.g. *V. sepium, L. pratensis*, Table 5-2) tend to be less persistent in the soil (Bekker et al. 1998). Therefore, without an annual delivery of seeds, the soil seed bank eventually will be depleted, and this will reduce population size.

Reproduction is a highly sensitive endpoint (Marrs et al. 1989; Marrs et al. 1991; Kjaer et al. 2006a,b; Carpenter & Boutin 2010; Strandberg et al. 2012). For example, herbicide effects on flower formation were observed up to a distance of 10 m from the field edge in the study of Marrs et al. (1989). In addition, Kjaer et al. (2006a) found a 100 % berry reduction at simulated drift rates of 5 % of the application rate of metsulfuron sprayed at the bud stage on hawthorn (*Crataegus monogyna*), a common shrub species in agricultural landscapes. Generally, for most plants it is not possible to recover from reproductive effects in one growing season, in contrast to damage effects such as chlorosis or leaf reduction. This is consistent with the concept of resource exploitation – increasing resource depletion or stress at first limits reproduction, then affects individual growth, and finally leads to death (Smith & Smith 2009).

Reproductive endpoints, such as flowering or seed production, are not considered in the current phytotoxicity tests performed for the herbicide risk assessment. However, the present study revealed that reproductive endpoints are probably a more sensitive endpoint than biomass. Strandberg et al. (2012) also found that seed production was a more sensitive endpoint than biomass, irrespectively of plant species, lifespan (annual, biennial, or perennial), and the life stage at the time of exposure (vegetative and reproductive). Therefore, herbicide effect assessments that only focus on effects on biomass are presumably underestimating the full herbicide effect.

The negative effects of the fertilizer and herbicide treatments on plant frequencies due to changes in competitiveness, as well as the effects of the herbicide treatments on reproductive capacity, contribute together to the long-term reduction of plants. Insecticide applications did not directly affect the plant frequencies or reproductive capacities of the four study species. Although annual applications of insecticides could reduce plant populations indirectly by reducing the density of pollinators (Potts et al. 2010), we observed no indirect effects of the insecticide on the four plant species. Our study was only three seasons, which may be too short to quantify such effects.

The herbicide used in the present study is only one product selected out of 574 registered herbicide products in Germany (BVL 2013c). To date, little is known about effects on the reproductive capacity of most herbicides that are applied in agricultural landscapes. Thus, there is a need to test additional wild plant species at their reproductive stage with different herbicides.

5.5 Conclusion

Plant frequencies of the four study species were significantly reduced by herbicide and fertilizer treatments. The effects became stronger over time and, therefore, long-term field studies are particularly important when estimating effects of agrochemical applications on the plant community in non-target habitats. Interaction effects between fertilizer and herbicide treatments were observed in the third experimental season, which caused an additive effect on the plant frequency of *V. sepium* and *R. acetosa*. In addition, the reproductive capacities of *R. acris*, *V. sepium*, and *L. pratensis* were significantly reduced by the herbicide treatment but not by the fertilizer treatment.

So far, interaction effects between fertilizers and herbicides, as well as reproductive effects, are not considered in risk assessment procedures for pesticides. However, these effects could lead to plant community shifts and cause the disappearance of the affected plants if field margins are exposed to repeated agrochemical applications over several years.

Thus, appropriate risk mitigation measures (e.g. in-field buffer for the application of fertilizers and herbicides) are needed to protect the vegetation of field margins from agrochemical misplacements. In addition, we recommend investigating reproduction effects of herbicides with different modes of action on a range of wild plant species to improve the current risk assessment of herbicides.

6 Assessing the risk of herbicides to terrestrial non-target plants using higher-tier studies

Paper IV

This chapter presents the author`s final version of the manuscript:

Schmitz, J., Stahlschmidt, P., Brühl, C.A.: Assessing the risk of herbicides to terrestrial non-target plants using higher tier studies.

Abstract – Herbicide risk assessment for non-target plants is based on single species phytotoxicity tests. This approach, however, may not reflect relevant ecological processes in terrestrial ecosystems. The current risk assessment scheme is based on endpoints measured at the species level and the assessment of ecological effects relies on the extrapolation from one species to another or from a single species to a community. This extrapolation contains many uncertainties that may be reduced by adopting more realistic testing approaches. However, thus far, higher-tier studies with non-target plants are not obligatory in the herbicide risk assessment and thus, no standard protocols are available. We reviewed the published literature concerning higher-tier tests and found that potential higher-tier approaches for terrestrial non-target plants are extremely limited. Sixteen studies were found that assessed the effects of herbicides on non-target plants by performing microcosms, mesocosms, or field studies. These studies showed that microcosms might provide useful data and help to reduce uncertainties associated with single-species tests. However, due to the limited number of available studies, much work is required to develop appropriate testing methods for regulatory processes. In addition, field experiments are necessary to establish baseline knowledge concerning the effects of herbicides on natural plant communities and to compare data generated in tiered testing approaches with data obtained from natural systems.

Keywords – Herbicide, Risk Assessment, Non-target plants, Microcosms, Mesocosms, Field studies

6.1 Introduction

Herbicides are used to control undesirable plants in agricultural fields that compete with crop plants for resources (weeds). However, during field applications, terrestrial non-target plants (NTP) in off-field habitats directly adjacent to the field may also be exposed to herbicides via overspray and spray drift (Füll et al. 2000) The misplacement of herbicides in off-field habitats (e.g., field margins) can cause changes in plant community composition and can reduce plant species diversity (Marrs et al. 1991a; Jobin et al. 1997; Kleijn & Snoeijing 1997; Marrs & Frost 1997; Gove et al. 2007). Because plants are primary producers and form the energetic basis of terrestrial ecosystems it is extremely likely that other non-target organisms (e.g., herbivorous arthropods, pollinators, and predators) are also adversely affected by herbicides due to altered habitats and food sources (indirect effects). Therefore, herbicide risk assessment is important for protecting not only plants, but also other organisms in the habitat (Brown et al. 2009).

Risk assessment (RA) aims at identifying and characterizing risks associated with the application of pesticides. At present, the RA for NTP, as described in the Guidance Document on Terrestrial Ecotoxicology, follows a tiered testing approach with three different steps (Tier I, Tier II and Tier III) (European Commission 2002). The concept of tiered approaches is to start with a simple conservative assessment of effects in the laboratory and then progress, if necessary, toward higher tiers with more realistic conditions. In general, lower tiers require less effort than higher tiers, whereas higher tiers provide more realistic risk estimations.

The first tier (Tier I) in the RA for NTP is a preliminary assessment, which can also be described as an initial screening, with at least 6 plant species tested once at the highest nominal application rate (European Commission 2002). However, this screening test can be skipped for herbicides and plant growth regulators because such compounds will inevitably require testing in the second tier. The second tier (Tier II) is a quantitative risk assessment following a TER (toxicity exposure ratio) approach. In this step, the risk for terrestrial NTP is assessed using emergence or vegetative vigor tests of single plant species (usually 6 crop species) grown in pots under standardized conditions in the greenhouse.

For the seedling emergence test, seeds are placed in soil treated with the herbicide and observed for emergence, visual phytotoxicity and mortality following 14 to 21 days after 50 % emergence of the seedlings in the control group. At the end of the test, the percent emergence and biomass of surviving plants are recorded. The vegetative vigor test begins at young development stages (usually the 2-6 leaf stage) and ER_{50}-values (= application rate causing 50 % effects) for mortality and biomass are determined after 21 to 28 days. Tier II tests are strictly defined, with precise methodologies and clear procedures for using the results in a regulatory context. The test guidelines were developed by the Organization for Economic Co-operation and Development (OECD) (OECD 2006).

In the risk assessment scheme, a higher-tier non-target plant study (Tier III) is required when a potential risk at the lower Tier II level is identified. However, semi-field and field studies (Tier III) are time-consuming and expensive, and therefore, the Guidance Document recommends determining whether there are options for the refinement of exposure and/or effects of the herbicide in the field (European Commission 2002). Accordingly, a Tier III study is not required if the risk based on the Tier II level could be managed by risk mitigation measures, such as in-field buffer distances to terrestrial off-field habitats or the usage of low-drift-nozzles during the pesticide application (European Commission 2002).

To date, the result of this policy is that primarily Tier II studies are performed for NTP risk assessment and for herbicide registration (Olszyk et al. 2004; UBA 2012). Moreover, because higher-tier studies (Tier III) are not obligatory, no standard protocols and guidelines are available. However, in recent years, it has been recognized that current phytotoxicity testing under greenhouse conditions may not be sufficiently protective for the entire non-target plant community (Dalton & Boutin 2010). The present RA scheme has been criticized in different terms, such as the number and types of plant species used in Tier II studies, phenological stages of plants, and assessment endpoints (survival, growth and biomass) (Boutin et al. 2012). Currently, no tests are required to assess effects on reproduction, although herbicides are often applied in the field at a time when plants are close to flowering. Effects of herbicides on the reproduction of NTP have been observed under field conditions (for example Marrs et al. 1991b, Fletcher et al. 1996, Marrs et al. 1993, Kjaer et al. 2006a,b; Boutin et al. 2012; Strandberg et al. 2012) and it was therefore suggested that ecological RA should include reproductive endpoints (Boutin et al. 2012 and 2014).

One further issue that continues to arise is whether laboratory tests are valid substitutes for field trials (Pfleeger et al. 2011). The RA scheme described above is based on endpoints measured at the species level and the assessment of ecological effects relies on the extrapolation from one species to another or from a single species to a community or even to an ecosystem (Sanchez-Bayo & Goka 2012). This extrapolation contains many uncertainties that can most likely be reduced by adopting more realistic testing approaches that consider endpoints (lethal and sublethal) at the community or ecological level (Sanchez-Bayo & Goka 2012).

The aim of this paper was to review the current published literature regarding higher-tier approaches for terrestrial NTP and to provide an overview of these studies. In addition, the test designs of the investigated studies were evaluated with regard to their realism and applicability for higher-tier testing in risk assessment procedures.

6.2 Material and methods

The publicly available literature was searched using ISI Web of Knowledge, OvidSP and Google Scholar. Multiple search terms were used, e.g., “non-target plant”, “field margin”, “herbicide drift”, “phytotoxicity test”, “greenhouse experiment”, “microcosm”, “field study” and/or e.g. “plant community”, “margin”, “pesticide”, “herbicide”, and “agriculture”. The resulting hits were screened and the cited sources and the articles in which this literature had been cited were also analyzed.

Relevance was based on papers describing methodologies for higher-tier tests, including non-standard laboratory tests, mono-species field and multispecies greenhouse or field tests, and field experiments.

6. 3 Results

Overview of published literature

The literature search revealed that potential higher-tier approaches for terrestrial NTP are limited and not well documented in the scientific literature. Sixteen studies were found that assessed the effects of herbicides on NTP by performing microcosm or mesocosm experiments, semi-field, or field studies.
Because tiered testing approaches suggest increasing the scale and realism from single-species tests to microcosms to mesocosms to field studies, the reviewed studies were grouped and arranged accordingly. Tables 6-1 and 6-2 provide an overview of the studies, their test designs and their main results.

Group 1 - single species to mesocosm studies

In this group, studies that primarily used one or several plant species for their investigations are summarized (Table 6-1). In total, twelve studies were assigned to this group. These studies were divided into three categories:

1 a) The four studies listed in this subcategory used ***realistic drift*** with in-situ bioassays during an herbicide application in a crop field. ***Single plant species*** at young development stages (seedlings in pots [= one species per pot]) were placed at different distances from a treated field. Thus, the plants received different spray drift rates at each distance. After application, the test plants were transferred to a holding area in the field or greenhouse and were monitored for the development of phytotoxicity effects and biomass reduction.

1 b) The second subcategory consists of five ***microcosm*** experiments. In microcosm studies, the realism compared with single-species tests increases and therefore, more than one plant species per ***pot or planting tray*** are used in microcosm experiments to investigate interaction effects. These studies were performed in the greenhouse or field, and microcosms were exposed to realistic or simulated herbicide spray drift (direct overspray).

1 c) The third subcategory consists of three ***mesocosm*** experiments. In mesocosm studies the realism is further increased by conducting the studies under field conditions on small experimental ***plots***. Experimental plant communities were exposed to a simulated herbicide spray drift (direct overspray). No artificial test system was used and consequently, the test designs of these studies varied.

Table 6-1: Overview of literature data concerning higher-tier studies with terrestrial non-target plants (**group 1**). Studies are divided in three categories: a) *realistic drift studies* with single species, b) *microcosm experiments* exposed to a realistic or to a simulated spray drift in the greenhouse or field, and c) *mesocosm experiments* in the field (simulated drift). DAT, WAT, MAT, YAT = days, weeks, months, years after treatment, n.d. = no data, NTP = non-target plants, LDist50-value = distance to the treated field where 50 % mortality occurred.

	Source	Test design	Sampling endpoints (time of assessment)	Main results
a) realistic drift studies with single plant species	Marrs et al. (1989)	single-species tests (seedlings*), pots placed at different distances from the treated field (0-20 m), 5 replicates per species and distance	sublethal, lethal effects (WAT-MAT; exact data not given)	lethal effects up to 6 m from the treated field, effects on flowering up to 10 m → buffer zones of 5-10 m were suggested to protect NTP
	Marrs et al. (1991a)	single-species tests (different development stages: seedlings[a], or established plants*), pots placed at different distances from the treated field (0-4 m), 5 replicates per species and distance	plant biomass, lethal effects (20 WAT)	lethal effects up to 2 m from the treated field, effects on biomass up to 4 m, young plants showed a higher sensitivity than the old ones → buffer zones of 5-10 m were suggested to protect NTP
	Marrs et al. (1993)	**a)** 1 species in trays (140-250 seedlings[a]/tray) placed at different distances from the treated field (0-20m), 4 replicates per distance	sublethal and lethal effects (28 DAT)	10 % mortality occurred at 10 m distance from the treated field
		b) single-species tests (seedlings[a]), pots placed at different distances from the treated field, different number of replicates per species (between 20 &120)	sublethal and lethal effects LDist50-value (28 DAT)	wide range of species responses, 1 seedling had a LDist50 value of 15-20 m → buffer zones of 20 m were suggested to protect NTP
	De Jong & Haes (2001)	single-species tests (plants approx. 2 weeks old), single species in separate compartments of multi-compartment trays placed at different distances from the treated field (0-20m), 20-30 seedlings were used as replicates per distance	plant biomass (21 DAT)	significant effects (50% biomass reduction) were found regularly up to a distance of 6 meters from the treated field, and in one experiment even at 16 m → they suggested that such a test setup is suitable to assess herbicide effects in the field
b) microcosm experiments simulated drift	Reuter and Siemoneit-Gast (2007); Siemoneit et al.(2007)	**a)** greenhouse: 4 dicotyl + 2 monocotyl species (4-6 leaf stage) in trays (17 cm x 17 cm, filling height 5 cm), plant density: 8 individuals per species and tray (48 plants/tray), 5 herbicide rates, 4 replicates **b)** single-species tests in greenhouse, 4 replicates	plant biomass, foliar injury (14, 28, 42 DAT)	species respond differently in pots and microcosms, 2 species showed a higher sensitivity 42 DAT than 28 DAT → some species showed a higher sensitivity in microcosms than in single-species test
	Riemens et al. (2008)	**a)** greenhouse: 4 dicotyl + 4 monocotyl species (4-6 leaf stage) in 5 L pots, plant density: 8 individuals per species and pots (64 plants/microcosm), 5 herbicide rates, 8 replicates	plant biomass, visual effects (28 DAT)	dicotyledons showed a higher sensitivity than monocotyledons, species respond differently in pots and microcosms due to inter- and intraspecific interferences and shielding effects in mixture
		b) single-species tests under field and greenhouse conditions, 8 replicates		greenhouse grown plants were more sensitive than field grown plants → results from single-species tests cannot be translated into effects in mixture
	Dalton & Boutin (2010)	**a)** greenhouse + outdoor: 7 or 9 dicotyl species (4-6 leaf stage) in 5 L pots, plant density: 1 individual per species and pot (7-9 plants/pot), 5 herbicide rates, 6 replicates	plant biomass (28 DAT)	species in greenhouse microcosms were more sensitive than species in single-species tests and species in outdoor microcosm experiments → sensitivity is dependent on interactions between species and test conditions (light intensity, humidity, temperature)
		b) greenhouse microcosm experiments with extended test durations, 5 herbicides, 6 replicates		
		c) single-species tests in greenhouse, 5 herbicide rates, 6 replicates	plant biomass (60-70 DAT)	

[a]Information on leaf stage not given.

Table 6-1 Continued.

	Source	Test design	Sampling endpoints (time of assessment)	Main results
b) microcosm experiments realistic drift	Marrs et al. (1991b)	2-year field study: 8 dicotyl + 1 monocotyl species[a] in microcosms (trays: 27 cm diameter, 12 cm depth) were placed (once each year) at different distanced from the treated area (0-8 m), plant density: 1 individual plant per species and tray (9 plants/microcosm), 5 replicates per distance	sublethal (flowering) and lethal effects (28 DAT), plant biomass (3 MAT)	phytotoxic effects up to 4 m from the treated field in the first year and second year, but effects on flowering were first detected in the second year up to 2 m from the treated field → buffer zones between 6-10 m are adequate to protect established plants in field margins
	Marrs & Frost (1997)	3-year field study: same test design as used by Marrs et al. (1991b)	sublethal, lethal effects, plant biomass (3 MAT), flower number, seed production (1 and 2 YAT)	effects (reduced biomass, flower suppression) became stronger over the years, the composition of species were affected from the second year of exposure → buffer zones of 8 m are adequate to protect NTP
c) mesocosm experiments simulated drift	Pfleeger at al. (2012)	**a)** study site: different fields on 2 farms, 2 monocotyl + 2 dicotyl species (21 days old) were transplanted in small test plots (60 cm x 60 cm) in April, plant density: 1 individual per species and plot (4 plants/plot), randomized design, plants were treated approx. 4 weeks after transplanting in the plots, 3 herbicide rates, 10-14 replicates	plant growth (measured every 2 weeks during the growing season (May-July)	the most sensitive species in the field was *Cynosurus echinatus*, the most sensitive species in the greenhouse was *Prunella vulgaris* → species showed different reactions in single-species tests and microcosms, mixed relationships between field and greenhouse responses
		b) single-species tests with the 4 species used at the field site, greenhouse vs. field	plant growth (12 DAT)	
	Gove et al. (2007)	study site: woodland margins, 6 woodlands species grown in pots were treated with 5 herbicide rates in the greenhouse and were transplanted to $1m^2$ field plots, plant density: 30 plants/plot; 20 replicates, half of the plots were treated with fertilizer	number of flowers and seeds, plant biomass (1 YAT)	herbicide drift rates increased mortality, reduced biomass and fecundity for all species, fertilizer treatment did not significantly alter flowering → buffer zones of 5 m are suggested to protect NTP
	Perry et al. (1996)	study site: simulated field margin, 3 dicotyl + 3 monocotyl species sown and grown in field plots (2 x 3 m), plant density: n.d., randomized block design, 4 replicates, plants were grown for 11 months and then the plots were treated with 4 herbicide and 3 fertilizer rates	plant cover abundance (first assessment approx. 2 month before herbicide treatment, then every month up to 2 MAT)	fertilizer and herbicide treatment reduced the cover of species significantly → fertilizer and herbicide affected the plant community, effects could potentially become stronger in the long term

[a]Information on leaf stage not given.

Group 2 **-** *field studies with natural plant communities*

In addition to the above-described studies using one or several plant species, we found four complex ***field studies*** investigating the effects of herbicides on natural plant communities. These field experiments were long-term studies where herbicide effects on plants were evaluated over several growing seasons in natural systems. Although the test designs of the field studies were similar (e.g., randomized block design), some differences could be observed (see Table 6-2 for details).

Table 6-2: Overview of literature data concerning *field studies* that have investigated herbicide effects on natural plant communities (**group 2**). DAT, WAT = days, weeks after treatment, n.d. = no data.

	Source	Test design	Agrochemicals	No. of species	Measurements (time of assessment)	Main results
herbicide effects	De Snoo et al. (2005)	3-year experiment: 4 different study sites: 2 road verges and 2 ditch banks, randomized block design, plot size: 25 m^2 (1 m x 25 m), 5 herbicide rates, 20 replicates	Liberty, 2 treatments/year	natural community (species no. n.d)	phytotoxic effects (10 DAT), assessments of vegetation composition (May and August), plant biomass (August)	significant effects on biomass and species composition were observed at high drift rates, lower herbicide rates resulted mainly in phytotoxic effects → effects on species composition found at 30% of the field rate or higher
herbicide and fertilizer effects	Kleijn & Snoeijing (1997)	**a)** 3-year experiment: study site: meadow, randomized block design, plot size: 2 m x 2 m, 2 fertilizer rates, 3 herbicide rates, all treatment combinations, 1 control, 4 replicates (= 48 plots in total)	Starane 200 NPK fertilizer 1 treatment/year	natural community (approx. 44 species)	assessments of vegetation composition (once a year in May/June), biomass (August)	fertilizer decreased the species richness significantly , only slight herbicide effects → fertilizer effects became stronger over the years
		b) 3-year experiment: study site: fallow arable field, same test design as in experiment a)	Starane 200 NPK fertilizer 1 treatment/year	30	assessments of vegetation composition (twice a year: May and September), biomass (August)	fertilizer and herbicide affected the species richness significantly → herbicide and fertilizer effects were additive (reduction of species no. by approx. 35%)
		c) single-species tests with species used in the field, greenhouse conditions, 3 herbicide rates, 1 control, 4 replicates	Starane 200 1 treatment	18	plant biomass (6 WAT)	results differed from the field results → extrapolation of the results of single-species tests to natural plant communities is inappropriate
	Strandberg et al. (2012); Damgaard et al. (2011)	long-term experiment (start 2001): study site: fallow field, randomized block design, plot size: 7 m x 7 m, 3 herbicide rates, 2 fertilizer rates, all treatment combinations, 1 control, 10 replicates (= 120 plots in total)	Roundup Bio nitrogen fertilizer 1 treatment/year	31	plant cover, vertical density (3 times a year: before treatment, 2 WAT and at the end of the growing season)	fertilizer and herbicide affected the species number negatively → interaction effects of fertilizer and herbicide were demonstrated
	Schmitz et al. (2013), 2014a,b)	3-year experiment: study site: meadow, randomized block design, plot size: 8 m x 8 m, 1 herbicide rate, 1 fertilizer rate, 1 insecticide rate, all combinations, 1 control, 8 replicates (= 64 plots in total)	Atlantis WG, Karate Zeon, NPK fertilizer 1 treatment/year	natural community (approx. 50 species)	plant frequency (every year in May and June), flower intensity of one species (every year in May), seed production of four species in the 3. year	fertilizer and herbicide decreased plant frequencies of several species, herbicide reduced flower intensity and the seed production → effects became stronger over the years, fertilizer and herbicide lead to community shifts, interaction effects of fertilizer and herbicide were detected

6.4 Discussion

Group 1a) realistic drift studies with single species

For studies were found that assessed herbicide effects on single plant species in the field. The test designs of these studies are similar to Tier II studies but include exposure under realistic drift conditions in the field instead of a treatment in the laboratory (Table 6-1). With the proposed test setup of De Jong & Haes (2001), only short-term effects up to 21 days can be assessed on individual plants at young development stages. Effects on reproduction are not addressed, although higher-tier studies should also include such sublethal endpoints.

Group 1 b) microcosm experiments

The five microcosm studies used similar test systems (e.g., 17 cm x 17 cm planting trays or 5 L pots) small enough to be used in dose-response experiments with an appropriate number of replicates. All studies contained multi-species assemblages (6 - 9 species), and four of the five microcosm studies used a mix of broadleaf species and grasses (Table 6-1). Most herbicides have a specific mode of action and are specifically designed to control mono- or dicotyledons. Therefore, a mix of plant species (mono- and dicotyledons) seems appropriate for microcosm studies, particularly when NTP communities should be simulated. Moreover, Marrs et al. (1997) detected that the presence or absence of monocotyledons in microcosms can influence the response of dicotyledons, perhaps due to different levels of interception of herbicides by plant species and densities.

Plant densities (individuals per test system) in the microcosm experiments (9-64 individuals per test system; see Table 6-1 for details) differed from each other. Using an appropriate plant density is required to study competition effects between species. A higher plant density can generally increase competition between plants. Thus, plants in microcosms may be under increased stress compared with species grown individually, and this stress may lead to a higher sensitivity toward herbicides (see Table 1; experiments performed by Reuter & Siemoneit-Gast (2007), Riemens et al. (2008), Dalton & Boutin (2010)). In contrast, some species can also benefit from neighboring plants. Species with a relative small stature (e.g., *Stellaria media*) can be shielded from herbicide exposure by the leaves of taller-growing plants (Riemens et al. 2008). The authors concluded that these interaction effects (intra- and interspecific interferences and shielding effects) are the reason why results from single-species tests cannot easily be translated to effects on the same species grown in mixture (Riemens et al. 2008).

The studies performed by Reuter & Siemoneit-Gast (2007), Riemens et al. (2008), and Dalton & Boutin (2010) used test durations of 14 to 70 days after treatment. Marrs et al. (1991b, 1997) conducted long-term (2-3 years) microcosm experiments in the field that were designed to investigate the effects of yearly herbicide applications (Table 6-1). Thus, the effects on population dynamics and reproduction could be assessed because the entire life-cycles of species were considered (Marrs et al.

199b, 1997). As a result, Marrs and co-workers observed phytotoxicity and lethal effects during the first year; however, the effects on species composition and reproduction (flowering, seed production) were first noted after the second year of exposure (Table 6-1). The authors also concluded that such perennial studies are perhaps the most efficient method of investigating cumulative effects on plant communities exposed to spray drift (Marrs & Frost 1997). However, these experiments are time consuming and labor intensive.

Another difference between the studies of Marrs and co-workers and the three other microcosm experiments is the method of herbicide application. In the greenhouse experiments conducted by Reuter & Siemoneit-Gast (2007), Riemens et al. (2008), and Dalton & Boutin (2010), the microcosms were exposed to a simulated spray drift (overspray with a specific drift application rate). In contrast, Marrs and co-workers (Marrs et al. 1991b, Marrs et al. 1997) placed the microcosms at different distances from a treated field and investigated the effects of the resulting spray drift (realistic drift scenario). Real drift in the field often consists of smaller droplets with possibly higher concentrations of the pesticide than droplets from direct spray (overspray) (Koch et al. 2004). Moreover, overspray droplets are larger and may have a higher penetrability on the vegetation than spray drift (Koch et al. 2004). Drift from the field is also influenced by meteorological conditions (e.g., wind speed, temperature, and relative humidity) and by technical factors (e.g., boom height, driving speed, and nozzles) that can vary from application to application and that may produce different effects. Conversely, the advantage of overspraying is that the application can be performed under controlled and repeatable conditions as appropriate for testing herbicide effects on NTP.

A further point that arose is that test conditions (greenhouse or field conditions) can also influence species sensitivity. Dalton & Boutin (2010) found that species grown in greenhouse microcosms were more sensitive than species grown in outdoor microcosms (Table 6-1). This sensitivity is most likely a result of differences in environmental conditions (higher temperature, higher relative humidity and light intensity in greenhouses than in the field), which increased the translocation of the herbicide in the greenhouse plants. Moreover, plants grown in the field had smaller leaves and thicker cuticles that may have contributed to decreased herbicide adsorption in the field (Dalton & Boutin 2010).

Fraser & Keddy (1997) published a review concerning the role of experimental microcosms in ecological research and suggested some general guidelines. Although these guidelines were not focused on studies for ecotoxicological research, their suggestions can be useful for developing microcosm studies for assessing the risks of herbicides to NTP. We also developed recommendations for the design and performance of microcosm studies, which, however, should be further validated (Table 6-3).

Table 6-3: Factors and recommendations for the design and performance of a microcosm experiment with terrestrial non-target plants.

Factors to consider	Recommendations
Species	Vegetation of non-target areas (e.g., field margins) consists of dicotyledons and monocotyledons, and annual and perennial species. Therefore, a mix of species seems to be appropriate. Considering the traits of each plant species is also important.
Number of species	The number of species used in the evaluated microcosm studies ranged from 6-9. We recommend using a minimum of 6 species. However, plant communities have many species; thus, the more plants used in a study, the greater the realism (Fraser and Keddy (1997).
Individuals per species and microcosm	The number of individual plants per species is dependent on the size of the test system and on the number of species used in the experiment. In the evaluated microcosms, up to 8 individuals per species were used. An appropriate plant density is important for investigating interaction effects. More information on this topic is required.
Development stage of the test species	In addition to using young developed plant species (2-6 leaf stage), it seems extremely important to also use plant species in older phenological stages, e.g., directly before flowering. Recent studies showed that herbicides could affect the reproductive capacity of wild plant species. Therefore, effects on reproduction should also be assessed.
Test duration	Test durations of 28 days, as used in standard Tier II tests, can underestimate effects, particularly when effects on reproduction and on plant composition are to be assessed. Therefore, it would be valuable to extent the assessment period after treatment to e.g., the time of seed maturity. This assessment period is species dependent and must be decided on a case-by-case basis.
Size of the test system	The size of the test systems is related to the size of the test plants and their phenological stages. The evaluated microcosm studies used test systems of 5 L pots or 17 cm x 17 cm trays. Fraser and Keddy (1997) recommend using areas no smaller than 25 cm x 50 cm for microcosm experiments.
Number of replicates	The number of replicates in the evaluated microcosm experiments ranged from 4-8. Because community analyses are complex, replication should be increased whenever possible (Fraser and Keddy 1997).
Pest infestations	Plants in microcosms can be infested by pests (e.g., aphids, spider mites, and fungus gnats). Pest populations that occur during the experiment can be managed with biological pest control. When a biological control is used, all treatments should be treated equally.
Fertilization	The amount of fertilization depends on the used soil/substrate and on the duration of the test. However, over-fertilization can also influence the species sensitivity; therefore, it is extremely important to establish general regulations for fertilization in studies. More information regarding this aspect is required.

Group 1c) mesocosm experiments

We found only three published studies that used small experimental plots in the field to assess herbicide effects on NTP. The number of species used in these studies ranged from four to six; whereas the plant density was relatively low (see Table 6-1). For example, Pfleeger et al. (2012)

planted only one individual of each test species (4 species in total) in 60 cm x 60 cm test plots. This seems to be a very small number of plant species and individuals for a mesocosm study, particularly considering that more species (6 - 9 species) and individuals were used in the previously presented microcosm experiments. To increase realism compared with the microcosm studies, it would be necessary to use an appropriate number of plant species that are representative of natural plant communities. However, more information regarding this topic is required to make appropriate recommendations.

The test designs used in the experiments could be easily managed because only small plots (60 x 60 cm, 1 m², and 2 m x 3 m) were used. It was demonstrate that when plant species are first cultured in greenhouses and then transferred to the field, standardized plots can be established. To increase plant density, the plots can also be sown with common grass species, as was done by Marrs et al. (1991b). Moreover, Perry et al. (1996) suggested that when an artificial plant community should be established, it might be necessary to prepare field plots sufficiently early (e.g., 1 year before the treatment of the plots begins). Another possibility would be to perform a mesocosm study directly on a meadow that is not contaminated with agrochemicals. Nonetheless, because insufficient experience with plant testing on this scale is currently available, much more research is required to obtain a solid knowledge to develop and perform a successful mesocosm approach.

Group 2 - field studies

Only four studies are available that investigated the effects of herbicides on natural plant communities in the field. This finding itself is surprising because field studies examining the effects on other non-target organisms, such as arthropods, are regularly performed (e.g., Langhof et al. (2005), Kühne et al. (2002), Davis et al. (1993)).

Three of the four reviewed studies were field experiments on a meadow or a fallow field (Kleijn & Snoeijing 1997, Strandberg et al. 2012, Schmitz et al. 2013), and one study was conducted on two road verges and two ditch banks (de Snoo et al. 2005) (Table 6-2). Hence, de Snoo et al. (2005) have combined and compared the data from four different locations. The combining of data from different sites may be problematic because the vegetation composition is different at all sites. In addition, the effects of a test substance on different vegetation can vary.

All field experiments used study sites that were not treated with herbicides or fertilizers before the experiments started to be able to assign plant community changes over time to the treatments (Kleijn & Snoeijing 1997). Moreover, all field studies had chosen a randomized block test design, whereas their plot sizes differed from each other (plot size ranged from 2 m x 2 m to 8 m x 8 m; see Table 6-2). Generally, the size of a test plot is dependent on the size of the study site, the number of species, and, in particular, the distribution of the species over the study site. Plant communities can be heterogeneous and dynamic and therefore, it seems necessary to increase the size of the test plots and their replications whenever possible, particularly when sub-samples (several plant community assessments per plot) are to be conducted.

The three field experiments performed by Kleijn & Snoeijing (1997), Strandberg et al. (2012), and Schmitz et al. (2013, and 2014) studied not only herbicide effects on the plant community but also fertilizer effects. This aspect is important because they observed that, in addition to relevant herbicide drift rates, low fertilizer rates also negatively affected the plant community composition (Kleijn & Snoeijing 1997, Strandberg et al. 2012, Schmitz et al. 2014). Fertilizer applications caused a relatively immediate measurable decrease in plant species diversity because fertilizers increased the availability of nutrients in soil and promoted plants with high nutrient uptake. Herbicide treatments caused mortality in certain plant species and resulted in sublethal effects (phytotoxic effects and flower suppression) that reduced the abundance of certain species over time (Table 6-2). In all field studies, herbicide and fertilizer effects became stronger with each year of application and therefore, long-term field studies are particularly important to assess all herbicide effects on NTP communities. In addition, the field experiments demonstrated that effects under field conditions are complex and that interaction effects between agrochemicals (e.g., herbicide and fertilizer) and between plant species occurred. Changes in the population of one species may affect the dynamics of other species in the same ecosystem and thus, the overall effects are usually a result of both, the agrochemical effects and interaction effects between species (Sanchez-Bayo & Goka 2012).

Furthermore, in addition to grassy and herbaceous plant species, it is likely that other non-target plants adjacent to agricultural fields, such as woody plant species are also exposed to herbicide spray drift. This exposure is not considered in the RA of herbicides, although effects on these species might have ecological effects on higher trophic levels. Kjaer et al. (2006a and 2006b) investigated herbicide effects on hedgerows near agricultural fields and found a 100 % berry reduction at drift rates of 5 % of the field rate for hawthorn (*Crataegus monogyna*). This effect reduces not only the fitness of the shrub but also may influence other non-target organisms, such as berry-eating birds (Kjaer et al. 2006a).

In general, field studies with natural plant communities as described above would probably not be practical for routine use in a tier RA procedure due to the long test duration over several vegetation seasons; however, these studies are required to establish values for extrapolation approaches.

6.5 Conclusion

Currently, the RA for terrestrial non-target plants is based on the results from standard greenhouse studies in which short-term effects on test species under relatively homogenous conditions are assessed (OECD 2006). However, recent studies have raised concerns regarding whether these short-term tests and their test conditions (e.g., tested species, the phenological stages of plants, and assessment endpoints) are protective for wild plant species in off-field habitats (Boutin & Rogers 2000, Brown et al. 2009, Strandberg et al. 2012, Boutin et al. 2012). Plants in the field undergo additional adverse conditions, such as competition, wind, rain, and chemical mixture and therefore, the extrapolation from greenhouse tests to natural ecosystems involves many uncertainties (Sanchez-Bayo & Goka 2012). Higher-tier testing approaches, such as microcosms (several species growing in pots) or mesocosms (small experimental plots with artificial plant communities), may help to increase the realism in contrast to single-species tests. However, to date, higher-tier tests with NTP are not obligatory in the RA, and no standard protocols and guidelines are available for higher-tier tests.

The present review found only 16 studies in the scientific literature that assessed the effects of herbicides on NTP with the use of microcosms, mesocosms or field studies. Twelve of these studies could be assigned to a higher-tier testing approach. These studies showed that microcosms might be useful testing systems for measuring the effects of a particular herbicide on several plant species grown in mixture. Although such studies represent an oversimplification of natural systems, they can increase realism compared with single-species tests and can act as a bridge between single-species-based laboratory tests and field studies (Marrs & Frost 1997, Fraser & Keddy 1997, Dalton & Boutin 2010). However, due to the limited number of available and comparable studies, much work is required to develop and establish appropriate testing methods for regulatory processes. It would also be important to investigate differences that may exist between data produced in single-species tests compared with multiple-species tests (micro- or mesocosms) and data generated in field experiments with natural plant communities (Brown et al. 2009).

To date, little research has been conducted regarding the effects of herbicides on NTP communities; only four perennial field studies could be found. Field experiments are generally required to establish baseline knowledge concerning the effects of herbicides on natural plant communities and the related consequences for the terrestrial ecosystem. This expertise, in combination with specific research programs to develop higher-tier testing approaches, seems to be crucial for refining and improving the tiered RA of herbicides for NTP and their habitats. Modeling approaches could also be integrated to characterize and quantify herbicide effects on NTP and their communities (Schad 2013). With an appropriate model, it may be possible to estimate the competitive growth, survival and establishment of selected species and to test whether these species are affected by herbicide applications (Damgaard et al. 2011).

The development and realization of higher-tier assessment schemes, such as microcosms, mesocosms, and modeling remains a considerable challenge; however, a linkage of different methods and approaches may be an important step in getting closer to the reality.

7 Summary and general discussion

7.1 Effects of herbicides, insecticides and fertilizers on a natural plant community

Although it is often assumed that the vegetation of field margins is affected by pesticides and fertilizers applied to the crop field (Boutin & Jobin 1998), there are only a few field studies that have assessed such effects on natural plant communities (only two field studies could be found: Kleijn & Snoeijing 1997, Strandberg et al. 2012). The reason for this lack of studies is most likely the assumption that background variations (e.g., fluctuations in species abundance) in natural plant communities across a field site will make it difficult to detect effects of agrochemicals on individual species and on the plant community structure (Marrs & Frost 1997; Egan et al. 2014). However, the results of the present study revealed that experiments with natural plant communities can be performed and designed in a successful manner (see also Kleijn & Snoeijing 1997; Strandberg et al. 2012). For the present field experiment, a meadow not influenced by agrochemicals was used, and the overall natural distribution of plant species across the meadow was homogenous. Obviously, some of the species were naturally more abundant than others (26 species; see Paper I) and therefore, only these species were used for detecting agrochemical effects (statistical analysis). In addition, the experimental design (randomized block design), the high number of replicates (including sub-samples in each replicate) and the time-span of three years allowed for the clear detection of differences caused by the treatments (Köhler et al. 2002; van Emden 2008). In general, such field experiment approaches are required to reveal the range of potential plant responses under realistic environmental conditions (Egan et al. 2014) and to understand how subtle agrochemicals may affect plant species in a natural community. The present field study provided insights into these effects and demonstrated the complexity of such effects on plant biodiversity. In total, 20 of the 26 abundant species on the study site were significantly affected after three years of agrochemical applications (Paper I). As suspected, the pesticides and fertilizers used in this study affected different species in different ways:

The separate **fertilizer treatment** caused significant effects on the frequencies of 17 plant species, with 15 species negatively affected and 2 species positively affected in the third year of the experiment (Table 3-1 and Table 3-2 in Paper I). The results showed that generally small and subordinate species (e.g., the common bugle *A. reptans*, and the germander speedwell *V. chamaedrys*) and species with low Ellenberg nitrogen values (e.g., the yellow rattle *R. alectorolophus*) respond negatively to fertilizer application because these species were easily replaced and overtopped by faster-growing species and by species with a high leaf canopy (e.g., grasses such as the cock`s foot *D. glomerata* and the tall oatgrass *A. elatius*) (Paper I). Thus, these results support the well-documented theory that an increase in nutrient resources in a grassland community increases the biomass production of a few species with a high nutrient uptake, consequently enhancing the effects of shading and leading to an increase in competition for light (Kleijn & Snoeijing 1997; Wilson 1999; Hautier et al. 2009). Species that could not adapt to the new habitat conditions in the field experiment were at a competitive disadvantage and ultimately became less abundant (e.g., *A. reptans* showed a frequency reduction of more than 75 % compared with the control) or disappeared almost entirely (e.g., *R. alectorolophus*) in the fertilizer treatment (F) (Table 3-1 in Paper I).

The separate **herbicide treatment** significantly affected the frequencies of 13 species, with 12 species negatively affected and 1 species positively affected after three years of application (Table 3-1 and Table 3-2 in Paper I). Three of the negatively affected species (the yellow rattle *R. alectorolophus*, the grass-like starwort *S. graminea*, and the sweet vernal grass *A. odoratum*) seem to have the highest sensitivity toward the herbicide used in this study (Paper I). *R. alectorolophus* disappeared completely in the herbicide-treated plots (H treatment), and *S. graminea* and *A. odoratum* showed frequency reductions of 83 % and 91 % compared with the control plots in 2012 (Tables 3-1 and 3-2 in Paper I). Similar reductions were also observed during the first and second years of the experiment.

For the 9 other negatively affected species, sublethal effects, such as phytotoxic and reproduction effects were primarily observed following the herbicide applications. Several leaves in the herbicide-treated plots were slightly yellow or brown during vegetation assessments, and plant growth was inhibited, as also indicated by the biomass samples (Fig. 3-4 (biomass) and Fig. 3-5 (vegetation height), Paper I). The affected species were presumably more vulnerable and sensitive to natural stress, and this may hinder a plant`s ability to compete with those species that are not affected (Carpenter et al. 2013), such as the hedge bedstraw *G. mollugo* (showed no response to the herbicide application) or the tall fescue grass *F. arundinacea* (significantly increased its frequency in the herbicide-treated plots). The repeated herbicide applications intensified the herbicide effects, which significantly reduced the abundance of the affected species over the three years of the experiment.

Moreover, herbicide effects on the reproductive capacity (flowering intensity) of the common buttercup *R. acris* were investigated during the field experiment. The herbicide reduced the flowering intensity by 85 % compared with the control plots in 2011, whereas the frequency (abundance) of this plant species was not significantly affected by the herbicide treatment during the first and second years (Fig. 7-1) (Paper II). In the third experimental season (2012), the effects of the herbicide application on the seed production (number of mature seeds) of *R. acris* and of three other plant species (the meadow vetchling *L. pratensis*, the bush vetch *V. sepium* and the common sorrel *R. acetosa*) were additionally studied. Significant reductions in the seed production of *R. acris* (average reduction of 94 %), *L. pratensis* (average reduction of 96 %) and *V. sepium* (average reduction of 44 %) were observed in the herbicide-treated plots (H, H+I, F+H, F+H+I) compared with the control plots in 2012 (Table 5-2 in Paper III). Such reductions in fruit sets most likely had negative effects on the habitat´s seedbank, and this reduced the population sizes of these species over the years. In the third year of the experiment, the plant frequencies of all four species were significantly decreased in the herbicide-treated plots. The populations of these species may be completely replaced by tolerant, reproductive species in the long term (Paper III).

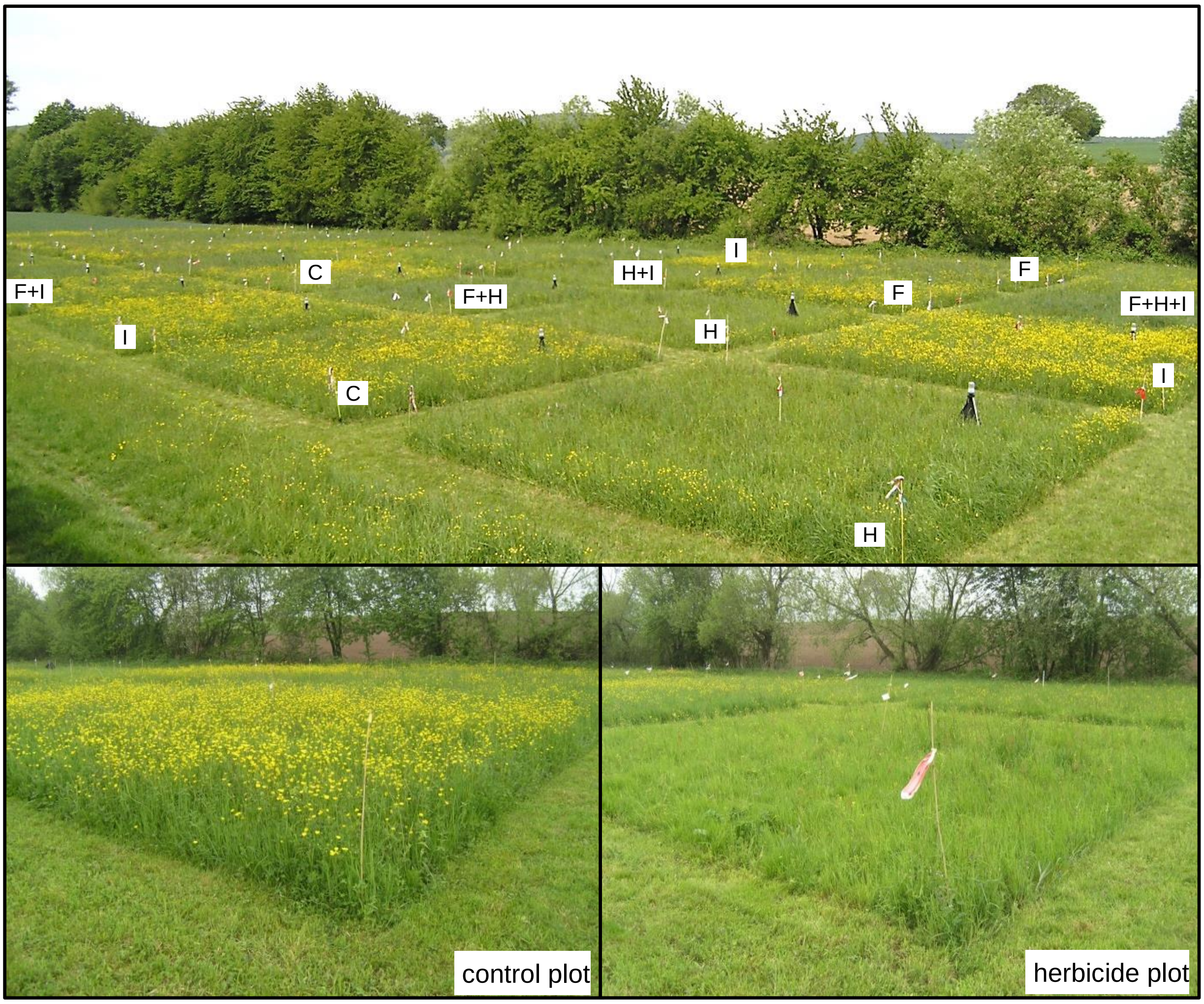

Fig. 7-1: Comparisons of the flowering intensity of *Ranunculus acris* in study site plots. The flowering intensity was significantly suppressed in plots treated with 30 % of the herbicide Atlantis WG. C = control, H = herbicide, F = fertilizer, I = insecticide, Photos taken at a period (26 April 11) when *R. acris* was the first and only yellow flowering plant species on the meadow.

The **treatment combinations of fertilizer and herbicide** (F+H and F+H+I) caused significant herbicide-fertilizer interaction effects for four species (*L. pratensis*, *A. reptans*, *R. alectorolophus* and *A. odoratum*) (Table 3-1 and Table 3-2 in Paper I). However, it seems that interaction effects between fertilizer and herbicide also occurred for other species, even if these effects were not significant. For example, the herbicide neutralized the negative fertilizer effect in plots treated with fertilizer and herbicide in combination (F+H and F+H+I) for 4 species (the germander speedwell *V. chamaedrys*, the narrowleaf plantain *P. lanceolata*, the colonial bentgrass *A. capillaris* and the tall fescue grass *F. arundinacea*). In addition to these interaction effects, additive effects of the fertilizer and herbicide were observed for three species: The frequencies of the ground ivy *G. hederacea*, the bush vetch *V. sepium*, and the common sorrel *R. acetosa* were significantly negatively affected by the single fertilizer and single herbicide treatment, whereas their frequencies were twice as strongly reduced by the treatment combinations of fertilizer and herbicide (F+H and F+H+I). Such interaction and additive effects could not be extrapolated from the individual fertilizer or herbicide effects found in the present study (Paper I). This finding explains why it is difficult to distinguish between the effects of fertilizer and herbicide on certain plants in field margins which are simultaneously exposed to both agrochemicals. Changes in the abundance of different species in natural plant communities are generally not only determined by the applied agrochemicals, but also by the effects of these agrochemicals on the growth and reproduction of neighboring plants (Strandberg et al. 2012). The effects of agrochemicals on the intensity of species competition also depend on species density in the plant community (Damgaard & Fayolle 2010).

For all affected species, the herbicide and fertilizer **effects** (individual and combined) **became stronger over time.** This finding is not surprising because treatment effects in a stable plant community of an unaffected meadow (or an unaffected field margin) build up more gradually, and the species composition in these habitats changes slowly following low fertilizer or herbicide application rates, such as those used in this study. With each year of application, the effects on the frequencies of individual species became stronger, and after three years, the composition of the plant community was altered by the fertilizer and herbicide treatments (single and in combination) (Fig. 3-3 (NMDS) in Paper I). Fig. 7-2 and 7-3 show exemplary photos of a control plot and a plot treated with fertilizer, herbicide and insecticide in combination (F+H+I). The F+H+I treatment is assumed to be the most realistic field margin scenario because a field margin of a conventionally arable field is exposed to all three agrochemicals.

The mean number of species was significantly reduced in the F+H and F+H+I treatments after three years of application (Fig. 3-1 in Paper I). Three species (*R. alectorolophus, S. graminea* and *A. odoratum*) completely disappeared or were recorded with only one individual in the F+H or F+H+I treatment in 2012 (Table 3-1 and Table 3-2 in Paper I). These findings are also of great concern because one of these species (*R. alectorolophus*) is on the Red List “V” in Germany (V = vulnerable), which indicates that this species is likely to become endangered in the near future. However, the frequencies of the other affected species were also highly significantly reduced three years after the start of the treatments, although they were not completely eliminated from the community. For example, the plant frequencies of *L. pratensis* and *R. acris* were reduced by almost 80 % and 45 %, respectively, in the F+H+I treatment compared with the control plots in 2012.
Due to these severe effects of the fertilizer and herbicide applications on the frequencies of several species, significantly lower species diversities in all fertilizer and herbicide treatments (F, H, F+I, H+I, F+H and F+H+I) (species diversity reductions up to approx. 15 %) than in the control were detected in 2012 (Fig. 3-2 (species diversity) in Paper I).

The results of the field experiment suggest that continuous annual applications of fertilizers and herbicides on the study site potentially cause further plant community shifts and would likely lead to a disappearance of certain affected plants because these plants will be replaced by tolerant species through interspecific competition (Carpenter et al. 2013). The most robust and least susceptible species to fertilizer and herbicide applications (predominantly tall grasses such as *D. glomerata* and *F. arundinacea*, and a few species of dicotyledons reproducing vegetatively, such as *G. mollugo*) may dominate the plant community after several years (Paper I).

Fig. 7-2: Photos of a control plot (plot number 18). Photos taken on 25 May 12.

Fig. 7-3: Photos of a plot treated with fertilizer, herbicide and insecticide (F+H+I) (plot number 14). Photos taken on 25 May 12.

Another concern involves the **insecticide** applications. Because insecticides are not directly designed to influence the vegetation, their effects on plants are generally not considered and investigated. However, the insecticide application in this field experiment significantly affected the frequencies of two plant species (1 was positively affected – the grass-like starwort *S. graminea*, and 1 was negatively affected – the meadow foxtail *A. pratensis*). The insecticide treatment increased the frequency of *S. graminea* by a factor of 3.6 and reduced the frequency of *A. pratensis* by a factor of 3 compared with the control. These effects might have resulted from effects on plant-insect interactions. For example, the insecticide used in the present study (pyrethroid) may have had toxic or repellent effects on insects (Gist and Pless, 1985; Blair, 1991, Hahn et al., in prep) such as the micro-moth *Coleophora striatipennella*, which uses the flowers of *S. graminea* for oviposition, and the fruits/seeds as food for their larvae (Database of Insects & their Food Plants, 2013). In such a case, the insecticide may have reduced herbivorous and seed-eating insects (direct effect), and this reduction, in turn, had positive effects on the plant (indirect effect). In contrast, *A. pratensis* probably has mutualistic relations with specific arthropods, and the plant species is negatively affected (indirect effect) through the direct effect of the insecticide on such arthropods. These possibilities of **indirect effects of an insecticide application on plants** are only speculations and require further investigations.

However, it is generally known that insecticide inputs in field margins can cause direct effects on the abundance of arthropods. In the same field experiment, with the help of diploma students, I annually collected the arthropod communities in the plots of the study site using various sampling techniques (i.e., vacuum sampler, sweep nets, and photoeclectors) (for details of the methods used each year, see Appendix II). The results indicated that the insecticide affected the abundance of certain cicadas, spiders, caterpillars and grasshoppers. For example, 2.5 weeks after the insecticide application in 2010, two cicada species, the greenish-yellow leafhopper *Arthaldeus pascuellus* and the meadow froghopper *Philaenus spumarius*, showed significantly reduced abundances of approximately 50 % and 66 %, respectively, in plots receiving an insecticide treatment compared with the control (Felix 2011; see Appendix II for details).

Another insecticide effect, for example, could be found for grasshoppers of the genus *Chorthippus* (the meadow grasshopper *Chorthippus parallelus* and the steppe grasshopper *Chorthippus dorsatus)* two days after the insecticide application in 2012. Their abundances in the insecticide-treated plots (I, H+I, F+I, and F+H+I) were reduced by an average of 65 % compared with the control plots (Bauer 2013; for more information concerning insecticide effects on arthropods on the study site, see Appendix II).

However, whether these negative effects of insecticide applications on arthropods also have negative effects on plants is less studied (Crawley 1989, Miller et al. 2009). To quantify such effects and to make accurate statements concerning possible plant-insect interaction effects, an observation period longer than three years may be required. In addition, for such investigations, specific plant-insect monitoring studies, which were not performed in this field experiment, would be required.

7.2 Indirect effects of herbicides and fertilizers on arthropods

In the present field study, the plant species composition significantly changed in the herbicide- and fertilizer-treated plots. However, in addition to the effects on plants, the **fertilizer and herbicide applications may have indirectly affected certain arthropod species**. As primary producers, plants form the basis of any food web in an ecosystem. Therefore, **herbivores** were most likely also adversely affected. Some insects groups, such as butterflies or cicadas, show high host-plant specificity. Thus, a loss or a frequency reduction of plant species may also have a negative effect on the population size of certain herbivorous insects (Freemark & Boutin 1995; Longley & Sotherton 1997; Wilson et al. 1999). **Pollinators** may be negatively affected due to a reduced flowering intensity. Such a reduction in flowering was studied in the present field experiment for *R. acris*, *L. pratensis* and *V. sepium* (Papers II and III). This food source decrease might be particularly severe for specialist pollinators like the solitary bee *Chelostoma florisomne*, which depends entirely on *Ranunculus* pollen (Westrich 1989). However, the pollen of *R. acris* is also consumed by many other insects. Weiner et al. (2011) recorded 117 flower-visiting insects on this plant species in Germany.

Fig. 7-4: Examples of flower-visiting insects on the common buttercup *Ranunculus acris*. Photos were taken in May 2012 at the study site.

In addition to the reduced food availability, herbicides may also affect the **food quality** of plants. For herbivorous insects, plant quality is essential for growth and reproduction. A few studies reported that some Lepidoptera species seem to be negatively affected by herbicide-treated host plants (Agnello et al. 1986a 1986b; Kjaer & Elmegaard 1996; Hahn et al. submitted). For example, Hahn et al. (submitted) studied the effects of two herbicides on the quality of different host plant species of the cabbage moth *Mamestra brassicae*. Caterpillars feeding on one plant-herbicide combination (*R. acris* treated with 10 % of the field rate of a sulfonylurea herbicide) showed a mortality of 30 %, whereas less than 5 % of the caterpillars in the control died. In addition, the development time from caterpillars to moths significantly increased by a factor of 1.1 (corresponding to an extended development time of 6 days) (Hahn et al. submitted). In a field situation, a longer development time would most likely result in a higher predation risk for the caterpillar. Some herbicides may increase the biosynthesis of plant defenses in certain plant species, which might negatively affect herbivores via toxic or repellent effects (Kjaer et al. 1996; Hahn et al. submitted). However, such effects seem to be highly species-specific and have rarely been studied thus far (Hahn et al. submitted).

On the other hand, it is also possible that arthropods with a very wide food spectrum (e.g., grasses) may be positively affected. For example, in the first year of the field experiment, we observed a significantly higher abundance (4.3 times higher) of the polyphagous cicada species meadow spur-hopper, *Javesella pellucida*, in fertilizer-treated plots compared with control plots (Felix 2011; see Appendix II for details). A nutrient supply can increase the developmental rates of cicadas; therefore, the fertilizer treatment may have caused a faster growth of juvenile cicadas (Nickel & Hildebrandt 2003), which may explain the increased abundance of *J. pellucida* in the fertilizer-treated plots (Felix 2011).

However, not only pollinators and herbivorous insects are likely to be adversely affected by a loss of plant species or a reduced flowering intensity, but also carnivorous arthropods, such as spiders. For example, crab spiders (Thomisidae, also called flower spiders) use flowers for hunting purposes. Crab spiders remain motionless on or beside flowers until their prey (small arthropods) arrives, and then they catch it (Morse 2007). In the second year of the field experiment, spiders were caught with sweep nets in all plots of the study site, and a significantly reduced density of crab spiders was observed in all treatments compared with the control in June 2011. The lowest density of crab spiders was recorded in the insecticide treatment (approximately 40 % fewer individuals than in the control). However, an average reduced crab spider density of approximately 30 % compared with the control was also observed in plots treated with the herbicide and/or fertilizer. These reductions might have been caused by changes in the vegetation/habitat structure and by the reduced flowering intensity in the herbicide-treated plots (Metz 2013; see Appendix II for details).

Therefore, in contrast to insecticides, herbicides or fertilizers do not directly affect arthropods, but may affect them indirectly by changing plant species composition, habitat structure and host plant availability and quality. Thus, repeated herbicide and fertilizer applications in field margins over several years may have ecological consequences for higher trophic levels and, thus, may affect the biodiversity of agricultural landscapes. However, until now, only limited attempts to study such effects through the food web have been made (Boutin et al. 2012).

7.3 Field margins in Germany

Narrow field margins adjacent to arable fields in Germany are most likely similarly exposed to pesticides and fertilizers, as simulated in the present study. This exposure occurs because field margins less than 3 m in width are not considered as terrestrial non-target areas in Germany (as already noted in the introduction); therefore, a farmer does not have to use drift-reducing technologies or to keep a distance to adjacent field margins during field application (Kühne et al. 2000). As a consequence, the pesticide application on arable land is conducted directly up to the border of the field, and thus, the neighboring margin receives not only spray-drift but also a partial overspray. Moreover, no distance requirements exist for fertilizer applications next to field margins, which could prevent fertilizer misplacements.

The results of the field experiment suggest that long-term misplacements of fertilizers and herbicides in field margins lead to a species-poor plant community in which a few robust and least susceptible species make up the main part of the vegetation. These results correspond well with monitoring data from field margins in agricultural landscapes. Field margins tend to develop vegetation particularly dominated by grasses and a few dicotyledonous plants. Flowering plants were only rarely observed (Jobin et al. 1998; de Snoo & van der Poll 1999; Kleijn & Verbeek 2000; Tarmi et al. 2002; Roß-Nickoll et al. 2004; Hovd & Skogen 2005). These findings were also supported by own monitoring studies in the proximity of the study site where the presence and absence of *R. acris* in field margins at 10 m intervals along a stretch of 11 km was recorded (Paper II). During this monitoring, *R. acris* was only observed in 7 % of the monitoring points in field margins adjacent to cereal fields, whereas the species was recorded frequently in margins next to meadows (where usually no agrochemicals are applied) or orchards (where herbicides and fertilizers are directly applied to the stem base) (in 85 % and 30 %, respectively, of the monitoring points; Paper II). Furthermore, two other monitoring studies near the study site found that field margins in the intensively used agricultural landscape are primarily characterized by grasses such as *D. glomerata* and *A. elatius* and a few robust dicotyledons such as *G. mollugo* and the common nettle *Urtica dioica* (Bakanov 2011; Schäfer 2013).This finding is also consistent with the results of the present field experiment, where *D. glomerata* increased in abundance in plots treated with fertilizer and herbicide in combination and *G. mollugo* was not affected by the fertilizer or herbicide treatment (Paper I). Because agrochemicals have been applied for the last five to six decades (since their introduction after the Second World War), it can be assumed that today, only these species that are least sensitive to pesticides and fertilizers remain in narrow field margins directly adjacent to an arable field (Fig. 7-5).

Fig. 7-5: Field margin adjacent to a cereal field (left) and a field margin adjacent to a tree row (orchard), which usually receives less or no exposure to fertilizer or herbicide misplacements (right).

7.4 Risk assessment of herbicides for non-target plants

The herbicide risk assessment (RA) aims to protect non-target plants in off-field habitats, such as field margins, from adverse effects (European Commission 2002). Although OECD testing guidelines allow the use of non-crop species (OECD 2006), the standard RA uses crop plants for testing procedures. However, recent studies have expressed concern regarding whether the tested species are protective of wild plant species in off-field habitats (Boutin & Rogers 2000; Strandberg et al. 2012). So far, available databases concerning the sensitivity of **crops versus wild plant** species are highly restricted and thus, much uncertainty about the use of crop plants as surrogates for non-crop or native plant species exists (Schmitz et al., 2013: unpublished report commissioned by the German Federal Environment Agency [Umweltbundesamt, UBA]).

Furthermore, currently, no tests are required to assess herbicide effects on **reproduction** because only young plant species (2-6 leaf stage) are used. However, for some species, reproductive endpoints (flowering and seed production) may be more sensitive to herbicide applications than vegetative endpoints (biomass) (Paper II, Strandberg et al. 2012; Boutin et al. 2014). This is especially true when plants are exposed at later development stages in the field when growth has ceased (Boutin et al. 2014 and references therein) or when the plants are in the budding stage immediately before flowering (Kjaer et al. 1996a, b). These negative effects on flowering and seed production result in negative effects on reproduction and, potentially, on the population development of the affected species in the long term (Carpenter et al. 2013). Therefore, herbicide effect assessments focusing only on effects on biomass are presumably underestimating the full herbicide effects. It can be assumed that effects on biomass and reproduction are highly dependent on the herbicide product (formulation, and active ingredients), the tested species and its development stage. However, for most herbicides and wild plant species, the effect on reproductive stages is not known, and thus, there is growing uncertainty by what factor the biomass endpoint differs from the reproductive endpoint.

In addition, with the standard test methods currently performed in the RA, interaction effects, such as intra- and interspecific competition, which can be altered by exposure to herbicides, are not considered. More realistic testing approaches (**higher-tier studies,** such as microcosms, mesocosms and field studies) seem to be an effective way to investigate interactions among species and to increase our understanding of natural processes by simplifying the complexity of the natural environment (Fraser & Keddy 1997; Marrs & Frost 1997; Dalton & Boutin 2010) (Paper IV). Such studies may have the potential to reduce uncertainties concerning the extrapolation from single-species tests to field situations. However, no appropriate and validated test systems are available and therefore, much work is required to develop and to establish appropriate testing methods for regulatory processes.

The RA of pesticides is performed for only one specific compound, and therefore, the **combined effects of pesticides and interaction effects with fertilizers** are not considered and represent a further uncertainty concerning the protection of wild plant species in the field. Plants in field margins are exposed to repeated pesticide and fertilizer applications with alternating pesticides during the growing season every year. This might lead to additive or synergistic effects. However, studies investigating the effects of pesticides and/or fertilizers on natural plant communities of field margins are scarce (Paper IV).

Considering the above-mentioned points, it seems that the currently performed RA provides insufficient protection for non-target plants and their habitats. To improve the RA of herbicides, investigating reproduction effects of herbicides with different modes of action on a range of wild plant species is recommended. Moreover, to get a more precise estimate about the uncertainties in the current RA, it is important to compare the sensitivity of wild and crop species in one study set with exactly matching conditions and to increase our knowledge about the extrapolation from single-species tests performed in the greenhouse to field situations.

8 Conclusion and outlook

The present field experiment demonstrated that herbicide and fertilizer misplacements in field margins are major factors that affect the natural plant communities of these habitats. Both agrochemicals (individually and in combination) reduced the frequencies of several plant species and significantly affected the plant species composition and species diversity. These effects of fertilizers and herbicides are particularly severe when field margins are exposed to repeated agrochemical misplacements over three or more successive years. It can be assumed that such long-term misplacements in intensively used agricultural landscapes are common practice and therefore, fertilizers and herbicides have likely contributed to the species impoverishments in field margins in recent decades. The negative effects on the vegetation of field margins may also involve detrimental effects for other non-target organisms (e.g., herbivores, pollinators, and predators) due to altered habitats and food sources (indirect effects). Thus, the entire biodiversity of agricultural landscapes might be negatively affected.

To preserve biodiversity in agro-ecosystems, it is necessary to protect the vegetation of margins from adverse effects. To achieve such protection, adaptations and improvements of the current RA to better evaluate the effects of herbicides on non-target plants are crucial. In addition, developing of general risk mitigation measures (RMM) for the application of pesticides is warranted. Here, it would be especially important not to exclude narrow field margins (0-3 m wide) because these margins are the last remaining habitats for wildlife in farmlands in Germany. RMM can include drift-reducing technologies or unsprayed buffer-zone distances to the off-crop habitat. For example, van de Zande et al. (2012) developed a matrix approach that combines drift-reducing spray-nozzles (50 %, 75 %, and 95 % drift reduction class) and the width of unsprayed buffer zones to calculate the spray-drift depositions in off-crop habitats. The required width of an unsprayed buffer zone to protect off-field habitats (e.g., field margins) can be determined with this method (van de Zande et al. 2012). The use of an end-nozzle is also an effective way to reduce spray-drift and to prevent overspray of field margins (van de Zande et al. 2007). In addition to such RMM, it will be important to develop regulations for the application of fertilizers next to field margins.

However, the changes in species composition and the loss of flowering plants in field margins as a result of the agricultural intensification over the last several decades cannot be reversed by only minimizing the risks of herbicides and fertilizers to terrestrial non-target plants. In addition, landscape management approaches with a focus on preserving and enhancing (in quality or size) the existing field margins would be valuable. To enhance the habitat quality of grassy field margins, a seed mixture of meadow plant species can be added (Bokenstrand et al. 2004). This method is usually a simple way of providing a given level of diversity of attractive and desirable species quickly (Meek et al. 2002; Pywell et al. 2004; Olson & Wäckers 2007). According to Vickery et al. (2009), field margins should also be managed in conjunction with adjacent boundary features, especially hedgerows, to create complex structures and network complexity that maximize nesting opportunities for birds and a range of invertebrates at the landscape scale.

Such risk mitigation measures and management schemes could help to increase and conserve plant diversity in field margins in agricultural landscapes and to ensure the food resources for the entire terrestrial food web. This would also benefit “Target 3” of the “EU Biodiversity Strategy for 2020”, which aims to increase the contribution of agriculture to the preservation of biodiversity and to improve the conservation status of species and habitats that depend on or that are affected by agriculture (European Commission 2011). However, the design of field margin management schemes and their implementation represent a complex challenge and thus, applied research projects are required to develop appropriate management schemes that are accepted by farmers and landscape planners alike.

9 References

Aebischer, N.J., Blake, K.A. (1994). Field Margins as Habitat for Game. In Boatman, N (Ed.): Field Margins: Integrating Agriculture and Conservation, pp. 95-104. BCPC Monograph No 58. Proceedings of a symposium organized by the British Crop Protection Council in association with the British Ecological Society and the Association of Applied Biologists and held at the University of Warwick, Coventry on 18-20 April 1994.

Agnello, A.M., Bradley, J.R., Van Duyn, J.W. (1986). Plant-mediated effects of postemergence herbicides on *Pseudoplusia includens*. *Journal of agricultural entomology* 3: 61-65.

Agnello, A.M., Van Duyn, J.W., Bradley, J.R. (1986). Influence of postemergence herbicides on populations of bean leaf beetle, *Cerotoma trifurcata* (Coleoptera: Chrysomelidae), and corn earworm, *Heliothis zea* (Lepidoptera: Noctuidae), in soybeans. *Journal of Economic Entomology* 79 (1): 261-265.

Anderson, M.J., Gorley, R.N., Clarke, K.R. (Eds.) (2008). Permanova+ for Primer: Guide to software and statistical methods. Primer-E Ltd, Polymouth, UK.

Andreasen, C., Streibig, J.C. (2011). Evaluation of changes in weed flora in arable fields of Nordic countries - based on Danish long-term surveys. *Weed Reseach* 51 (3), 214-226.

Asteraki, E.J., Hart, B.J., Ings, T.C., Manley, W.J. (2004). Factors influencing the plant and invertebrate diversity of arable field margins. *Agriculture, Ecosystems & Environment*. 102 (2), 219-231.

Bäckman, J.P.C., Tiainen, J. (2002). Habitat quality of field margins in a Finnish farmland area for bumblebees (*Hymenoptera : Bombus and Psithyrus*). *Agriculture, Ecosystems & Environment* 89 (1-2): 53-68.

Baessler, C., Klotz, S. (2006). Effects of changes in agricultural land-use on landscape structure and arable weed vegetation over the last 50 years. *Agriculture Ecosystems & Environment* 115 (1-4): 43-50.

Ball, D.A. (1992). Weed seedbank response to tillage, herbicides, and crop rotation sequence. *Weed Science* 40 (4), 654-659.

Bakanov, N. (2011). Floristische Artenzusammensetzung in anthropogen beeinflussten Saumstrukturen. *Fallstudie*. Institut für Umweltwissenschaften, Universität Koblenz-Landau, Campus Landau.

Bauer, P. (2013): Der Einfluss von Dünger, Pestiziden und ihrer Kombination auf Orthopteren und Lepidopteren in Feldsäumen. *Fallstudie*. Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau.

Bekker, R.M., Bakker, J.P., Grandin, U., Kalamees, R., Milberg, P., Poschlod, P., Thompson, K., Willems, J.H. (1998). Seed size, shape and vertical distribution in the soil: indicators of seed longevity. *Functional Ecology* 12 (5), 834-842.

Benton, T.G., Vickery, J.A., Wilson, J.D. (2003). Farmland biodiversity: is habitat heterogeneity the key? *Trends In Ecology & Evolution* 18 (4): 182-188.

Berendse, F., Chamberlain, D., Kleijn, D., Schekkerman, H., 2004. Declining biodiversity in agricultural landscapes and the effectiveness of agri-environment schemes. *Ambio* 33 (8), 499-502.

Blair, B.W. (1991). The concentration of lamdacyhalothrin and its effect on the cutworm *Agrotis segetum* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 81 (02): 143-145.

BMELV (2013). Landwirtschaft verstehen - Fakten und Hintergründe. Berlin, Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV), Berlin.

Boatman, N.D. (1994). The impact of nitrogen fertilisers on field margin flora. In Boatman, N. (Ed.): Field Margins: Integrating Agriculture and Conservation, pp. 209-214. BCPC Monograph No 58. Proceedings of a symposium organized by the British Crop Protection Council in association with the British Ecological Society and the Association of Applied Biologists and held at the University of Warwick, Coventry on 18-20 April 1994.

Bokenstrand, A., Lagerlof, J., Torstensson, P.R. (2004). Establishment of vegetation in broadened field boundaries in agricultural landscapes. *Agriculture Ecosystems & Environment* 101 (1): 21-29.

Boutin, C., Aya, K.L., Carpenter, D., Thomas, P.J., Rowland, O. (2012). Phytotoxicity testing for herbicide regulation: shortcomings in relation to biodiversity and ecosystem services in agrarian systems. *Science of the Total Environment* 415: 79-92.

Boutin, C., Lee, H.B., Peart, E.T., Batchelor, P.S., Maguire, R.J. (2000). Effects of the sulfonylurea herbicide metsulfuron methyl on growth and reproduction of five wetland and terrestrial plant species. *Environmental Toxicology and Chemistry* 19 (10), 2532-2541.

Boutin, C., Jobin, B. (1998). Intensity of agricultural practices and effects on adjacent habitats. *Ecological Applications* 8 (2): 544-557.

Boutin, C., Rogers, C.A. (2000). Pattern of sensitivity of plant species to various herbicides - An analysis with two databases. *Ecotoxicology* 9 (4): 255-271.

Boutin, C., Strandberg, B., Carpenter, D., Mathiassen, S.K., Thomas, P.J. (2014). Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications? *Environmental Pollution* 185: 295-306.

Boutin, C., White, A.L., Carpenter, D. (2010). Measuring variability in phytotoxicity testing using crop and wild plant species. *Environmental Toxicology and Chemistry* 29 (2): 327-337.

Breeze V, Thomas G, Butler R. (1992). Use of model and toxicity data to predict the risks to some wild plants species drom drift of four herbicides. *Annals of Applied Biology* 121:669-677.

Brown K, Tomlinson J, Duncan J, Hinchcliffe A (2009). Literature reviews on ecotoxicology of chemicals with special focus on plant protection products. Reference: CFT/EFSA/PPR/2008/01. Exponent International Limited, Harrogate, Yorkshire, UK

Bunce, R.G.H., Howard, D.C., Barr, C.J., Cummins, C.P., French, D. (1994). Botanical diversity in British hedgerows. In Boatman, N. (Ed.): Field Margins: Integrating Agriculture and Conservation, pp- 43-52. BCPC Monograph No 58. Proceedings of a symposium organized by the British Crop Protection Council in association with the British Ecological Society and the Association of Applied Biologists and held at the University of Warwick, Coventry on 18-20 April 1994.

Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft (2006). Richtlinien für die sachgerechte Düngung. BMLFUW, Wien.

Bundschuh, R., Schmitz, J.,Bundschuh, M., Brühl, C.A. (2012): Does insecticide drift adversely affect grasshoppers (Orthoptera: Saltatoria) in field margins? A case study combining laboratory acute toxicity testing with field monitoring data. *Environmental Toxicology and Chemistry* 31(8): 1874-1879.

Bührer, W. (2011). 50 Jahre Düngemittelstatistik in Deutschland. Statistisches Bundesamt. *Wirtschaft und Statistik* 5: 367-371.

BVL (2013a). Absatz an Pflanzenschutzmitteln in der Bundesrepublik Deutschland - Ergebnisse der Meldung gemäß § 19 Pflanzenschutzgesetz für das Jahr 2012. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Braunschweig,

BVL (2013b). Kodeliste für Kennzeichnungstexte und sonstige Auflagen zum Pflanzenschutzmittel-verzeichnis. Last accessed August 2013, see http://www.bvl.bund.de/DE/04_Pflanzenschutzmittel/01_Aufgaben/02_ZulassungPSM/01_ZugelPSM/01_OnlineDatenbank/psm_onlineDB_node.html.

BVL (2013c). "Verzeichnis zugelassener Pflanzenschutzmittel - Standardsuche", Last access July 2013 see. https://portal.bvl.bund.de/psm/jsp/ListeMain.jsp?page=1&ts=1375103957718.

Carpenter, D., Boutin, C. (2010). Sublethal effects of the herbicide glufosinate ammonium on crops and wild plants: short-term effects compared to vegetative recovery and plant reproduction. *Ecotoxicology* 19 (7): 1322-1336.

Carpenter, D., Boutin, C., Allison, J. (2013). Effects of chlorimuron ethyl on terrestrial and wetland plants: Levels of, and time to recovery following sublethal exposure. *Environmental Pollution* 172: 275–282.

Carreck, N.L., Williams, I.H. (2002). Food for insect pollinators on farmland: insect visits to flowers of annual seed mixtures. *Journal of Insect Conservation* 6 (1): 13-23.

Chamberlain, D.E., Fuller, R.J., Bunce, R.G.H., Duckworth, J.C., Shrubb, M. (2000). Changes in the abundance of farmland birds in relation to the timing of agricultural intensification in England and Wales. *Journal of Applied Ecology* 37 (5): 771-788.

Clark, C.M., Tilman, D. (2008). Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* 451 (7179), 712-715.

Cooper J, Dobson H. (2007). The benefits of pesticides to mankind and the environment. *Crop Protection* 26 (9):1337–1348.

Crawley, M.J. (1989). Insect herbivores and plant population dynamics. *Annual Review of Entomology* 34: 531–564.

Dalton, R.L., Boutin, C. (2010). Comparision of the effects of glyphoste and atrazine herbicides on non-target plants grown single and in microcosms. *Environmental Toxicology and Chemistry* 29 (10): 2304-2315.

Damgaard, C., Fayolle, A. (2010). Measuring the importance of competition: a new formulation of the problem. *Journal of Ecology* 98 (1): 1-6.

Damgaard, C., Mathiassen, S.K., Kudsk, P. (2008). Modeling effects of herbicide drift on the competitive interactions between weeds. *Environmental Toxicology and Chemistry* 27 (6): 1302-1308.

Damgaard, C., Strandberg, B., Mathiassen, S.K., Kudsk, P. (2011). The combined effect of nitrogen and glyphosate on the competitive growth, survival and establishment of *Festuca ovina* and *Agrostis capillaris*. *Agriculture, Ecosystems & Environment* 142 (3–4): 374-381.

Damgaard, C., Strandberg, B., Mathiassen, S.K., Kudsk, P. (2013). The effect of nitrogen and glyphosate on survival and colonisation of perennial grass species in an agro-ecosystem: Does the relative importance of survival decrease with competitive ability? *Plos One* 8 (4).

Database of Insects and their Food Plants, 2013. Last assessed October 2013, see http://www.brc.ac.uk/dbif/hostsresults.aspx?hostid=5408

Davis BNK, Lakhani KH, Yates TJ, Frost AJ, Plant RA (1993). Insecticide drift from ground-based, hydraulic spraying of peas and brussels-sprouts - Bioassays for determining buffer zones. *Agriculture, Ecosystems & Environment* 43:93-108.

De Cauwer B, Reheul D, Nijs I, Milbau A. (2006). Effect of margin strips on soil mineral nitrogen and plant diversity. *Agronomy for Sustainable Development* 26:117-126.

de Jong FMW, de Haes HAU (2001). Development of a field bioassay for the side-effects of herbicides on vascular plants using Brassica napus and Poa annua. *Environmental Toxicology and Chemistry* 16:397-407.

de Snoo, G.R. (1999). Unsprayed field margins: effects on environment, biodiversity and agricultural practice. *Landscape and Urban Planning* 46 (1-3), 151-160.

de Snoo, G.R., Tamis, W.L.M., van de Brink, P.J., van der Poll, R.J. (2005). Non target plant field study: effects of glufosinate-ammonium on off crop vegetation. Leiden University, Institute of Environmental Sciences (CML), Leiden.

de Snoo, G.R., van der Poll, R.J. (1999). Effect of herbicide drift on adjacent boundary vegetation. *Agriculture Ecosystems & Environment* 73 (1): 1-6.

Deckers, B., Hermy, M., Muys, B. (2004). Factors affecting plant species composition of hedgerows: relative importance and hierarchy. *Acta Oecologica* 26: 23-27.

Dennis, P., Fry, G.L.A. (1992). Field margins - can they enhance natural enemy population-densities and general arthropod diversity on farmland? *Agriculture Ecosystems & Environment* 40 (1-4): 95-115.

Dover, J.W. (1994). Arable field margins: factors affecting butterfly distribution and abundance. In: Boatman, N. (Ed.): Field Margins: Integrating Agriculture and Conservation, pp. 59-66. BCPC Monograph No 58. Proceedings of a symposium organized by the British Crop Protection Council in association with the British Ecological Society and the Association of Applied Biologists and held at the University of Warwick, Coventry on 18-20 April 1994.

Egan, F., Bohnenblust, E., Goslee, S., Mortensen, D., Tooker, J. (2014). Herbicide drift can affect plant and arthropod communities. *Agriculture Ecosystems & Environment* 185: 77-87.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulißen, D. (1992). Zeigerwerte der Pflanzen in Mitteleuropa. Göttingen, Verlag Erich Goltze KG.

Elzinga, C.L., Salzer, D.W., Willoughby, J.W. (Eds.), 1998. Measuring and monitoring plant populations. Bureau of Land Management, National Business Center, Denver, Colorado.

European Commission (2002). Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC, Working Document Sanco/10329/2002 Rev. 2 final, October 17, 2002. Luxembourg.

European Commission (2009). Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union. 309.

European Commission (2011). The EU Biodiversity Strategy to 2020. Luxembourg, Publications Office of the European Union.

European and Mediterranean Plant Protection Organization (EPPO) (2003). EPPO Standards: Environmental risk assessment scheme for plant protection products. EPP/EPPO Bulletin 33: 239-244.

Eurostat (2007). The use of plant protection products in the European Union. Data 1992-2003. Office for Official Publications of the European Communities, Luxembourg.

Felix, T. (2011). Untersuchung zum Einfluss des Dünger-, Herbizid- und Insektizideintrags auf die Biodiversität von Feldsäumen am Beispiel von Zikaden (Auchenorrhyncha). *Diplomarbeit.* Institut für Umweltwissenschaften, Universität Koblenz-Landau, Campus Landau.

Finch-Savage, W.E., Leubner-Metzger, G. (2006). Seed dormancy and the control of germination. *New Phytologist* 171 (3), 501-523.

Firbank, L.G., Petit, S., Smart, S., Blain, A., Fuller, R.J. (2008). Assessing the impacts of agricultural intensification on biodiversity: a British perspective. *Philosophical Transactions Of The Royal Society B-Biological Sciences* 363 (1492): 777-787.

Fletcher, J.S., Pfleeger, T.G., Ratsch, H.C., Hayes, R. (1996). Potential impact of low levels of chlorsulfuron and other herbicides on growth and yield of nontarget plants. *Environmental Toxicology and Chemistry* 15 (7), 1189-1196.

Flohre, A., Fischer, C., Aavik, T., Bengtsson, J., Berendse, F., Bommarco, R., Ceryngier, P., Clement, L.W., Dennis, C., Eggers, S., Emmerson, M., Geiger, F., Guerrero, I., Hawro, V., Inchausti, P., Liira, J., Morales, M.B., Onate, J.J., Part, T., Weisser, W.W., Winqvist, C., Thies, C., Tscharntke, T. (2011). Agricultural intensification and biodiversity partitioning in European landscapes comparing plants, carabids, and birds. *Ecological Applications* 21 (5): 1772-1781.

Fraser, L.H., Keddy, P. (1997). The role of experimental microcosms in ecological research. *Trends in Ecology & Evolution* 12 (12): 478-481.

Freemark, K., Boutin, C. (1995). Impacts of agricultural herbicide use on terrestrial wildlife in temperate landscapes - A review with special reference to North-America. *Agriculture Ecosystems & Environment* 52 (2-3): 67-91.

Freier B, Pallutt B, Jahn M, Sellmann J, Gutsche V, Zornbach, W. (2008). Netz Vergleichsbetriebe Pflanzenschutz: Jahresbericht 2007. Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen.

Fuchs, M. (2012). Stickstoff-Düngung im Spannungsfeld zwischen Ertragssicherung und Umweltwirkungen, Fachtagung Düngung 2012, SKW Stickstoffwerke Piesteritz GmbH.

Füll, C., Jung, S., Schulte, C. (2000). Plant protection products: assessing the risk for terrestrial plants. *Chemosphere* 41 (4): 625-629.

Ganzelmeier, H., Rautmann, D., Spangenberg, R., Streloke, M., Hermann, M., Wenzelburger, H.-J., Friedrich, H.-W. (1995). Untersuchungen zur Abdrift von Pflanzenschutzmitteln. BBA, Berlin.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Part, T., Bretagnolle, V., Plantegenest, M., Clement, L.W., Dennis, C., Palmer, C., Onate, J.J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hanke, S., Fischer, C., Goedhart, P.W., Inchausti, P. (2010). Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology* 11 (2): 97-105.

Gist, G.L., Pless, C.D. (1985). Ovicidal activity and ovipositional repellent properties of synthetic pyrethroids to the fall armyworm, *Spodoptera frugiperda*. *The Florida Entomologist* 68 (3): 462-466.

Gove, B., Power, S.A., Buckley, G.P., Ghazoul, J. (2007). Effects of herbicide spray drift and fertilizer overspread on selected species of woodland ground flora: comparison between short-term and long-term impact assessments and field surveys. *Journal of Applied Ecology* 44 (2): 374-384.

Hahn, M., Geisthardt, M., Brühl, C.A. (2014). Effects of herbicide treated host plants on the development of *Mamestra brassicae* (L.) caterpillars. *Environmental Toxicology and Chemistry* 33 (11): 2633–2638.

Hahn, M., Lenhardt, P.P., Brühl, C.A. (2014). Characterization of field margins in intensified agro-ecosystems – why narrow margins should matter in terrestrial pesticide risk assessment and management. *Integrated Environmental Assessment and Management* 10 (3): 456-462.

Hahn, M., Schotthöfer, A., Schmitz, J., Franke, L., Brühl., C.A.: (submitted): Insecticide inputs in field margins – how moths (Lepidoptera) can be affected. *Agriculture Ecosystems & Environment.*

Hautier, Y., Niklaus, P.A., Hector, A. (2009). Competition for light causes plant biodiversity loss after eutrophication. *Science* 324 (5927): 636-638.

Hejcman, M., Klaudisova, M., Schellberg, J., Honsova, D. (2007). The Rengen Grassland Experiment: Plant species composition after 64 years of fertilizer application. *Agriculture Ecosystems & Environment* 122 (2), 259-266.

Holzschuh, A., Steffan-Dewenter, I., Tscharntke, T. (2009). Grass strip corridors in agricultural landscapes enhance nest-site colonization by solitary wasps. *Ecological Applications* 19 (1): 123-132.

Hovd, H., Skogen, A. (2005). Plant species in arable field margins and road verges of central Norway. *Agriculture Ecosystems & Environment* 110 (3-4): 257-265.

Jobin, B., Boutin, C., DesGranges, J.L. (1997). Effects of agricultural practices on the flora of hedgerows and woodland edges in southern Quebec. *Canadian Journal of Plant Science* 77 (2): 293-299.

Jumpponen, A., Mulder, C.P.H., Huss-Danell, K., Högberg, P. (2005). Winners and losers in herbaceous plant communities: insights from folier carbon isotope composition in monocultures and mixtures. *Journal of Ecology* 93, 1136-1147.

Kindt, R., Coe, R. (2005). Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi.

Kjær, C., Elmegaard, N. (1996). Effect of herbicide treatment on host plant quality for a leaf-eating beetle. *Pesticide Science* 47 (4): 319-325.

Kjær, C., Strandberg, M., Erlandsen, M. (2006a). Metsulfuron spray drift reduces fruit yield of hawthorn (*Crataegus monogyna* L.). *Science of the Total Environment* 356 (1–3): 228-234.

Kjær, C., Strandberg, M., Erlandsen, M. (2006b). Effects on hawthorn the year after simulated spray drift. *Chemosphere* 63 (5): 853-859.

Kleijn, D., Kohler, F., Baldi, A., Batary, P., Concepcion, E.D., Clough, Y., Diaz, M., Gabriel, D., Holzschuh, A., Knop, E., Kovacs, A., Marshall, E.J.P., Tscharntke, T., Verhulst, J. (2009). On the relationship between farmland biodiversity and land-use intensity in Europe. *Proceedings of the Royal Society B-Biological Sciences* 276 (1658): 903-909.

Kleijn, D., Snoeijing, G.I.J. (1997). Field boundary vegetation and the effects of agrochemical drift: Botanical change caused by low levels of herbicide and fertilizer. *Journal of Applied Ecology* 34 (6): 1413-1425.

Kleijn, D., Verbeek, M. (2000). Factors affecting the species composition of arable field boundary vegetation. *Journal of Applied Ecology* 37 (2): 256-266.

Klotz, S., Kühn, I., Durka, W., 2002. BiolFlor - Datenbank biologisch-ökologischer Merkmale der Flora von Deutschland. Umweltforschungszentrum Leipzig-Halle GmbH.

Koch H, Weißer P (2004). A proposal for a higher tier Investigation of pesticide drift exposure to non-target organisms (NTO) in field trials. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 56:180-183.

Köhler, W., Schachtel, G., Voleske, P. (2001). Biostatistik - Eine Einführung für Biologen und Agrarwissenschaftlter. Berlin, Heidelberg, New York, Springer-Verlag.

Kühne, S., Enzian, S., Jüttersonke, B., Freier, B., Forster, R., Rothert, H. (2000). Beschaffenheit und Funktion von Saumstrukturen in der Bundesrepublik Deutschland und ihre Berücksichtgung im Zulassungsverfahren im Hinblick auf die Schonung von Nichtzielarthropoden. Mitteilung der Biologisches Bundesanstalt für Land- und Forstwirtschaft, Vol. 378: 1-128, Berlin-Dahlem.

Kühne, S., Freier, B. (2001). Saumbiotope in Deutschland - ihre historische Entwicklung, Beschaffenheit und Typisierung. Biozönosen in Saumbiotopen in landwirtschaftlichen Einflussbereichen: Beeinflussung durch Pflanzenschutzmitteleinträge? Mitteilung der Biologisches Bundesanstalt für Land- und Forstwirtschaft, Vol. 387: 24-29. Berlin-Dahlem, Germany.

Kühne S, Freier B, Kaul P, Jütersonke B, Schenke D, Forster R, Baier B, Moll E. (2002). Auswirkung der Abdrift von Insektiziden in einem Saumbiotop. Bern, Hannover.

Lamoureaux SL, Bourdot GW. (2007). A review of the ecology and management of *Ranunculus acris* subsp. acris in pasture. *Weed Research* 47:461-471.

Langhof M, Gathmann A, Poehling H-M (2005). Insecticide drift deposition on noncrop plant surfaces and its impact on two beneficial nontarget arthropods, *Aphidius colemani* viereck (Hymenoptera, Braconidae) *and Coccinella septempunctata* L. (Coleoptera, Coccinellidae). *Environmental Toxicology and Chemistry* 24:2045-2054.

Longley, M., Sotherton, W. (1997). Factors determining the effects of pesticides upon butterflies inhabiting arable farmland. *Agriculture, Ecosystems and Environment* 61 (1): 1-12.

Ma, M.H., Tarmi, S., Helenius, J. (2002). Revisiting the species-area relationship in a semi-natural habitat: floral richness in agricultural buffer zones in Finland. *Agriculture Ecosystems & Environment* 89 (1-2): 137-148.

Marrs, R.H., Frost, A.J. (1997). A microcosm approach to the detection of the effects of herbicide spray drift in plant communities. *Journal of Environmental Management* 50: 369-388.

Marrs, R.H., Frost, A.J., Plant, R.A., (1991a). Effects of herbicide spray drift on selected species of nature conservation interest - the effects of plant-age and surrounding vegetation structure. *Environmental Pollution* 69, 223-235.

Marrs RH, Frost AJ, Plant RA (1991b). Effect of mecoprop drift on some plant-species of conservation interest when grown in standardized mixtures in microcosms. *Environmental Pollution* 73:25-42.

Marrs RH, Frost AJ, Plant RA, Lunnis P (1993). Determination of buffer zones to protect seedlings of nontarget plants from effects of glyphosate spray drift. *Agriculture Ecosystems & Environment* 45:283-293

Marrs, R.H., Williams, C.T., Frost, A.J., Plant, R.A. (1989). Assessment of the effects of herbicide spray drift on a range of plant species of conservation interest. *Environmental Pollution* 59 (1): 71-86.

Marshall, E.J.P., 2009. The impact of landscape structure and sown grass margin strips on weed assemblages in arable crops and their boundaries. *Weed Research* 49 (1), 107-115.

Marshall, E.J.R., Moonen, A.C. (2002). Field margins in northern Europe: their functions and interactions with agriculture. *Agriculture Ecosystems & Environment* 89 (1-2): 5-21.

Marshall, E.J.P., Smith, B.D. (1987). Field margin flora and fauna; interaction with agriculture. Field Margins. BCPC Monograph No. 35. Proceedings of a Workshop organized by the British Crop Protection Council and held in London on 11th March, 1986. J. M. Way and P. W. Greig-Smith. Thornton Heath, The British Crop Protection Council: 23-33.

Meek, B., Loxton, D., Sparks, T., Pywell, R., Pickett, H., Nowakowski, M. (2002). The effect of arable field margin composition on invertebrate biodiversity. *Biological Conservation* 106 (2): 259-271.

Metz, M. (2012): Quantifizierung der Effekte von Pflanzenschutzmitteln und Dünger auf die Spinnendiversität (Araneae) eines Feldsaums. Diploma thesis. Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau.

Miller, T.E.X., Louda, S.M., Rose, K.A., Eckberg, J.O. (2009). Impacts of insect herbivory on cactus population dynamics: experimental demography across an environmental gradient. *Ecological Monographs* 79 (1): 155-172.

Morse, D. (2007). Predator upon a flower: life history and fitness in a crab spider, In Harvard University Press 2007 Cambridge.

Nentwig, W. (2000). Die Bedeutung von streifenförmigen Strukturen in der Kulturlandschaft. In: Nentwig, W. (Ed.): Streifenförmige ökologische Ausgleichsflächen in der Kulturlandschaft: Ackerkrautstreifen, Buntbrache, Feldränder, pp. 11-40. Verlag Agrarökologie**,** Bern, Hannover.

Nickel, H., Hildebrandt, J. (2003). Auchenorrhyncha communities as indicators of disturbance in grasslands (Insecta, Hemiptera) - a case study from the Elbe flood plains (northern Germany). *Agriculture Ecosystems & Environment* 98 (1-3): 183-199.

Oksanen, J., 2013. Multivariate analysis of ecological communities in R: vegan tutorial. R Foundation for Statistical Computing, 1-43.

Olson, D.M., Wackers, F.L. (2007). Management of field margins to maximize multiple ecological services. *Journal of Applied Ecology* 44 (1): 13-21.

Olszyk DM, Burdick CA, Pfleeger TG, Lee EH, Watrud LS (2004). Assessing the Risks to Non-Target Terrestrial Plants from Herbicides. *Journal of Agricultural Meteorology* 60:221-242.

Olszyk, D., Pfleeger, T., Lee, E.H., Burdick, C., King, G., Plocher, M., Kern, J. (2008). Selecting and evaluating native plants for region-specific phytotoxicity testing. *Integrated environmental assessment and management* 4 (1): 105-117.

Organistion for Economic Cooperation and Development (OECD) (2006). Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test, OECD Publishing.

Perry, N.H., Chaney, K., Wilcox, A., Boatman, N.D. (1996). The effect of fertiliser and herbicide application on herbaceous field margin communities. In: Vegetation Management in Forestry, Amenity and Conservation Areas: Managing for Multiple Objectives; Aspects of Applied Biology, Association of Applied Biologists, Wellesbourne. 44**:** 339-344.

Pfiffner, L., Luka, H. (2000). Overwintering of arthropods in soils of arable fields and adjacent semi-natural habitats. *Agriculture, Ecosystems and Environment* 78: 215-222.

Pfleeger T, Blakeley-Smith M, King G, Henry Lee E, Plocher M, Olszyk D (2012). The effects of glyphosate and aminopyralid on a multi-species plant field trial. *Ecotoxicology* 1-17

Pfleeger T, Olszyk D, Lee EH, Plocher M (2011). Comparing effects of low levels of herbicides on greenhouse- and field-grown poatoes (*Solanum tuberosum* L.), Soybeans (*Glycine max* L.), and Peas (*Pisum sativum* L.). *Environmental Toxicology and Chemistry* 30:455-468

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends In Ecology & Evolution* 25 (6): 345-353.

Power, A.G. (2010). Ecosystem services and agriculture: tradeoffs and synergies. *Philosophical Transactions of the Royal Society B-Biological Sciences* 365 (1554): 2959-2971.

Pywell, R.F., Warman, E.A., Sparks, T.H., Greatorex-Davies, J.N., Walker, K.J., Meek, W.R., Carvell, C., Petit, S., Firbank, L.G. (2004). Assessing habitat quality for butterflies on intensively managed arable farmland. *Biological Conservation* 118: 313-325.

Rautmann, D., Streloke, M., Winkler, R. (2001). New basic drift values in the authorization procedure for plant protection products. Workshop on Risk assessment and Risk Mitigation Measures (WORMM), 27.-29. September 1999. Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Vol. 383, Berlin-Dahlem.

Rew, L.J., Froud–Williams, R.J., Boatman, N.D. (1995). The effect of nitrogen, plant density and competition between *Bromus sterilis* and three perennial grasses: the implications for boundary strip management. *Weed Research* 35 (5): 363-368.

Reuter S, Simoneit-Gast, S. (2007). Entwicklung einer weiterführenden Methode zur Bewertung des Risikos für terrestrische Pflanzen durch Exposition mit Pflanzenschutzmitteln und ihren Wirkstoffen, Umweltforschungsplan des Bundesministeriums für Umwelt, Naturschutz und Reaktorsicherheit, Förderkennzeichen (UFOPLAN) 202 67 431. Unpublished reprot. Im Auftrag des Umweltbundesamtes, Desssau.

Riemens, M.M., Dueck, T., Kempenaar, C. (2008). Predicting sublethal effects of herbicides on terrestrial non-crop plant species in the field from greenhouse data. *Environmental Pollution* 155 (1): 141-149.

Riester, R., Huber, J. (2013). Betriebsmittel. Agrarmärkte Jahresheft 2013. Schriftenreihe der Landesanstalt für Entwicklung der Landwirtschaft und der Ländlichen Räume (LEL) und der Bayerischen Landesanstalt für Landwirtschaft (LfL): 310-320.

Riffel, M., Dietzen, C., Künast, C., Day, P., Schiansky, J. (2009). Agriculture and Biodiversity, Editors: European Landowners Organisation (ELO), European Crop Protection Association (ECPA), BASF SE, RIFCON GmbH**.**

Roberts, H.A., Neilson, J.E., (1981). Changes in the soil seed bank of four long-term crop/herbicide experiments. *Journal of Applied Ecology* 18 (2), 661-668.

Robinson, R.A., Sutherland, W.J. (2002). Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology* 39 (1): 157-176.

Roß-Nickoll, M., Lennartz, G., Fürste, A., Mause, R., Ottermanns, R., Schäfer, S., Smolis, M., B., T.e., Toschki, A., Ratte, H.T. (2004). Die Arthropodenfauna von Nichtzielflächen und die

Konsequenzen für die Bewertung der Auswirkungen von Pflanzenschutzmitteln auf den terrestrischen Bereich des Naturhaushaltes. F+E Bericht, Umweltbundesamt, Berlin.

Sánchez-Bayo, F., (2011). Impacts of agricultural pesticides on terrestrial ecosystems. In: Sánchez-Bayo, F., Mann, R.M. (Ed.): Ecological impacts of toxic chemicals, Bentham Science Publishers.

Sanchez-Bayo, F., Goka, K. (2012). Evaluation of suitable endpoints for assessing the impacts of toxicants at the community level. *Ecotoxicology* 21 (3): 667-680.

Sarukhan J. (1974). Studies on plant demography: *Ranunculus repens*., *R. bulbosus* L. and *R. acris* L. *Journal of Ecology* 62 (1):151-177.

Schad T (2013). Xplicit – A Modelling Framework for Ecological Risk Characterisation at Landscape-scales in Regulatory Risk Assessment and Risk Management of Plant Protection Products. *Doctoral Thesis*. Institut für Umweltwissenschaften, Universitity Koblenz-Landau, Campus Landau.

Schäfer, K. (2013). Auswirkungen von feldsaumrelevanten Herbizid- und Düngereinträgen auf das Vorkommen, die Blütenbildung und die Reproduktion verschiedener Nichtzielpflanzen. *Diplomarbeit.* Institut für Umweltwissenschaften, Universität Koblenz – Landau, Campus Landau.

Schmitz, J., Schäfer, K., Brühl, C.A. (2013). Agrochemicals in field margins - Assessing the impacts of herbicides, insecticides, and fertilizer on the common buttercup (*Ranunculus acris*). *Environmental Toxicology and Chemistry* 32 (5): 1124-1131.

Schmitz, J., Schäfer, K., Brühl, C.A. (2014a). Agrochemicals in field margins - Field evaluation of plant reproduction effects. *Agriculture Ecosystems & Environment* 189: 82-91.

Schmitz, J., Hahn, M., Brühl, C.A. (2014b). Agrochemicals in field margins - An experimental fiel study to assess the impacts of pesticides and fertilizers on a natural plant community. *Agriculture Ecosystems & Environment*. 193: 60-69.

Schmitz, J., Stahlschmidt, P., Brühl, C.A. (2013). Protection of terrestrial non-target plant species in the regulation of environmental risks of pesticides (Gutachten erstellt im Auftrag des Umweltbundesamt, Dessau (see refernce UBA 2012)), unpublished report.

Siemann, E., Tilman, D., Haarstad, J., Ritchie, M. (1998). Experimental tests of the dependence of arthropod diversity on plant diversity. *American Naturalist* 152 (5): 738-750.

Siemoneit-Gast S, Reuter S, Kubiak R, Höllrigl-Rosta A (2007). Development of an extended method for assessing the risk to terrestrial plants exposed to plant protection products and their active ingredients. Paper presented at the 13th Symposium Pesticide Chemistry, Environmental fate and ecological effects of pesticides 2007, 3-6 September. Piacenza, Italy.

Smart, S.M., Bunce, R.G.H., Firbank, L.G., Coward, P. (2002). Do field boundaries act as refugia for grassland plant species diversity in intensively managed agricultural landscapes in Britain? *Agriculture Ecosystems & Environment* 91 (1-3): 73-87.

Smart, S.M., Firbank, L.G., Bunce, R.G.H., Watkins, J.W. (2000). Quantifying changes in abundance of food plants for butterfly larvae and farmland birds. *Journal of Applied Ecology* 37: 398-414.

Smith, T.M., Smith, R.L., (2009). Ökologie. Boston, Mass. [u.a.] : Pearson Studium München.

Socher, S.A., Prati, D., Boch, S., Müller, J., Baumbach, H., Gockel, S., Hemp, A., Schöning, I., Wells, K., Buscot, F., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.-D., Weisser, W.W., Fischer, M. (2013). Interacting effects of fertilization, mowing and grazing on plant species diversity of 1500 grasslands in Germany differ between regions. *Basic and Applied Ecology* 14 (2): 126-136.

Sparks, T.H., Parish, T. (1995). Factors affecting the abundance of butterflies in field boundaries in Swavesey Fens, Cambridgeshire, UK. *Biological Conservation* 73: 221-227.

Statistisches Bundesamt (2011). Agrarstrukturen in Deutschland. Einheit in Vielfalt - Regionale Ergebnisse der Landwirtschaftszählung 2010. Stuttgart, Statistische Ämter des Bundes und der Länder.

Steinbach K, Gottsberger G. (1994). Phenology and pollination biology of five *Ranunculus* species in Giessen, Central Germany. Phyton 34 (2): 203-218.

Stoate, C., Baldi, A., Beja, P., Boatman, N.D., Herzon, I., van Doorn, A., de Snoo, G.R., Rakosy, L., Ramwell, C. (2009). Ecological impacts of early 21st century agricultural change in Europe - A review. *Journal of Environmental Management* 91 (1): 22-46.

Stoate, C., Boatman, N.D., Borralho, R.J., Carvalho, C.R., de Snoo, G.R., Eden, P. (2001). Ecological impacts of arable intensification in Europe. *Journal of Environmental Management* 63 (4): 337-365.

Strandberg, B., Mathiassen, S.K., Bruus, M., Kjær, C., Damgaard, C., Andersen, H.V., Bossi, R., Løfstrøm1, P., Larsen, S.E., Bak, J., Kudsk, P. (2012). Effects of herbicides on non-target plants: How do effects in standard plant test relate to effects in natural habitats?, Danish Environmental Protection Agency.

Tarmi, S. (2011). Plant communities of field margins - The effects of management and environmental factors on species composition and diversity. *Doctoral Thesis.* Department of Agricultural Sciences/Agroecology, University of Helsinki.

Tarmi, S., Tuuri, H., Helenius, J. (2002). Plant communities of field boundaries in Finnish farmland. *Agricultural and Food Science in Finland* 11 (2): 121-U121.

Tew, T.E. (1994). Farmland hedgerows: habitat, corridors or irrelevant? A small mammals perspective, pp. 80-94. In: Watt T. A., Buckley G. P. (Ed.): Hedgerow management and nature conservation. Wye College Press.

Thompson, K., Band, S.R. & Hodgson, J.G., (1993). Seed size and shape predict persistence in soil. *Functional Ecology* 7 (2), 236–241.

Travers, S.E., Fauske, G.M., Fox, K., Ross, A.A., Harris, M.O., (2011). The hidden benefits of pollinator diversity for the rangelands of the Great Plains: Western prairie fringed orchids as a case study. *Rangelands* 33 (3), 20-26.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., Thies, C. (2005). Landscape perspectives on agricultural intensification and biodiversity - ecosystem service management. *Ecology Letters* 8 (8): 857-874.

Tscharntke, T., Steffan-Dewenter, I., Kruess, A., Thies, C. (2002). Characteristics of insect populations on habitat fragments: A mini review. *Ecological Research* 17 (2): 229-239.

Tsiouris, S., Marshall, E.J.P. (1998). Observations on patterns of granular fertiliser deposition beside hedges and its likely effects on the botanical composition of field margins. *Annals of Applied Biology* 132: 115-127.

UBA (2012). Leistungsbeschreibung: Gutachten „Erarbeitung von Empfehlungen zur Weiterentwicklung des derzeitigen Verfahrens der Risikobewertung für terrestrische Nichtzielpflanzen als Grundlage für die Revision des ´EU guidance documents on terrestrial ecotoxicology´“. Umweltbundesamt, Dessau.

U.S. Environmental Protection Agency. (1996). Ecological effects test guidelines: Terrestrial Plant Toxicity - Vegetative Vigor. OPPTS 850.4150 and 4250. EPA 712–C–96–163. Washington, DC.

van Assche, J., Van Nerum, D., Darius, P., (2002). The comparative germination ecology of nine *Rumex* species. *Plant Ecology* 159 (2), 131-142.

van de Zande, J.C., Holterman, H.J., Huijsmans, J.F.M. (2012). Spray drift for the assessment of exposure of aquatic organisms to plant protection products in the Netherlands : part 1: field crops and downward spraying. *Research Report 419.* Plant Research International Wageningen UR.

van de Zande, J.C., Michielsen, J.G.P., Stallinga, H. (2007). Spray drift and off-field evaluation of agrochemicals in the Netherlands. *Research Report 149.* Plant Research International Wageningen UR.

van Emden, H. (2008). Statistics for terrifed biologists. USA, UK, Australia, Blackwell Publishing.

van Liedekerke, P., Tijskens, E., Bravo, C., Ramon, H. (2008). Study of the granular fertilizers and the centrifugal spreader using Discrete Element Method (DEM) simulations. *IAgrE Landwards e-Xtra* 63 (1): 1-8.

Vickery, J.A., Feber, R.E., Fuller, R.J. (2009). Arable field margins managed for biodiversity conservation: A review of food resource provision for farmland birds. *Agriculture Ecosystems & Environment* 133 (1-2): 1-13.

Weiner, C.N., Werner, M., Linsenmair, K.E., Blüthgen, N. (2011). Land use intensity in grasslands: Changes in biodiversity, species composition and specialisation in flower visitor networks. *Basic and Applied Ecology* 12: 292-299.

Westrich, P. (1989). Die Wildbienen Baden-Württembergs, Vol. I and II. Stuttgart, Ulmer Verlag.

White, A.L., Boutin, C., Dalton, R.L., Henkelman, B., Carpenter, D. (2009). Germination requirements for 29 terrestrial and wetland wild plant species appropriate for phytotoxicity testing. *Pest Management Science* 65 (1): 19-26.

Willems, J.H., Peet, R.K., Bik, L. (1993). Changes in Chalk-Grassland Structure and Species Richness Resulting from Selective Nutrient Additions. *Journal of Vegetation Science* 4 (2): 203-212.

Wilson, J.D., Morris, A.J., Arroyo, B.E., Clark, S.C., Bradbury, R.B. (1999). A review of the abundance and diversity of invertebrate and plant foods of granivorous birds in northern Europe in relation to agricultural change. *Agriculture Ecosystems & Environment* 75 (1-2): 13-30.

Wilson, P.J. (1999). The effect of nitrogen on populations of rare arable plants in Britain, pp. 93-100. In: Boatman, N.D., Davis, B.N.K., Chaney, K., Feber, R., De Snoo, G. R., Sparks, T. H. (Ed.): Field Margins and Buffer Zones: Ecology, Management and Policy, Vol. 54. The Association of Applied Biologists, c/o Horticulture Research International, Wellesbourne, Warwick, UK.

Appendices

Appendix I Teaching involvement during my PhD study

During my PhD study at the University Koblenz-Landau, Campus Landau, I co-supervised several research projects (10 case studies and 5 diploma theses). The research studies were integrated into my PhD project, and all of these studies were supervised by Dr. Carsten Brühl.

A list of theses finished with my assistance is provided below. For some of these studies (that dealt with arthropods), a short description is provided in Appendix II.

In addition, I was involved in teaching and co-supervising students in the course "Terrestrische Systeme" in the years 2010, 2011, and 2012.

2010

Annalena Schotthöfer: „Bodenchemische Untersuchung auf einer Wiesenfläche". SS 2010. *Fallstudie.*

Roman Szabo: „Auswirkungen landwirtschaftlicher Tätigkeiten auf bodenchemische Parameter". SS/WS 2010. *Fallstudie.*

Rosaly Richter: „Bewertung des Einflusses von Pflanzenschutzmitteln und Dünger auf die Vegetation von Feldsäumen". SS 2010. *Fallstudie.*

Nikita Bakanov: „Floristische Artenzusammensetzung in anthropogen beeinflussten Saumstrukturen". SS 2010. *Fallstudie.*

Marcus Metz: „Auswirkungen von Dünger auf terrestrische Nicht-Ziel-Pflanzen - Effekte auf Wachstum, Biomasse und Entwicklungsstadium bei Zugabe eines Volldüngers". WS 2010. *Fallstudie.*

Timo Felix: „Untersuchung zum Einfluss des Dünger-, Herbizid- und Insektizideintrags auf die Biodiversität von Feldsäumen am Beispiel von Zikaden (Auchenorrhyncha)". Freilandsaison 2010. *Diplomarbeit.*

2011

Philipp Uhl: „Subletale Effekte des Herbizides Atlantis WG auf *Ranunculus acris*". SS 2011. *Fallstudie.*

Karoline Schäfer: "Auswirkungen des Eintrags von Pflanzenschutzmitteln und Dünger auf den in

Feldsäumen vorkommenden *Ranunculus acris*". SS 2011. *Fallstudie.*

Katharina Schmücking: " Auswirkungen des Eintrags von Pflanzenschutzmitteln und Dünger in Feldsäumen auf Heuschrecken". SS 2011. *Fallstudie.*

Annalena Schotthöfer: „Untersuchung zur Eignung von Feldsäumen verschiedener landwirtschaftlicher Kulturen als Entwicklungshabitat für Schmetterlingsraupen (Lepidoptera) unter Berücksichtigung der Auswirkungen von Agrarchemikalieneinträgen - eine quantitative Analyse". SS 2011. *Diplomarbeit.* (note: further co-supervisor: Melanie Hahn)

Marcus Metz: „Quantifizierung der Effekte von Pflanzenschutzmitteln und Dünger auf die Spinnendiversität (Araneae) eines Feldsaums". SS 2011. *Diplomarbeit.*

2012

Karoline Schäfer: „Auswirkungen von feldsaumrelevanten Herbizid- und Düngereinträgen auf das Vorkommen, die Blütenbildung und die Reproduktion verschiedener Nichtzielpflanzen". SS 2012. *Diplomarbeit.*

Lisa Ressl: „Untersuchung der Abdrift von Pflanzenschutzmitteln und Dünger auf terrestrische Wildpflanzen in Monokultur und Gemeinschaft – Tests von zwei verschiedenen Entwicklungsstadien". SS 2012. *Diplomarbeit.*

Philipp Bauer: „Der Einfluss von Dünger, Pestiziden und ihrer Kombination auf Orthopteren und Lepidopteren in Feldsäumen". SS 2012. Fallstudie.

2013

Revina-Rosa: „Seed bank of field margins". SS 2013. *Fallstudie.*

Appendix II Overview of sampling methods used in 2010, 2011 and 2012 for collecting arthropods on the study site and the main results of these investigations

Background: Field margins are refuges and corridors for arthropods in agricultural landscapes. In addition, they can act as valuable habitats for many beneficial insects and spiders, which move into adjoining arable fields and provide ecosystem services and natural pest control. However, arthropods in field margins may also be negatively affected by agrochemical inputs.

Methods: To investigate the effects of the agrochemical applications on the arthropod community, various sampling techniques (vacuum sampler, sweep nets, and photoeclectors) were used during the three years of the field experiment. The arthropods were collected with the help of students who performed their diploma theses or their research projects in the framework of my PhD study.

In the following table, the different sampling methods used each year (2010, 2011, and 2012) are listed (Table A-1). Then, a short description of the sampling methods, the collected arthropods, and selected results are represented. Please see the corresponding diploma or bachelor theses for more details because the description presented in this thesis only provide a general overview and, therefore, are not exhaustive. The theses are listed below the results section on the following pages. The supervisor of all studies was Dr. Carsten Brühl, and I co-supervised the studies.

Table A-1: Sampling methods used in 2010, 2011 and 2012 for collecting arthropods on the study site.

	Arthropods	Sampling method	Students
2010	cicadas	suction sampler	Timo Felix (diploma thesis)
	soil emerging arthropods	photoeclectors	–
2011	spiders, caterpillars of butterflies and moths, and grasshoppers	sweep nets	Marcus Metz (diploma thesis), Annalena Schotthöfer (diploma thesis), Katarina Schmücking (research project)
	soil emerging arthropods	photoeclectors	Corinna Kupfer (bachelor thesis)*, Alisa Schreiber (bachelor thesis)*
2012	grasshoppers and caterpillars of butterflies and moths	sweep nets	Philipp Bauer (research project)

* These students were not involved in the field work. They were only responsible for the arthropod identification in the lab. These two students were supervised by Dr. Jörn Buse.

1) Vacuum sampling in 2010:

In 2010, a vacuum sampler and a biocoenometer (a modified rain barrel with an area of 0.5 m^2) were used for a quantitative collection of arthropods. The suction sampler was a modified leaf suction machine (SH 85, Stihl AG, Waiblingen; 0.8 kW; 625 m^3 air flow/h), equipped with a sampling tube. The mouth of the tube was covered with a nylon collection net, and when the tube was placed over vegetation, insects were sucked into the collecting net. Suction traps are primarily used for collecting above-ground grassland invertebrates in field studies.

We focused on one specific group of arthropods, namely the **cicadas** (Auchenorrhyncha). Cicadas are a highly diverse group of phytophagous insects with a wide range of specific ecological strategies. In grasslands, cicadas function as herbivores and prey for higher trophic levels. Because cicadas usually have a high abundance, these insects are frequently analyzed for conservation studies and monitored for habitat changes.

The diploma student Timo Felix assessed the effects of the herbicide, insecticide and fertilizer applications in 2010 (first field season) on the cicada community (Auchenorrhyncha). Therefore, in each plot of the experimental study site, arthropods were collected with the suction sampler (Fig. A-1). Three subsamples were collected per plot. The sampling time for each subsampling was 2 minutes, totaling 6 minutes per plot.

The arthropod collection occurred at three different times during the field season in 2010:

a) one sampling was performed 1 day before the insecticide application (3 June 10)
(all 64 plots were sampled),

b) one sampling was performed 2 days after the insecticide application (6 June 10)
(all plots treated with the insecticide and the control plots were sampled = 40 plots), and

c) one sampling was performed 20 days later (23 June10)
(all 64 plots were sampled).

→ Thus, 504 samples were collected = (64+40+64)*3.

Fig. A-1: Timo Felix and Juliane Schmitz with the suction sampler and the biocoenometer. Photo taken on 3 June 10.

The samples were stored in plastic bags in the freezer. All collected arthropods were sorted, all individuals were determined to the order level, and the Auchenorrhyncha were identified to the species level by Timo Felix.

Results of the diploma thesis of Timo Felix (Felix 2011):

In all samples, Timo Felix found 38 613 cicadas, which included 12 605 adult cicadas of 49 species and 26 008 nymphs.

Significant effects on the total abundance of individuals could not be detected. However, in the first sampling (which was collected 1 day before the insecticide application), a significant effect on the mean number of species was found: in the fertilizer-treated plots, a significantly higher number of species were detected (Fig. A-2). In addition, one species, the meadow spur-hopper *Javesella pellucida*, significantly increased its abundance (by a factor of 4.3) in the fertilizer-treated plots compared with the control.

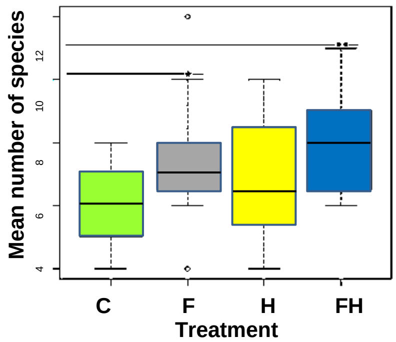

Fig. A-2: Mean number of species in the different treatments. C: control, F: fertilizer, H: herbicide. Sampling date: 3 June 10. At this sampling date, no insecticide treatment had occurred on the study site; therefore, the number of replicates for each treatment was 16 and not 8 (for instance, the eight insecticide plots were not yet treated; thus, these eight plots could be seen as control plots etc.) The data were square-root transformed. * $p < 0.05$, ** $p < 0.01$ (Dunnett`s test). Figure modified after Felix, T. (2011).

A nutrient supply can increase the developmental rates of cicadas; therefore, it is possible that the fertilizer caused a faster growth of the juvenile cicadas. This may explain the increased abundance of *J. pellucida* in the fertilizer-treated plots. This species is a pioneer with no high ecological demands on the habitat and an extremely wide food spectrum (e.g., grasses) (Felix 2011).

Contrary to expectations, the cicadas showed no acute effect to the insecticide treatment: no significant effect on the mean number of species or on individuals could be detected 2 days after the insecticide treatment (second sampling: 6 June10).

However, 20 days later, two species (the greenish-yellow leafhopper *Arthaldeus pascuellus* and the meadow froghopper *Philaenus spumarius*) showed significantly reduced abundances of approximately 50 % and 66 %, respectively, in plots receiving an insecticide treatment (I: *A. pascuellus* = 96 individuals; *P. spumarius* = 23 individuals) compared with the control (C: *A. pascuellus* = 187 individuals; *P. spumarius* = 69 individuals) (Fig. A-3).

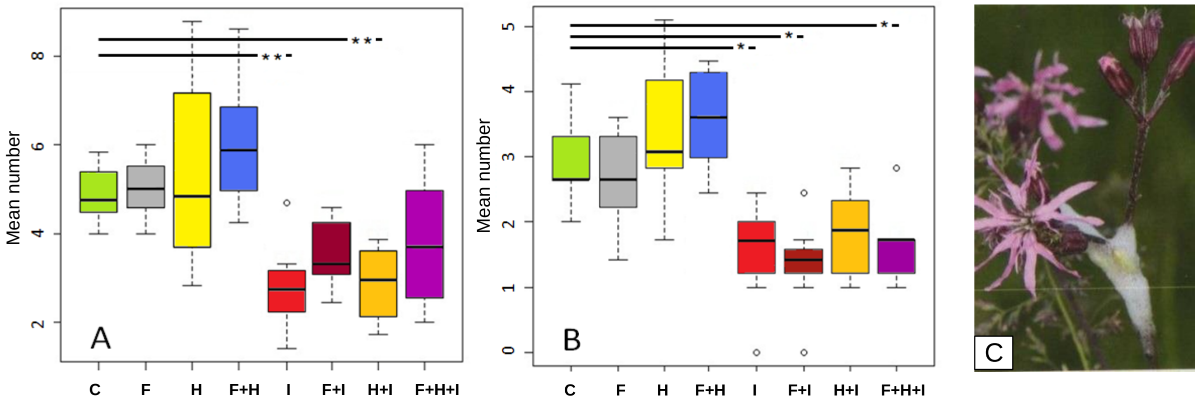

Fig. A-3: Abundance of *Arthaldeus pascuellus* (A) and *Philaenus spumarius* (B) in all eight treatments (C: control, F: fertilizer, H: herbicide, I: Insecticide, F+H, F+I, H+I, F+H+I, n = 8). The data were square-root transformed. Sampling date: 23 June 2010. * p<0.05, ** p<0.01 (Dunnett`s test). Figure modified after Felix, T. (2011). Figure 3 C: Foam nests of *P. spumarius* on the plant *Lychnis flos-cuculi*.

Both species are known to use predominately higher vegetation strata; therefore, these species were most likely affected due to direct contact with the insecticide. Moreover, the application of the insecticide was performed during the larval stage of *P. spumarius*. The foam nests of this species were possibly affected by the insecticide application (Fig. A-3C).

Discussion: The results showed that cicadas were affected by the agrochemical applications. The fertilizer had most likely increased the developmental rates of some cicadas, whereas the herbicide did not negatively affect the abundance of cicadas. In contrast, the insecticide affected two species, which use predominately higher vegetation strata (Felix 2011).

Source: Felix, T. (2011): Untersuchung zum Einfluss des Dünger-, Herbizid- und Insektizideintrags auf die Biodiversität von Feldsäumen am Beispiel von Zikaden (Auchenorrhyncha). *Diploma thesis*. Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau.

2) Sweep nets in 2011

In 2011, arthropods on the study site were collected with **sweep nets**. This collection focused on taxonomic groups of arthropods, which can easily and effectively be sampled with this method. Therefore, we focused on **spiders (a), caterpillars of moths and butterflies (b) and grasshoppers (c)**. Sweep nets are usually very robust and are composed of a heavy material (such as canvas) with short, thick handles. Thus, sweep nets can be dragged through dense vegetation without being damaged. Sweep net samples can be compared if the area swept per sweep and numbers of sweeps per sample are held constant among sample plots.
To assess the effects of the agrochemicals on the above-listed arthropods, two diploma theses and one research project were assigned (further details are provided on the next page).

The arthropod sampling was performed at three different times during the field season in **2011:**

a) the first sampling was conducted approximately two weeks after the insecticide application (7 June11) (only the control and insecticide plots were sampled = 16 plots)

b) the second sampling was conducted 34 days after the insecticide application (27 June 11) (all 64 plots were sampled), and

c) the third sampling was conducted approximately 3.5 months after the insecticide application (15 September 11) (all 64 plots were sampled).

→ Thus, 144 samples were collected = 16+64+64.

At each sampling date, the numbers of sweeps per plot were held constant and followed a specific scheme (Fig. A-4).

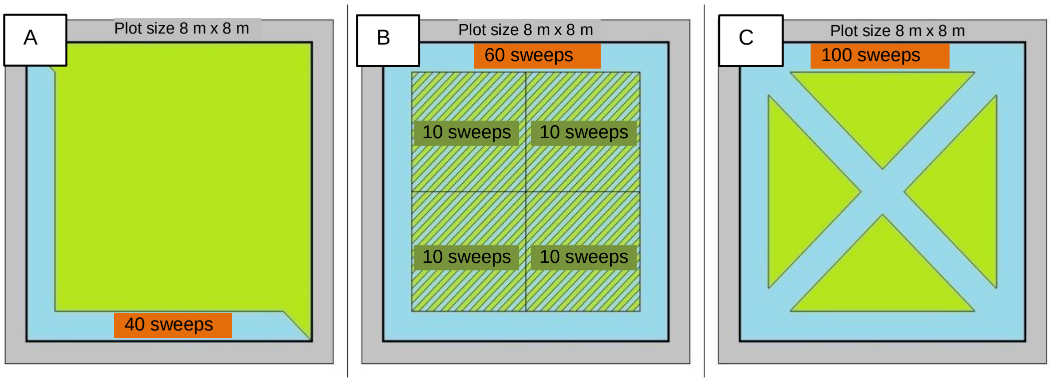

Fig. A-4: Numbers of sweeps per plot on A): 7 June 11; B): 27 June 11; C): 15 September 11. The arthropods were collected in the blue colored area. The numbers of sweeps per plot are listed. Figure modified after Metz, M. (2012).

The collected arthropods were transferred from sweep nets to labeled plastic bags and were frozen until identification in the lab. All collected spiders, grasshoppers and caterpillars were determined to order level or species level by Marcus Metz (Diploma Student; spiders), Annalena Schotthöfer (Diploma Student; caterpillars) and Katarina Schmücking (Master Student; grasshoppers).

Fig. A-5: Arthropod collection with sweep nets in 2011. From left to right: Picture 1: Marcus Metz; Picture 2: Annalena Schotthöfer; Picture 3: Melanie Hahn, Katarina Schmücking, Juliane Schmitz; Picture 4: Melanie Hahn, Annalena Schotthöfer. Photos taken on 27 June 11.

Results of the diploma thesis of Marcus Metz (Metz 2013):

a) Spiders

In all samples, Marcus Metz found 3400 spiders (individuals), which could be divided into 14 families, 47 genera and 55 species. The most common families on the study area were Thomisidae (crab spiders), Pisauridae (web spider), Philodromidae (running spiders) and Araneidae (orb-weaver spiders).

At the first sampling date (approximately two weeks after the insecticide application in 2011) and at the third sampling date (approximately 3.5 months after the insecticide application in 2011), no significant differences in the mean number of species or individuals between the treatments and the control were observed. However, the samples collected at the second sampling date (34 days after the insecticide application) showed significant differences. In particular, juvenile spiders of the family Thomisidae and the genus *Xysticus*, which are known to primarily use higher vegetation strata for hunting, were affected by the agrochemicals.

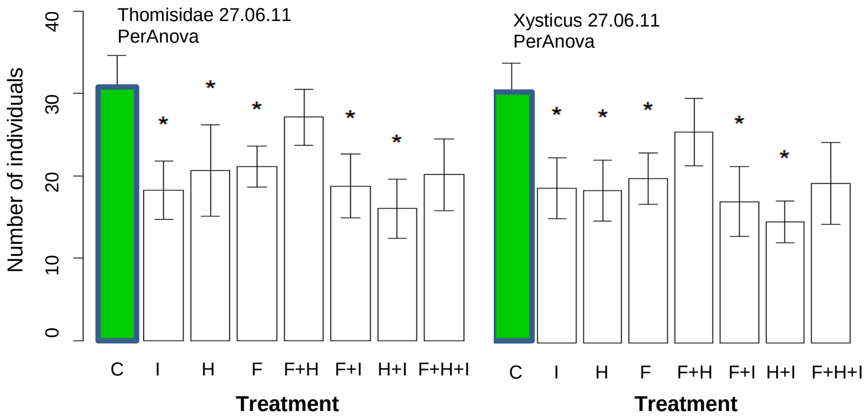

Fig. A-6: Mean number of Thomisidae and *Xysticus* [± SE] per plot and treatment. * Significantly different from the control, $p < 0.05$ [PerAnova]. Sampling date: 27 June11 (34 days after the insecticide application). Figure taken from Metz, M. (2012).

Significantly reduced densities of the crab spiders were observed in all treatments compared with the control (Fig. A-6). The lowest density of crab spiders was recorded in the insecticide treatment (40 % fewer individuals than in the control) and in plots treated with insecticide and herbicide in combination (H+I) (47 % fewer individuals than in the control). In the separate herbicide an fertilizer treatment (F and H), a significantly reduced abundance of approximately 30% compared with the control plots was observed.

Discussion: The results showed no acute insecticide effect for spiders. However, 34 days after the insecticide application, the crab spiders were significantly affected by the different treatments (Fig. A-6). Thus, it is possible that the insecticide application did not directly affect the abundance of crab spiders but may have reduced their reproduction capacity or possibly the hatching of juvenile spiders from the egg cocoon. Another possibility is that the food availability changed in the insecticide-treated plots; thus, the spiders moved to plots (e.g., control) with higher food resources. These possibilities are only speculations, and further investigations would be required to make accurate statements.

The fertilizer and herbicide applications most likely reduced the density of the crab spiders due to changed vegetation and habitat structure. The vegetation height increased in the fertilizer-treated plots but decreased in the herbicide-treated plots. Additionally, the reduced flowering intensity in the herbicide-treated plots could potentially be responsible for the reduced abundances of spiders because crab spiders use flowers for hunting purposes (Metz, 2012).

Source: Metz, M. (2012): Quantifizierung der Effekte von Pflanzenschutzmitteln und Dünger auf die Spinnendiversität (Araneae) eines Feldsaums. *Diploma thesis*. Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau.

Results of the diploma thesis of Annalena Schotthöfer (Schotthöfer 2012):

b) Caterpillars of moths and butterflies

In all samples, Annalena Schotthöfer found 318 caterpillars. Individual numbers per plot and sampling date were low; thus, analysis was based on the family level rather than on the species level.

The results of this study will be published in a scientific journal. Currently, a publication is in preparation (Hahn et al. in prep.); therefore, in the following section only some main results are presented.

The most frequent families on the study site were Noctuidae and Geometridae. Both families showed a statistically reduced abundance in the insecticide-treated plots (Fig. A-7).

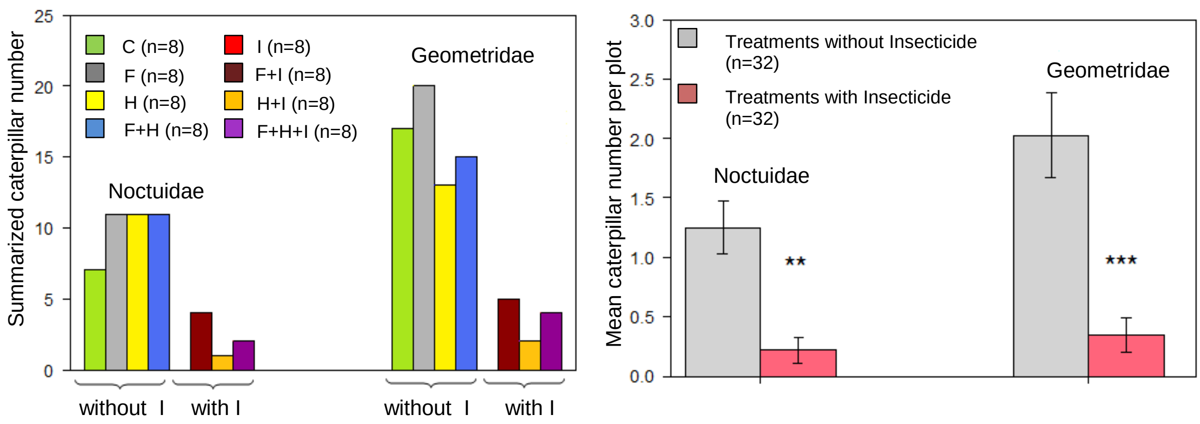

Fig. A-7: Summarized caterpillar number per treatment (left) and mean caterpillar number per plot (right, all plots treated with/without insecticide) of Noctuidae and Geometridae. The results are based on data from two sampling dates: 30 May 2011 and 27 June 2011. ** $p < 0.01$, *** $p < 0.001$ (permutational ANOVA, number of permutations: 999). Figure taken from Brühl et al. 2014 (UBA-Bericht).

Discussion: The results showed that the insecticide application negatively affected the abundance of caterpillars. Two possible explanations exist: This effect was caused either by a direct toxic effect of the insecticide on caterpillars or by a repellency effect on adult butterflies so that the butterflies avoided plots treated with the insecticide for egg deposition.

Source: Schotthöfer, A. (2012): Untersuchung zur Eignung von Feldsäumen verschiedener landwirtschaftlicher Kulturen als Entwicklungshabitat für Schmetterlingsraupen (Lepidoptera) unter Berücksichtigung der Auswirkungen von Agrarchemikalieneinträgen - eine quantitative Analyse -. *Diploma Thesis.* Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau.

Brühl, C.A., Alscher, A., Berger, G., Bethwell, C., Graef, F., Hahn, H., Schmidt, T., Weber, B. (2014; in press): Protection of Biodiversity in the Risk Assessment and Risk Management of Pesticides (Plant Protection Products & Biocides) with a Focus on Arthropods, Soil Organisms, and Amphibians. Environmental Research Plan of the German Federal Ministry for the Environment, Nature Conservation, Buildings and Nuclear Safety Research and Development Project Nr. 3709 65 421.

Hahn, M., Schotthöfer, A., Schmitz, J., Franke, L., Brühl., C.A.: (submitted): The effects of insecticides, herbicides, and fertilizers on moths (Lepidoptera) in field margin habitats. *Agriculture, Ecosystems and Environment*

c) Grasshoppers:

Grasshoppers sampled in 2011 were identified by Katarina Schmücking. The adult grasshoppers were identified to the species level, and the juvenile grasshoppers were identified to the order level. Unfortunately, thus far, no statistical analysis has been performed with the data. However, the data were entered in an Access database and are available for analysis.

3) Sweep nets in 2012

In 2012, grasshoppers and caterpillars of moths and butterflies were collected with sweep nets. The sampling was performed at two different times during the field season in 2012:

a) the first sampling was conducted two days after the insecticide application in 2012 (1 June 12) (all 64 plots were sampled), and

b) the second sampling was conducted approximately 1.5 months later (18 July 12) (all 64 plots were sampled)

→ Thus, 128 samples were collected = 64+64.

At each sampling date, the numbers of sweeps per plot were held constant and followed a specific scheme (Fig. A-8).

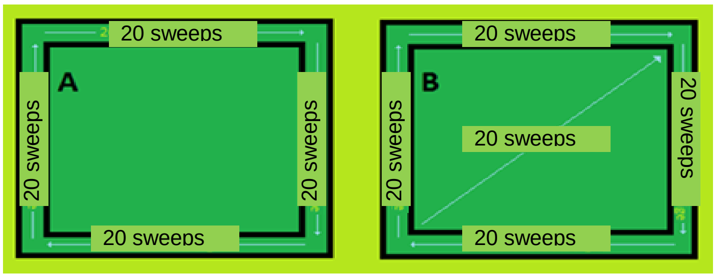

Fig. A-8: Sketch of the numbers of sweeps per plot at the two sampling dates. A): 1 June12; B): 18 July 12. The numbers of sweeps per plot are listed in the plots. Figure modified after Bauer, P. (2013).

The collected arthropods were transferred from sweep nets to labeled plastic bags and were frozen until identification in the lab. All collected grasshoppers and caterpillars were identified to the order level or to the species level by Philipp Bauer (research project).

Results of the research project of Philipp Bauer (Bauer 2013):

Overall, Philipp Bauer found 10 grasshopper species and 5 caterpillar families. The most frequent grasshoppers occurring at the study site were assigned to the genus *Chorthippus sp*. and to the species Roesel's bush-cricket *Metrioptera roeselii* and the Large marsh grasshopper *Stethophyma grossum*.

Chorthippus sp. showed a high sensitivity 2 days after the insecticide application: A significantly reduced number of *Chorthippus sp.* individuals (the meadow grasshopper *Chorthippus parallelus* and the steppe grasshopper *Chorthippus dorsatus)* were found in the insecticide-treated plots compared with the control plots (Fig. A.9): Their abundances in plots treated with the insecticide (I, H+I, F+I, F+H+I) were reduced by an average of 65 % compared with the control plots.

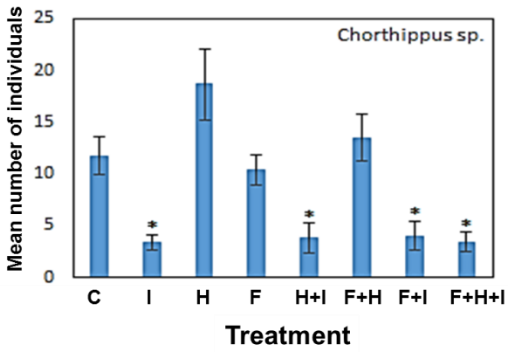

Fig. A-9: Mean number of individuals of *Chorthippus sp*. per plot and treatment. Sampling date: 1 June 12. * Significantly different from the control, $p < 0.05$ (Wilcoxon-test, p-values Bonferroni corrected). C = control, I = insecticide, F = fertilizer, H = herbicide. Figure taken from Bauer, P. (2013).

All other grasshopper species, as well as the caterpillars, were not significantly affected by the different treatments. No significant differences in the mean number of species or individuals between the treatments and the control were observed at the second sampling date.

Discussion: Grasshopper species in field margins may be negatively affected by insecticide inputs. The insecticide application most likely had a direct toxic effect on grasshoppers because the reduced abundance was observed two days after the insecticide application. However, oral exposure of the grasshoppers (ingestion of plant material containing insecticide residues) or a repellency effect of the insecticide may also be responsible for the observed effects.

Source: Bauer, P. (2013): Der Einfluss von Dünger, Pestiziden und ihrer Kombination auf Orthopteren und Lepidopteren in Feldsäumen. *Research Project.* Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau.

4) Photoeclectors in 2010 and 2011

In 2010 and 2011, **ground photoeclectors** (emergence traps) were used to collect emerging arthropods from the soil. Photoeclectors consisted of a circular frame, which was sunk into the ground. The upper part of the frame was covered with a black tent (Fig. A-10). The emerging arthropods were caught in clear plastic bottles on top of the photoeclectors. Such traps are very useful to collect ground dwelling arthropods and to verify the density of arthropod emergence from the soil.

Fig. A-10: Set up of the ground photoeclectors in 2011. One trap was placed in each plot.

One ground photoeclector (0.25 m^2) was placed in each plot between the center of the plot and the plot boundary to sample arthropods from the end of April/beginning of May to mid-June in 2010 and 2011. The traps were moved after approximately four weeks and emptied once per week or every two weeks (= four times in 2010 and 2011; April/May-June). The collected arthropods were transferred from the bottles to labeled plastic tubes, which were filled with ethanol for storage.

All arthropods collected in 2011 were identified to the order level by two students in 2012 and 2013 (Corinna Kupfer and Alisa Schreiber, Bachelor of Education students). The students were supervised by Dr. Jörn Buse (Ecosystem Analysis working group, University Koblenz-Landau, Campus Landau). These students focused primarily on Coleoptera. All Coleoptera were identified to the family level.

Results of the Bachelor theses of Corinna Kupfer and Alisa Schreiber (Kupfer 2013 and Schreiber 2013):

The results showed that the fertilizer treatment (F) increased the abundance of Coleoptera individuals compared with the control in May 2011 (sampling date: 6 May 11) (Fig. A-11A). The most common family on the study site was Staphylinidae (rove beetles) and these beetles were most likely responsible for the observed differences (Fig. A-11B).

The biomass of all Coleoptera individuals (particularly in June 2011, after the insecticide application on the study site) was affected by the treatments compared with the control, although these effects were not significant (Fig. A-12).

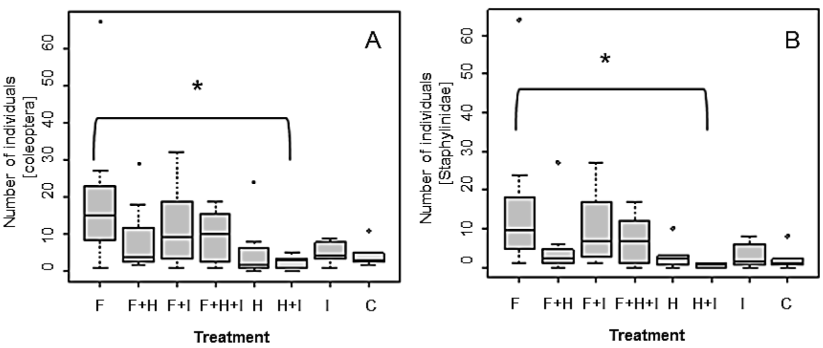

Fig. A-11: Mean number of individuals of Coleoptera (A) and Staphylinidae (B) per plot and treatment in May 2011 (sampling date: 6 May11). * Indicates significant differences between treatments, $p < 0.05$ (Wilcoxon-test). Figure modified after Schreiber, A. (2013).

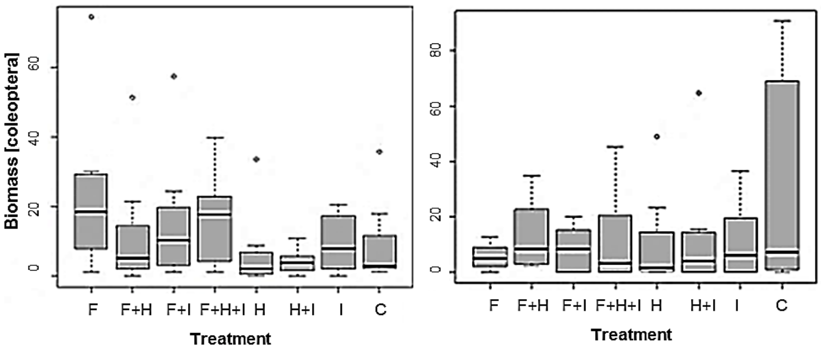

Fig. A-12: Mean biomass [mg] of all Coleoptera individuals (left: sampling date 6 May 11; right: sampling date: 20 June 11) per plot and treatment. Figure taken from Schreiber, A. (2013).

Discussion: A higher abundance of Coleoptera individuals was observed in the fertilizer-treated plots than in the control plots in May 2011. This finding may be caused by the higher plant biomass production in the fertilizer treatment, which increased the food availability for herbivorous organisms. In turn, a higher availability of herbivorous organisms most likely also had positive effects on carnivorous organisms. However, approximately 6 weeks later (June 2011), the mean biomass of all Coleoptera individuals was lower in the insecticide-, fertilizer- and herbicide-treated plots than in the control.

Because invertebrates provide food for higher trophic levels, their biomasses within grasslands and field margins may have important implications for other taxa in agricultural landscapes. To provide more accurate statements and conclusions concerning the effects of agrochemicals on Coleoptera, it would be valuable to identify individuals in the family Staphylinidae to the species level (Schreiber 2013).

Source: Kupfer, C. (2013): Einfluss von Düngemittel, Herbiziden und Insektiziden auf die Lebensgemeinschaft von Käfern. *Bachelor thesis*. Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau.

Schreiber, A. (2013): Einfluss von Pflanzenschutzmitteln (PSM) und Kunstdüngergaben auf Käfergemeinschaften in Ackerrandstrukturen. *Bachelor thesis.* Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau.

Appendix III Declaration

I, the undersigned, author of this work, declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

………………………………..   ……………………………………………..

date   signature

Appendix IV Curriculum Vitae

Personal Information

Name	Juliane Schmitz
Date of birth	21.07.1982
Place of birth	Mainz
Address	Zum Petersberg 10, 67725 Börrstadt
E-mail	Schmitz@uni-landau.de
Nationality	German

Work Experience

2009 – 2014	**PhD student,** Institute for Environmental Sciences, University Koblenz-Landau, Campus Landau Working group: Ecotoxicology and Environment Title of dissertation: "Assessing the effects of pesticides and fertilizers on a natural plant community of a field margin: An experimental field study"
2012 – 2013	**Freelance consultant** for the evaluation of herbicide effects on terrestrial non-target plants
2008	**Research assistant** at the University Koblenz-Landau, Campus Landau

Education

2008	**Diploma** in Environmental Sciences University Koblenz-Landau, Campus Landau
2002 - 2008	**Studies of Environmental Sciences** • Main subjects: Ecotoxicology, Applied Ecology and Geoecology • Minor subjects: Biodiversity & Sustainability, Environmental economics Title of diploma thesis: „Auswirkungen der Abdrift von Pflanzenschutzmitteln auf phytophage Insekten am Beispiel von Heuschrecken und Schmetterlingen"

Appendix V Publications and presentations at scientific conferences

Publications (doctoral thesis)

- **Schmitz**, J., Hahn, M., Brühl, C. (2014): Agrochemicals in field margins – An experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community. *Agriculture, Ecosystems and Environment* 193, 60-69.
- **Schmitz**, J., Schäfer, K., Brühl, C. (2014): Agrochemicals in field margins – Field evaluation of plant reproduction effects. *Agriculture, Ecosystems and Environment* 189, 82-91.
- **Schmitz**, J., Schäfer, K., Brühl, C. (2013): Agrochemicals in field margins – Assessing the impacts of herbicides, insecticides and fertilizer on the common buttercup *Ranunculus acris*. *Environmental Toxicology and Chemistry* 32 (5), 1124–1131.
- **Schmitz, J.**, Stahlschmidt, P., Brühl, C.A.: Assessing the risk of herbicides to terrestrial non-target plants using higher-tier studies. Manuscript.

Publications (diploma thesis)

- Bundschuh, R., **Schmitz**, J., Bundschuh, M., Brühl, C. (2012): Does insecticide drift adversely affect grasshoppers (Orthoptera: Saltatoria) in field margins? A case study combining laboratory acute toxicity testing with field monitoring data. *Environmental Toxicology and Chemistry* 31 (8), 1874–1879.

Publications (others)

- Hahn, M., Schotthöfer, A., **Schmitz,** J., Franke, L., Brühl, C. (submitted): The effects of insecticides, herbicides, and fertilizers on moths (Lepidoptera) in field margin habitats. *Agriculture Ecosystems & Environment.*

Presentations at scientific conferences:

- **Schmitz**, J., Brühl, C. (2013): Effects of herbicides and fertilizers on the plant community of field margins – Assessing reproduction effects. Oral presentation at the SETAC Europe 23rd Annual Meeting 2013, Glasgow, Scotland.
- **Schmitz**, J., Schäfer, K., Brühl, C. (2012): Risk assessment of herbicides for the common buttercup *Ranunculus acris* in field margins – an experimental field study. Oral presentation at the SETAC Europe 22nd Annual Meeting/6th SETAC World Congress 2012, Berlin, Germany.
- Hahn, M., Schotthöfer, A., Geisthardt, M., **Schmitz**, J., Lenhardt, P., Brühl, C. (2012): Caterpillars and protection goals: The role of field margins as habitats and the effects of pesticide applications. Poster presentation at the SETAC Europe 22nd Annual Meeting/6th SETAC World Congress 2012, Berlin, Germany.

- **Schmitz**, J., Schäfer, K., Uhl, P., Brühl, C. (2011): Auswirkungen des Herbizideintrags auf den Scharfen Hahnenfuß *Ranunculus acris* in Feldsäumen. Oral presentation at the SETAC GLB 16th Annual Meeting 2011, Landau, Germany.
- Felix, T., **Schmitz**, J., Brühl, C. (2011): Untersuchung zum Einfluss des Insektizideintrags in Feldsäumen am Beispiel von Zikaden (*Arthaldeus pascuellus* & *Philaenus spumarius*). Poster presentation at the SETAC GLB 16th Annual Meeting 2011, Landau, Germany
- **Schmitz**, J., Brühl, C. (2010): Auswirkungen des Eintrags von Pflanzenschutzmitteln und Dünger auf die Biodiversität von Feldsäumen. Poster presentation at the 4th joint Annual Meeting of the SETAC GLB and the GDCh (Section Environmental chemistry and Ecotoxicology) 2010, Dessau, Germany.
- **Schmitz**, J., Pierstorf, R., Brühl, C. (2009): Effects of plant protection products on grasshoppers in the agricultural landscape, Poster Presentation, Young Environmental Scientist Meeting (YES-Meeting), Landau.

Awards:

- Young Scientist Award for the best platform presentation at the annual SETAC GLB meeting (German Language Branch) in Landau (September 2011)

Reports

- Research report for the German Federal Environment Agency (Umweltbundesamt, UBA): Protection of terrestrial non-target plant species in the regulation of environmental risks of pesticides, FKZ 360 03 053, Ausschreibungstitel des Umweltbundesamtes: „Erarbeitung von Empfehlungen zur Weiterentwicklung des derzeitigen Verfahrens der Risikobewertung für terrestrische Nichtzielpflanzen als Grundlage für die Revision des ‚EU guidance documents on terrestrial ecotoxicology‘.

Workshops

- **SETAC Europe workshop:** Non-target terrestrial plant workshop, Wageningen, Netherlands, 1 – 3 April 2014.