Aquatic-terrestrial linkages and how they are affected by land use related stressors: The case of riparian spiders

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The history of life on earth has been a history of interaction between living things and their surroundings. To a large extent, the physical form and the habits of the earth's vegetation and its animal life have been molded by the environment. Considering the whole span of earthly time, the opposite effect, in which life actually modifies its surroundings, has been relatively slight. Only within the moment of time represented by the present century has one species—man—acquired significant power to alter the nature of his world.

Rachel Carson, Silent Spring

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SUMMARY

Streams are coupled with their riparian area. Emerging insects from streams can be an important prey in the riparian area. Such aquatic subsidies can cause predators to switch prey or increase predator abundances. This can impact the whole terrestrial food web. Stressors associated with agricultural land use can alter insect communities in water and on land, resulting in complex response patterns of terrestrial predators that rely on prey from both systems.

This thesis comprises studies on the impact of aquatic insects on a terrestrial model ecosystem (Objective 1, Chapter 2), the influence of agricultural land use on riparian spiders' traits and community (Objective 2, Chapter 3), and on the impact of agricultural land use on the contribution of different prey to spider diet (Objective 3, Chapter 4).

In chapter 2, I present a study where we conducted a mesocosm experiment to examine the effects of aquatic subsidies on a simplified terrestrial food web consisting of two types of herbivores (leafhoppers and weevils), plants and predators (spiders). I focused on the prey choice of the spiders by excluding predator immigration and reproduction. In accordance with predator switching, survival of leafhoppers increased in the presence of aquatic subsidies. By contrast, the presence of aquatic subsidies indirectly reduced weevils and herbivory.

In chapter 3, I present the results on the taxonomic and trait response of riparian spider communities to gradients of agricultural stressors and environmental variables, with a particular emphasis on pesticides. To capture spiders with different traits and survival strategies, we used multiple collection methods. Spider community composition was best explained by in-stream pesticide toxicity and shading of the stream bank, a proxy for the quality of the habitat. Species richness and the number of spider individuals, as well as community ballooning ability, were negatively associated with in-stream pesticide toxicity. In contrast, mean body size and shading preference of spider communities responded strongest to shading, whereas mean niche width (habitat preference for moisture and shading) responded strongest to other environmental variables.

In chapter 4, I describe aquatic-terrestrial predator-prey relations with gradients of agricultural stressors and environmental variables. I sampled spiders, as well as their aquatic and terrestrial prey along streams with an assumed pesticide pollution gradient and determined their stable carbon and nitrogen signals. Potential aquatic prey biomass correlated positively with an increasing aquatic prey contribution of *T. montana*. The contribution of aquatic prey to the diet of *P. amentata* showed a positive relationship with increasing toxicity in streams.

Overall, this thesis contributes to the emerging discipline of cross-ecosystem ecology and shows that aquatic-terrestrial linkages and riparian food webs can be influenced by land use related stressors. Future manipulative field studies on aquatic-terrestrial linkages are required that consider the quality of prey organisms, fostering mechanistic understanding of such cross-ecosystem effects. Knowledge on these linkages is important to improve understanding of consequences of anthropogenic stressors and to prevent further losses of ecosystems and their biodiversity.

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1. INTRODUCTION AND OBJECTIVES

1.1 Cross-ecosystem linkages

Traditionally, ecosystems were regarded as independent units. Their linkages were mainly determined locally by internal processes, where cross-ecosystem linkages were assumed to be relatively weak (Forbes, 1887). This simplified, narrow concept of ecosystems was useful to understand the linkages within ecosystems (e.g. Levin and Paine, 1974). In the 20th centura concepts suggested that energy flows from the more productive to the less productive system (e.g. Polis and Strong, 1996; Vannote et al., 1980). Later, the importance of movements of energy, organic and inorganic material across ecosystems was demonstrated, for both productive and less productive systems (Baxter et al., 2005; Richardson et al., 2010; Schulz et al., 2015). These results changed the concept of ecosystems from being independent from each other to being linked via multiple linkages of diverse strength (e.g. Henschel et al., 2001; Holt, 2008; Knight et al., 2005; Polis and Strong, 1996).

Ecosystems and their organisms are controlled by a vast number of factors that influence and interact directly and indirectly at many spatial and temporal scales (Maloney and Weller, 2011). Direct effects are defined in this thesis as linkages without mediating compartments or organisms, whereas indirect effects are defined as not directly connected but via an additional compartment or organism (e.g. food web). The major links between ecosystems across their boundaries are called subsidies (Richardson et al., 2010). Cross-ecosystem subsidies are defined by movement of biological matter or energy (e.g. detritus, invertebrates) from one (donor or source) to another system (recipient or sink) (Moyo et al., 2017). These subsidy flows can alter the productivity of recipient systems (Nakano and Murakami, 2001; Polis and Strong, 1996). The impact of such subsidies is determined by the ratio of subsidies to the comparable resources in the recipient system as well as its spatial and temporal extent (Marczak et al., 2007). The magnitude of aquatic insect output, varies spatio-temporally and is depending on different factors such as climate (Boulton et al., 2008; Freitag, 2004), geomorphology of the catchment (Iwata, 2007; Iwata et al., 2003), cover of riparian vegetation (Edwards and Huryn, 1995) and water flow (Polis et al., 2004). These linkages and subsidies across aquatic-terrestrial boundaries have been identified in different food webs like oceanic islands (Anderson and Polis, 1999), lakes (Jonsson and Wardle, 2009), rivers (Baxter et al., 2005; Kato et al., 2003; Nakano et al., 1999; Power et al., 2004) and wetlands (Regester et al., 2006, 2008). Especially riparian areas are coupled through reciprocal trophic cross-subsidies with freshwater ecosystems (Schulz et al., 2015).

Consumers can benefit from the dispersal of organisms across ecosystem boundaries, which can in turn have different responses in the recipient food web. Terrestrial leaves and invertebrates can fall into streams and provide a food source for aquatic shredders and fish, respectively. Conversely, emerging aquatic insects can be prey organisms for birds, bats and spiders (Baxter et al., 2005). Subsidies have effects on habitats at individual, population, community and ecosystem level (Baxter et al., 2005). Earlier studies have shown effects of terrestrial inputs to aquatic systems (Baxter et al., 2005). Current studies have started to focus more on the energy flow from aquatic to terrestrial systems (Schulz et al., 2015).

Aquatic insects are an important energy source for predators in the riparian area (Henschel et al., 2001; Kato et al., 2004; Nakano and Murakami, 2001; Paetzold et al., 2005) and can

influence both the predators directly (Krell et al., 2015) and the whole terrestrial system indirectly (Bultman et al., 2014; Henschel et al., 2001). Although the amount (biomass) of terrestrial to aquatic prey subsidies often is greater than the reverse, their overall contribution to the carbon budget of predators is similar (Bartels et al. 2012). The extent of cross-subsidies varies spatially and depends on different variables like climate (Boulton et al., 2008; Freitag, 2004), catchment geomorphology (Iwata, 2007; Iwata et al., 2003), the cover of the riparian vegetation (Edwards and Huryn, 1995) and the flow of water (Power et al., 2004; Schindler and Smits, 2017). These subsidies can range from 700 – 156 000 individuals / m² year (Jackson and Fisher, 1986), with an additional prey input ranging from 23 100 - 500 000mg / m² year (Baxter et al., 2005). Studies have shown that dipterans made up 25 – 99 % of aquatic subsidies biomass (Gray, 1989; Jackson and Fisher, 1986; Raitif et al., 2018), the remaining mass consisted mostly of Ephemeroptera, Plecoptera, Trichoptera, and Odonata (Baxter et al., 2005; Raitif et al., 2018) in different stream systems.

Indirect effects of such subsides depend on differences in the quality and mobility of prey species (Eubanks and Denno, 2000). A strong increase (e.g. seasonal change) of aquatic emergence can not only increase the abundance of riparian predators, but can also dominate riparian food webs (Dreyer et al., 2016; Henschel et al., 2001; Sabo and Power, 2002). These aquatic insects are often responsible for a higher density and diversity of consumers reported in riparian zones (Baxter et al., 2005; Nakano and Murakami, 2001). Due to the increasing number of predators, terrestrial prey organisms might be affected negatively (Holt and Lawton, 1994; Holt and Polis, 1997) and can in turn have a positive indirect effect on the prey (e.g. plants) of the terrestrial prey. On the other hand, a switch from terrestrial prey to subsidy prey, and by that reducing predation pressure on the terrestrial prey, can have indirect positive effects of aquatic subsidies on terrestrial prey organisms (Abrams and Matsuda, 1996; Dreyer et al., 2016). Studies on the role of aquatic insects for plants (Bultman et al., 2014; Henschel et al., 2001) and for trophic cascades including more than one prey type in an experiment (Dreyer et al., 2016) are scarce (Figure 1.1) **(Objective 1)**.

Aquatic insects contribute to the diet of riparian spiders, which in turn are a prey resource, e.g. for birds (Poulin et al., 2010). Spiders occur widely in riparian areas and can adapt to different habitats and have specialized hunting strategies (Roberts, 1996; Sanders and Entling, 2011). They can respond to changes in the aquatic insect emergence within short time by changing their habitat (Power et al., 2004). They feed on diverse prey organisms, but also other spiders, and also prey directly from the water surface (Graham et al., 2003; Marshall and Rypstra, 1999). Differences in spider composition and community characteristics (traits) can reflect changes in the environmental conditions (Dauber et al., 2005; Major et al., 2006; Schmidt et al., 2007; Vanbergen et al., 2005). Spiders profit from living in riparian areas, from the water by avoiding dehydration, and from subsidy prey organisms (Kato et al., 2004; Polis et al., 2004; Sanzone et al., 2003). Some species build orb webs on debris and vegetation next to streams to capture flying aquatic prey (Henschel et al., 2001). Ground-hunting species can obtain parts of their diet from aquatic prey, though they are often less dependent on aquatic prey than web-weaving spiders (Briers et al., 2005; Collier et al., 2002; Sanzone et al., 2003). Increasing aquatic prey availability increases aquatic prey contribution (Kraus et al., 2014) and can lead to higher spider densities in riparian areas (Henschel et al., 2001; Kato et al., 2004; Kraus et al., 2014). In contrast, an artificial reduction of aquatic insects by a greenhouse cover leads to a decrease of riparian web building spiders (Kato et al., 2003). Aquatic resources can range between 15 % to 90 % of riparian spiders diet (Baxter et al., 2005; Paetzold et al., 2005). In a field study, web building spiders built up approximately 61 % of their body carbon from aquatic prey insects,

whereas free-hunting spiders built up approximately 55 % (Collier et al., 2002). Differences in their diet represent changes in the environmental conditions, physico-chemical parameters and land use (Kraus et al., 2014; Paetzold et al., 2011). Consequently, land use can affect aquatic-terrestrial food webs along multiple pathways.

1.2 Effects of anthropogenic stressors

The current geological epoch has been referred to as "Anthropocene", which is characterized by human activities that affect major biogeochemical cycles (Crutzen, 2002; Steffen et al., 2011; Waters et al., 2016), and is defined by biotic changes. The decline in biodiversity, which is affecting the majority of species, suggests that the current epoch is characterized by the highest extinction rates on Earth since the Permian and Cretaceous periods (Ceballos et al., 2017). The loss of insects has an impact on ecosystems and their functioning, since they play an important role for many ecosystem processes including pollination (Öckinger and Smith, 2006; Ollerton et al., 2011), herbivory and detrivory (Mattson and Addy, 1975; Yang and Gratton, 2014), nutrient cycling (Yang and Gratton, 2014) and are important in food webs as prey for birds, mammals, amphibians and spiders (Baxter et al., 2005). The extinction rate of insects is higher than for other organisms, which might trigger indirect effects, via cascading effects, in many different ecosystems (Sánchez-Bayo and Wyckhuys, 2019; Thomas, 2004). Flying insects declined by 76 % in several German protected areas (Hallmann et al., 2017). It is suggested that approximately 10 000 - 20 000 freshwater species are at risk of extinction or already extinct worldwide (Strayer and Dudgeon, 2010). Responsible for the insect decline were made habitat changes (49.7 %), followed by pollution (25.8 %) and a variety of biological factors (17.6 %), in a review (Sánchez-Bayo and Wyckhuys, 2019). Disappearing aquatic species and their replacement by often non-native species represents a threat to freshwater biodiversity (Chandra and Gerhardt, 2008; Karatayev et al., 2009; Sala, 2000).

Responsible for that loss in biodiversity are anthropogenic stressors, like hunting, energy and resource extraction, as well as traffic and the development of infrastructure and habitat loss via deforestation, agricultural expansion and intensification, industrialisation and urbanisation (Ceballos et al., 2017; Maxwell et al., 2016; Millennium Ecosystem Assessment, 2005). In this regard the land surface has been modified to a large extent, chemical substances have been released in large quantities and the climate changed (Foley, 2005; Millennium Ecosystem Assessment, 2005). These stressors together affected 30-50 % of natural ecosystems at the end of the 20th century (Vitousek, 1997), among the ecosystems they also threaten stream ecosystem functions and biodiversity (Millennium Ecosystem Assessment, 2005).

Freshwater ecosystems make up only 0.01 % of the worlds water, and cover only 0.8 % of the Earth's surface but provide habitats for nearly 6 % of global biodiversity (Dudgeon et al., 2006). They host an important part of the global biodiversity (Balian et al., 2008; Strayer and Dudgeon, 2010), and provide important services to humans (Millennium Ecosystem Assessment, 2005). Especially smaller streams are important, since they host a high abundance (Downing, 2012) and high biodiversity of organisms (Davies et al., 2008), providing important ecosystem services (Biggs et al., 2016). However, it was shown that nearly half of European water bodies are at risk from organic toxicants with pesticides playing a major role (Malaj et al., 2014). Due to their connection with the riparian area and their low water volume, small streams are highly exposed to chemical pollution (Biggs et al., 2016).

Even though some studies suggest that habitat destruction and climate change have large impacts on ecosystems (Fox, 2013), other studies suggest that the impact of pesticides on

biodiversity is larger (Collier et al., 2002; Dudgeon et al., 2006; Millennium Ecosystem Assessment, 2005; Mineau and Whiteside, 2013). Agricultural intensification is considered to be a main driver of population declines in insectivorous mammals and insects (Sánchez-Bayo and Wyckhuys, 2019). This is due to many anthropogenic stressors, but the main reason is agricultural land use (Vörösmarty et al., 2004). The resulting changes in the microhabitat, like altered riparian vegetation complexity affect the diversity, abundance and density of organisms (Lambeets et al., 2008; Schindler and Smits, 2017). With an intensification of agriculture, pesticide use is increasing, as well as stream channelization, draining of wetlands, modification of floodplains and the riparian vegetation is removed with a subsequent removal of soil and nutrients. This leads to a homogenization of stream habitats and alteration of aquatic and riparian communities (Moyo et al., 2017; Schindler and Smits, 2017). Pesticides were made responsible for decline in aquatic insects (Beketov et al., 2013), birds, mammals and amphibians (Hallmann et al., 2017; Mineau and Whiteside, 2013). Pesticides are typically applied as mixtures, likely exceeding the effects of single pesticides (Brack et al., 2015). The intensification of land use also increases eutrophication and sedimentation in water systems, which can lead to a reduction in shredders and predators, while filterer species may be favoured (Burdon et al., 2013; Olson et al., 2016). Freshwater systems can suffer eutrophication due to an input of manure and dung and additional artificial fertilizers (Smith et al., 1999). Modifications of water flow destroy habitats and important structures like flood plains (Schindler and Smits, 2017) and cause unnatural fluctuations of river discharge. This leads to alterations of the local conditions and channel connectivity. In the 19th century Justus von Liebig showed a positive correlation between soil nutrients and the growth of terrestrial plants. However, fertilizers are not only enhancing growth of agricultural crop and forests, they can also enhance growth of aquatic algae and aquatic plants (Moss, 2008). The result of excess fertilizer in aquatic systems are growth but also the degradation of water resources, which can result in losses in the abundance and diversity of species as well as the loss of functions and services (Moss, 2008). Habitat change and pollution have been suggested as main drivers of species and diversity decline (Sánchez-Bayo and Wyckhuys, 2019). Other sources make the intensification of agriculture and the use of synthetic pesticides in the last six decades responsible as the main cause of the insect loss in recent times (Dudley and Alexander, 2017).

More than 100 000 chemicals are registered and in daily use (Schwarzenbach et al., 2010; Schwarzman and Wilson, 2009). Even though pesticides are intended to have a negative effect on pests and they are intentionally released, they have not been studied enough in the past in ecological studies of freshwater threats (Schäfer et al., 2016), because of that it is not clear how much they contribute to biodiversity loss (Persson et al., 2013; Rockström et al., 2009). Intensive agriculture includes a widespread and systematic use of pesticides to control crop pest (insecticides), competing weeds (herbicides) and fungal infection (fungicides) (Dudley and Alexander, 2017). Regarding toxicity, insecticides have the largest impact on insects and other arthropods, followed by fungicides, while herbicides have less to no impact on them (Mulé et al., 2017; Sanchez-Bayo and Goka, 2014). Declines can be observed specifically for aquatic insects, where agricultural pesticides represent a major cause of biodiversity loss (Beketov et al., 2013; Weston et al., 2014). These organisms are exposed not just to one pesticide, but to mixtures of pesticides, it is difficult to assign causality to individual toxic compounds (Sánchez-Bayo and Wyckhuys, 2019).

Insects represent the base of food webs (Sánchez-Bayo and Wyckhuys, 2019) and various groups of organisms depend on insects as prey (Baxter et al., 2005; Sánchez-Bayo and Wyckhuys, 2019). A change due to anthropogenic stressors, and especially agricultural land use, subsequently can alter diet patterns and in turn the adjacent food webs. However, there is a lack of information about the effects of agricultural land use and pesticides on cross-ecosystem linkages (Schulz et al., 2016).

1.3 Effects of anthropogenic land use on food webs

Several studies found a link between the environmental conditions including stressors and the diet across ecosystems (e.g. for birds) (Carlson et al., 2016; Jakob and Poulin, 2016; Krell et al., 2015; Poulin et al., 2010; Raitif et al., 2018). First observations of cascading effects were shown in England in 1952. Consequences for grey partridge populations were reproductive failure, caused by the use of insecticides and herbicides, leading to reduced insects and in turn to reduced prey for the chicks (Potts, 1986). Abundances and diversity of bats were lower in intensive agricultural land used fields than in less intense organic farms, due to a reduction of insects, which constitute a prey for bats, caused by the use of pesticides in intense agriculture (Wickramasinghe et al., 2004), and by an exposure to pesticides via the prey (Mispagel et al., 2004; Stahlschmidt and Brühl, 2012).

Agricultural land use in a forested landscape has an impact on abundances of prey and its consumption by consumers via its effects on the composition of emerging aquatic insects, rather than the overall abundance of the aquatic subsidy (Stenroth et al., 2015). Losses of insect biomass in Europe (Hallmann et al., 2017) and Puerto Rico (Lister and Garcia, 2018) correlate with a decline in dependent consumers (Hallmann et al., 2014; Jakob and Poulin, 2016; Lister and Garcia, 2018; Poulin et al., 2010; Wickramasinghe et al., 2003).

Riparian spiders represent a group of predators that rely on terrestrial and aquatic prey that may be impacted by pesticides and in turn respond to related impacts. Web-spinning behaviour and overall fitness of spiders was affected by substrate availability (Chan et al., 2009; Laeser et al., 2005; Vollrath et al., 1997) and by the diversity and quality of prey organisms (Mayntz and Toft, 2001; Sherman, 1994). Changes of aquatic prey by stressors has led to a reduction of riparian spiders in the past. Contaminants reduced aquatic prey and in turn riparian spiders (Kraus et al., 2014; Paetzold et al., 2011). Conversely, a meta-analysis by Lafage et al., (2019) showed, that increasing agricultural land use can cause an increased aquatic diet contribution in riparian spiders.

The composition of taxa and traits of spider communities can be shaped by stressors, such as those related to agricultural land use (Dauber et al., 2005; Major et al., 2006; Vanbergen et al., 2005). Spiders can be exposed to pesticides via multiple pathways, direct and indirect with lethal (fatal) and sublethal (not fatal) consequences, resulting in a change in species and trait composition of spider communities (Pekár, 2012).

Contaminants can also accumulate in consumers via higher aquatic prey consumption (Walters et al., 2008). Additionally, riparian spider species and their trait composition can be affected by the availability of aquatic and terrestrial prey (Pekár, 2012). These response traits (Violle et al., 2007), can be used to establish trait-stressor relationships. Such relationships might be transferable to areas with different taxonomic composition and can allow for explaining of changes in compositions as well as for testing hypotheses of relationships (Keddy, 1992; Lambeets et al., 2008; Le Viol et al., 2008; Pekár, 2012; Schirmel et al., 2012).

Disentangling the mechanisms by which land use affects streams is complicated due to the

numerous pathways between land use and ecosystems. These pathways are mostly indirect (e.g. via food web) and carry effects of past land use (Burcher et al., 2007). In the past, studies mainly focused on direct effects on streams, even though they are additionally influenced indirectly (Burcher et al., 2007; Maloney and Weller, 2011). Studies on the impact of pesticides have largely focussed on either aquatic (Schäfer et al., 2012) or terrestrial (Fogel et al., 2016; Gibbs et al., 2009) systems in the agricultural landscape ignoring potential effects for cross-ecosystem food webs (Schulz et al., 2015). Studies that focussed on the impact and response of riparian spiders only focussed on the effect of agricultural land use as a general stressor (Carlson et al., 2016; Krell et al., 2015; Raitif et al., 2018; Stenroth et al., 2015). Studies investigating possible land use related effects of pesticides on riparian organisms and their trait composition are missing. Pesticides in streams can change aquatic as well as terrestrial community compositions (Geiger et al., 2010; Liess and Von Der Ohe, 2005). These in-stream effects on emerging aquatic insects can propagate to terrestrial food webs, especially to consumers like spiders (Pekár, 2012). Nevertheless, studies on specifically in-stream pesticides riparian spiders and trait composition **(Objective 2)** (Figure 1.1) as well as their diet **(Objective 3)** (Figure 1.1) are scarce. Knowledge on aquatic terrestrial linkages and interactions associated with stressors is needed to protect ecosystems.

1.4 Objectives and structure of the thesis

The aim of this thesis is to contribute to a better understanding of cross-ecosystem linkages in the riparian area and how they are affected by agricultural land use.

In detail, the thesis addresses the following research objectives:

Objective 1: Examining the indirect impact of aquatic insect subsidies on a terrestrial model food web in a mesocosm study with two herbivores, which have different predator avoidance strategies, including plants and spiders. (Chapter 2)

Objective 2: Examining the impact of agricultural land use with a specific focus on pesticides on the taxonomic composition of riparian spiders, their number, species richness, traits of in-stream toxicity and other environmental variables in a field study. (Chapter 3)

Objective 3: Examining the impact of agricultural land on the aquatic prey biomass by in-stream pesticide toxicity and other agricultural stressors. Assessing the impact of in-stream pesticide toxicity and other agricultural stressors on the aquatic diet contribution of riparian spiders. (Chapter 4)
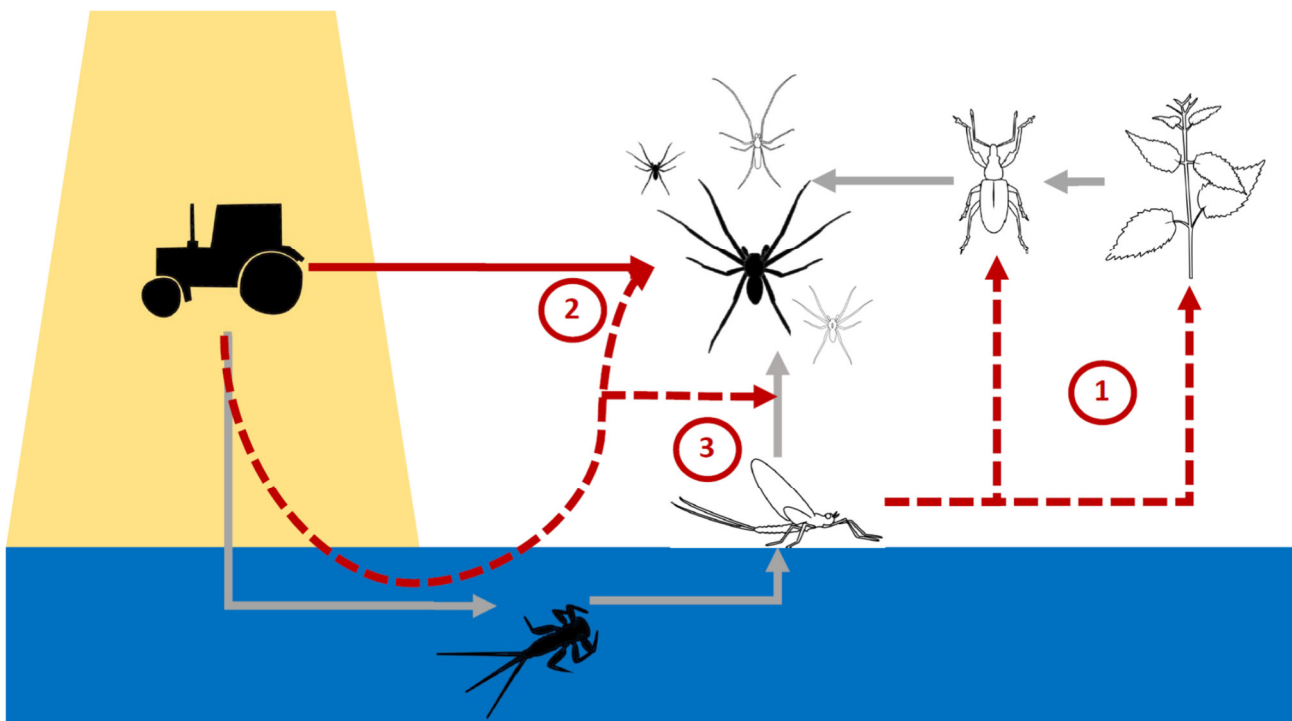
Figure 1.1: Overview of this thesis based on the thematic background in the introduction. Grey continuous arrows indicate direct interactions which were not investigated in the thesis. Red dashed arrows indicate indirect effects. Red continous arrows represent investigated effects in the three studies. (1) Indirect impact of aquatic insect subsidies on herbivores and plants via spiders (2) Impact of agricultural land use stressors on the taxonomic composition of riparian spiders, their number, species richness and traits (3) Impact of agricultural land use stressors on potential aquatic prey and the impact of agricultural stressors and biomass of potential aquatic prey on the aquatic diet contribution of riparian spiders.

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2. CONTRASTING EFFECTS OF AQUATIC SUBSIDIES ON A TERRESTRIAL TROPHIC CASCADE

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2.1 Abstract

Subsidies from adjacent ecosystems can alter recipient food webs and ecosystem functions, such as herbivory. Emerging aquatic insects from streams can be an important prey in the riparian zone. Such aquatic subsidies can enhance predator abundances or cause predators to switch prey, depending on the herbivores. This can lead to an increase or decrease of in situ herbivores and herbivory. We examined the effects of aquatic subsidies on a simplified terrestrial food web consisting of two types of herbivores, plants and predators (spiders). In our six-week experiment, we focused on the prey choice of the spiders by excluding predator immigration and reproduction. In accordance with predator switching, survival of leafhoppers increased in the presence of aquatic subsidies. By contrast, the presence of aquatic subsidies indirectly reduced weevils and herbivory. Our study shows that effects of aquatic subsidies on terrestrial predators can propagate through the food web in contrasting ways. Thereby, the outcome of the trophic cascade is determined by the prey choice of predators.

3. DO AGRICULTURAL PESTICIDES IN STREAMS INFLUENCE RIPARIAN SPIDERS?

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3.1 Abstract

Freshwater ecosystems are coupled with their riparian area. Emerging insects are prey for predators n the riparian zone, enriching the terrestrial ecosystem with energy and nutrients. Stressors associated with agriculture can alter insect communities in water and on land, resulting in complex response patterns of terrestrial predators relying on prey from both systems. Examining the effects from individual agricultural stressors such as pesticides is hampered in landscapes with intensive agriculture where multiple stressors like habitat degradation and typically co-occur. In rural regions of Eastern Europe, traditional low intensity agriculture based on working animals and human labour prevails alongside intensive, mechanised agriculture. Assuming that low-intensity agriculture relies on no or limited pesticide use, whereas fertilizer use is similar across different agricultural intensities, such regions may allow to study in-stream pesticide effects independent from other stressors, such as nutrient input or habitat degradation. We examined the taxonomic and trait response of riparian spider communities to gradients of agricultural stressors and environmental variables in the region around Cluj-Napoca, Romania. Pesticide sampling was done using passive samplers in the streams adjacent to spider sampling sites. To capture spiders with different traits and survival strategies, we used multiple collection methods. Community composition was best explained by in-stream pesticide toxicity and shading of the stream bank, a proxy for the quality of the habitat. Species richness and the number of spider individuals, as well as community ballooning ability, were negatively associated with in-stream pesticide toxicity. In contrast, mean body size and shading preference of spider communities responded strongest to shading, whereas mean niche width (habitat preference for moisture and shading) responded strongest to the other environmental variables. Our study suggests that in-stream pesticide toxicity can influence riparian communities. The identification of mechanisms requires further studies targeting the potential contributions of direct toxicity and indirect effects from reduced aquatic and terrestrial prey availability.

4. RELATIONSHIP BETWEEN AGRICULTURAL PESTICIDES AND THE DIET OF RIPARIAN SPIDERS

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4.1 Abstract

Background
Examining the effects of pesticides is difficult in regions such as Western Europe because of the relatively ubiquitous use of agrochemicals and the lack of unaffected areas. To obtain a gradient of agrochemical stress, we conducted a study in Central Romania, where traditional agriculture, which is assumed to use less agrochemicals, exists adjacent to intensive agriculture. We investigated potential effects of land use related stressors including pesticides on aquatic-terrestrial predator-prey relationships using stable isotope analysis. Therefore, we sampled spiders, as well as their aquatic and terrestrial prey along streams with a pesticide pollution gradient and determined spider and prey stable carbon and nitrogen signals.
Results
Aquatic prey contributed 40.8 to 55.4 % to the diet of the orb web weaving spider Tetragnatha sp. and 34.0 to 53.0 % to the diet of the ground-hunting Pardosa sp. The contribution of aquatic prey to the diet of the orb weaver was related to the biomass of potential aquatic prey and to a gradient representing stream characteristics and intensive agricultural land use. The contribution of aquatic prey to the diet of the ground-hunter was related to in-stream toxicity and a negative relation with a gradient representing riparian habitat and water variables and land use intensity.
Conclusion
Our study suggests that in-stream pesticide toxicity can impact the riparian food web in different ways. Future manipulative field studies on aquatic-terrestrial linkages are required to examine the causality of our findings and should also consider the quality of prey organisms to foster mechanistic understanding of such cross-ecosystem effects.

4.2 Background

Riparian areas are linked to streams via fluxes of dead material and living organisms (Baxter et al., 2005). Aquatic insects are an important prey source for riparian predators, subsidising the terrestrial ecosystem with energy and essential nutrients (Graf et al., 2017; Kato et al., 2004; Paetzold et al., 2005). Emerged aquatic insects constitute a major part of the diet of riparian spiders, which in turn are a prey resource for terrestrial organisms such as birds (Poulin et al., 2010).

The magnitude of aquatic emergence varies spatio-temporally and depends on factors such as climate (Boulton et al., 2008; Freitag, 2004), geomorphology of the catchment (Iwata, 2007; Iwata et al., 2003), cover of riparian vegetation (Edwards and Huryn, 1995) and water flow (Power et al., 2004). Stressors related to agricultural land use, such as excessive nutrient, sediment and pesticide inputs, can influence aquatic-terrestrial trophic relationships (Krell et al., 2015; Stenroth et al., 2015; Walters et al., 2008). Altered insect emergence may subsequently affect the diet of terrestrial predators and in turn components of the terrestrial food web. Indeed, several studies have shown a link between the environmental conditions including stressors and the diet of predators (e.g., birds) consuming stream-derived prey (Carlson et al., 2016; Krell et al., 2015; Poulin et al., 2010). In particular, land use related stressors can affect aquatic-terrestrial coupling with effects propagating into terrestrial food webs. To date, studies on the effects of pesticides have largely been limited to either aquatic (Schäfer et al., 2012) or terrestrial (Fogel et al., 2016; Gibbs et al., 2009) habitats in the agricultural landscape ignoring potential consequences for cross-ecosystem food webs (Schulz et al., 2015). Previous studies of such consequences and the response of spiders have mainly focused on agricultural land use as a general stressor (Carlson et al., 2016; Krell et al., 2015; Stenroth et al., 2015), whereas, to our knowledge, studies of the specific effects of pesticides on the diets of riparian organisms are lacking.

Riparian spiders represent a group of predators that relies on terrestrial and aquatic prey. Some spiders build orb webs on debris and vegetation next to surface water bodies to capture flying aquatic prey (Henschel et al., 2001). Ground hunting spiders along streams can obtain a considerable part of their diet from aquatic prey, though they are often less dependent on aquatic prey than web-weaving spiders (Briers et al., 2005; Collier et al., 2002; Sanzone et al., 2003). Higher aquatic prey availability increases the aquatic prey contribution to spider diet (Gergs et al., 2014; Kraus et al., 2014) and can lead to a higher spider density in riparian areas (Henschel et al., 2001; Kato et al., 2004; Kraus et al., 2014). Conversely, a change of aquatic prey by stressors can lead to a reduction of riparian spiders. For example, contaminants reduced aquatic prey and in turn riparian spiders (Kraus et al., 2014; Paetzold et al., 2011).

Similarly, a study by Graf et al. (Graf et al., 2019) found that reduced abundance and species richness, as well as changes in the community composition of riparian spiders correlated with in-stream pesticide toxicity. However, a recent meta-analysis (Lafage et al., 2019) showed, that the aquatic diet contribution of spiders increases with agricultural land use at local and landscape scale, which the authors attributed to an increase of nutrients due to fertilizers.

We conducted an explorative field study on the response of aquatic-terrestrial predator-prey relationships to agricultural stressors with a particular emphasis on pesticide toxicity in Central Romania. This region was selected because traditional, low-intensity agriculture persists next to areas with high-intensity agriculture (Fischer et al., 2012; Kovács-Hostyánszki et al., 2016), potentially allowing to capture a wider gradient of in-stream pesticide toxicity than in landscapes predominantly exhibiting high intensity agriculture, as for example in Western Europe (Schäfer et al., 2012). In a previous study, we found that changes in riparian spider

communities correlated with in-stream pesticide toxicity (Graf et al., 2019). Here, we focus on the response of the diet of two riparian spiders, with different foraging strategies (a web weaver and a ground hunter), to in-stream pesticide toxicity and other stream variables, to identify potential changes in the linkage between aquatic and terrestrial food webs. We expected that the diet responds to pesticide toxicity because it is well known that pesticides change the composition of aquatic invertebrate communities and consequently the composition of potential prey biomass (Schäfer, 2019). Given that several agricultural stressors often co-occur (e.g. excessive nutrients, pesticides), with potentially different effect directions on the potential prey biomass (e.g. nutrients can increase biomass, pesticides can decrease the biomass of sensitive organisms but increase the biomass of tolerant organisms), we did not formulate a specific hypothesis on the direction of the response of the spider diet. However, we expected a higher proportion of aquatic prey in the diet of the web weaving spider due to its stronger reliance on streams.

4.3 Material and Methods

4.3.1 Study area and sample site selection

The study was conducted in Central Romania around Cluj-Napoca. The landscape has been characterized as a mosaic of arable fields (15 % cover, less intense agriculture, low use of agrochemicals expected), settlements and other minor land uses (15 % cover), deciduous forests (30 % cover) and pastures (40 % cover, low-intensity grazing and mowing) (Fischer et al., 2012). In total we selected 19 sites where we sampled spiders and their potential prey, in-stream pesticides and other environmental parameters (see supporting information Table SI 6.1). All sampling sites were located along streams with adjacent agricultural fields. The fields included in the study comprised high-intensity agricultural fields that were expected to represent high pesticide use (e.g. large field sizes, mechanised agriculture) and low-intensity agricultural fields that were expected to represent low pesticide use (e.g. fields subdivided into many small parcels, agriculture based on human labour and working animals). They were selected to cover a gradient of high-intensity to low-intensity agriculture and, hence, were expected to provide gradients in habitat quality, physico-chemical parameters and in-stream pesticide toxicity.

4.3.2 Sampling of riparian spiders and their potential prey

Sampling took place in May and June 2016. At each site, transects of 20 m along the streams were defined for biological sampling. Tetragnatha sp. (Tetragnathidae, orb web weaver) and Pardosa sp. (Lycosidae, ground-hunter) were collected (see supporting information, Table S2 & S3) after sampling of aquatic and terrestrial prey. The web weaving spider Tetragnatha sp. and the ground-hunting spider Pardosa sp. were selected as they are common riparian spiders with different foraging strategies, which was expected to lead to different responses to changes in aquatic prey availability. Tetragnatha sp. builds orb webs on debris and vegetation next to the water to primarily capture flying prey (Roberts, 1996). In contrast, Pardosa sp. hunts a broad spectrum of terrestrial invertebrates in the riparian zone and aquatic prey directly from the water surface (Graham et al., 2003; Marshall and Rypstra, 1999; Nyffeler, 1999). To reduce variability, where feasible, only adult female spiders were analysed, because adult male spiders may abandon food intake (see details about specimens in supporting information, Table SI 6.2 & Table SI 6.3). All spiders were hand collected and kept individually in small containers.

We sampled the aquatic invertebrates listed as potential prey for spiders (Nyffeler, 1999; Nyffeler and Sunderland, 2003) (Table 4.1). As spider tissues need one to three weeks to enrich stable isotope signals from their contributed prey (Kato et al., 2004; Ostrom et al., 1997), we sampled aquatic prey over three weeks until one week before spider sampling. Aquatic emergence traps (Cadmus et al., 2016) with a basal area of 0.25 m2, which were emptied at least once a week, were used. Two traps were placed per stream, each with a bottle trap that was filled with an aqueous solution of 1 % (vol.) TWEEN® 80, a non-ionic detergent, and saturated with sodium chloride (for preservation purposes). Terrestrial prey organisms were sampled once, three weeks before the sampling of the spiders. Terrestrial prey was collected with a modified leaf blower (modified STIHL SH86 blower; Stihl). Vegetation and ground were vacuumed within a distance of 0 - 1.5 m to the stream 50 times each for 5 s. The vacuumed samples were sorted in the lab. All samples were transported at ~5 °C and later kept frozen (~-18 °C) until identification and analysis.

4.3.3 Preparation of spider and prey samples for stable isotope analysis

Spiders were identified to species level following Roberts (Roberts, 1996). Where feasible, aquatic and terrestrial prey were identified to family and genus and order and family level, respectively.

For isotope analysis, samples were dried at 60 °C for at least 24 h. Where size allowed, single organisms were used in the isotope analysis, otherwise multiple individuals of one taxon were pooled to ensure a sufficient amount of material for the analysis. Samples were homogenized and weighted into tin cups (approximately $0.5 - 1$ mg). Three to five replicates per site and organism group were analysed using a Flash 2000 HT elemental analyser coupled via a ConFlo IV interface to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific). Stable isotope ratios of carbon and nitrogen are presented in conventional δ notation (‰) relative to their respective international standards (Vienna Pee Dee Belemnite) and atmospheric N2. Repeated analyses of an internal standard (i.e. casein) typically resulted in an accuracy (± standard deviation (SD)) of 0.047 ‰ and 0.044 ‰ for carbon and nitrogen, respectively. Prey groups were combined for mixing models based on stable isotope analysis (SIA) according to taxon and in cases were stable isotope signatures of different insect groups overlapped (Phillips et al., 2014).

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Table 4.1: Origin of the prey organisms, prey groups used in the mixing model and prey groups used in the stable isotope analysis (SIA).

Prey origin (aquatic/terrestrial)	Prey groups in mixing model	Prey groups used in SIA
Aquatic	Diptera	Empididae
		Chironomidae
		Simuliidae
	Ephemeroptera	Ephemeroptera
	Trichoptera	*Hydropsyche*
		Hydroptilidae
		Trichoptera other
Terrestrial	Collembola	Collembola
	Hemiptera	Auchenorrhyncha
		Sternorrhyncha

4.3.4 Characterisation of environmental parameters and pesticide analysis

To determine the potential response of aquatic prey to stressors and in turn the propagation of effects to riparian spiders, we recorded the hydromorphological structure and physico-chemical parameters of the streams (Table 4.2).

The distances from the stream to adjacent fields on both sides were recorded and aggregated into a single field distance index (*fdi*) for each respective sampling site to decrease the number of explanatory variables. We calculated the *fdi* as follows:

$$fdi = \frac{\left(\left(1-\frac{x_L}{100}\right)+\left(1-\frac{x_R}{100}\right)\right)}{2} \; ; \text{ with } x_L \lor x_R = 99 \text{ if } x_L \lor x_R > 99 \qquad (4.1)$$

where x_L and x_R is the distance of the agriculturally used field on the left (L) and right (R) side of the stream, respectively. For further details and rationale see Graf et al., 2019.

To determine the ratio of land use in the upstream catchment of each sampling site, we overlaid the upstream catchment areas calculated by the ATRIC algorithm (Bhowmik et al., 2015) with a CORINE land cover vector layer (European Environment Agency, 2007).

Pesticide sampling was done using passive samplers in the streams adjacent to each sampling site with polydimethylsiloxane (PDSM) sheets and styrene-divinylbenzene (SDB) disks (for details see (Graf et al., 2019)). The in-stream toxicity of the 53 detected pesticides was assessed using the logarithmic sum of the toxic unit (referred to as sumTU, (Schäfer et al., 2013). The sumTU is based on the EC50 value of the most sensitive freshwater invertebrate species for each pesticide (i.e. the minimum EC50 value), which was compiled from the Pesticide Property Data Base (Lewis et al., 2016), reported toxicity values in Malaj et al. and data from the US EPA ECOTOX data base (Malaj et al., 2014; U.S. Environmental Protection Agency, 2018). A previous comparative analysis of different proxies for exposure showed, that the sumTU was among the indices that yielded the highest relationship with ecological responses (Beketov et al., 2013). In the analysis, we used the maximum sumTU across four pesticide sampling time points as a proxy for the maximum pesticide pollution, which may influence the export of aquatic prey to the spiders' habitats, in the sampling period. Hereafter, we will refer to the maximum sumTU as toxicity.

4.3.5 Estimation of potential aquatic prey biomass

The dry biomass (W) of the emerged aquatic insects was used as a proxy for the potential aquatic prey available to spiders. We estimated the potential aquatic prey biomass W using length-mass relationships (Sabo & Power, 2002). The length L of the aquatic prey was estimated using literature values (supporting information, Table SI 6.4; (Müller and Bährmann, 2015; Schaefer et al., 2010) and W was calculated as (Sabo and Power, 2002):

$$W = a * L^b \qquad (4.2)$$

where L is length (mm) and a and b are fitted parameters describing the allometric relationship between the dry mass and length. Finally, the biomass m of aquatic prey per day was calculated as:

$$m = \frac{\sum W_i}{t} \qquad (4.3)$$

where t is the sum of the number of days per emergence trap per stream during which aquatic prey taxon i was sampled.

4.3.6 Data analyses and statistics

To identify variables that may explain the potential aquatic prey biomass available at each site, we conducted variable selection using a linear model, given that the response exhibited normal distribution. We conducted stepwise backward model selection and used the Akaike information criterion (AIC) as a model goodness of fit measure (Akaike, 1974), corrected for small sample sizes, i.e. corrected AIC (AICc). Similarly, variables that may explain the aquatic contribution to the diet of spiders were identified with a stepwise backward model selection using a linear model for the ground-hunting spider, given that the response exhibited normal distribution, and a generalized linear model (GLM) with binomial distribution, as the normal distribution assumption was not met, for the web weaving spider with the AICc as model goodness of fit measure. The aquatic contribution to the spider diet was calculated with the R package MixSIAR (Stock et al., 2018) (version 3.1.7, run chain length 3,000,000). MixSIAR is based on Bayesian inference and estimates the most likely sources contributing to the spiders' assimilated diets (Moore & Semmens, 2008). We used the trophic enrichment factors for terrestrial (0.4 ± 0.17 ‰ for δ^{13}C; 2.3 ± 0.28 ‰ for δ^{15}N) and aquatic prey (0.5 ± 0.19 ‰ for δ^{13}C; 2.3 ± 0.24 ‰ for δ^{15}N) (McCutchan et al., 2003). To ensure the correct prey organisms were sampled for the spiders, only stable isotope data for the sites where at least 60 % of spiders were inside the mixing polygon were used following Parnell (2016) (for details see supporting information Figure SI 6.1, Figure SI 6.2, Table SI 6.2, Table SI 6.3).

Due to loss of emergence traps, absence of spiders in some sites and more than 40% of spiders' isotopic values in a site lying outside of the mixing polygon, only 10 sites for ground-hunter and 13 sites for web weaving spiders were available for the final analysis (details in supporting information, Figure SI 6.1 & Figure SI 6.2).

Toxicity was included as an individual variable in the data analysis, given our focus on the potential relationships of pesticides with spider diets. The intensity of the agricultural land use in the area surrounding each sampling site was not measured directly (see above) but is likely

reflected by several measured environmental variables (Table 4.2). The low ratio of sample size (i.e. number of sites) to the number of explanatory variables prohibited the inclusion of all explanatory variables in the statistical models. To capture most of the information from the variables, we used a sparse principal component analysis (SPCA) to reduce the number of variables in the models and generate orthogonal, i.e. independent, explanatory variables. SPCA improves interpretability through reducing the number of variables that load on an axis at the cost (typically minor) of capturing less of the total variance of all variables (Zou et al., 2006). The scores of each sampling site on the two sparse principal component axes were used in the subsequent regression analysis (principal component regression).

The statistical software R (R Development Core Team, 2011), with the package effects (Fox and Hong, 2009) for graphics, was used for statistical analyses. SPCA was done in R using the package pcaPP (Filzmoser et al., 2018). We provide all computer code and data under https://github.com/rbslandau/graf_spiderdiet .

4.4 Results

Thirteen variables were captured by the two SPCA axes, which explained 23.9 % and 12.3%, respectively, of the total variance of these environmental data. The first axis was primarily (loading > 0.3) composed of stream conditions such as pH, oxygen concentrations, % of riffles, stream width and upstream catchment size (Table 4.2). In addition, it represented the cover with arable and permanent crops in the catchment, indicating intensive agricultural land use (Table 4.2). The field distance index and the corridor meadows cover within 200 m of the stream loaded only weakly on the first SPCA axis and will not be considered in interpretation. High values on the first SPCA axis represent a higher pH, higher oxygen, greater width and a higher proportion of riffles in the stream reach as well as a larger catchment. Low values represent a higher amount of catchment land use type 1, indicating intensive agricultural use. The second SPCA axis was composed of sulphate concentrations in water and the riparian cover close to the streams as well as land use type 2, representing pastures and heterogeneous agricultural areas in the catchment, indicating less intense land use. Chloride, nitrite and phosphate concentrations in the streams as well as conductivity, the agricultural land use within 200 m of the stream and shading were not captured by the SPCA axes and consequently omitted from analysis (Table 4.2).

Table 4.2: Environmental variables used in data analysis with units and explanation. SD = standard deviation

Variable	Unit and explanation	Loading on SPCA axes	Value	
			Mean	SD
Toxicity	Maximum of log sum toxic unit for the most sensitive freshwater invertebrate	Excluded from SPCA	-0.56	0.52
Chloride[†]	[mg/L]	No loading	13.5	12.21
Conductivity[$]	[μS/cm]	No loading	0.74	0.19
Nitrate[†]	[mg/L]	No loading	0.79	0.51
Phosphate[†]	[mg/L]	No loading	0.29	0.21
Corridor land use agriculture	[%] agricultural land use within a 200 m distance to stream	No loading	37.31	26.35
Shading	[%] shading elements	No loading	52.31	35.86
pH[$]		First axis; 0.33	8.19	0.18
Oxygen[$]	[%] Dissolved oxygen in the stream	First axis; 0.45	75.99	8.95
Field distance index	Weighted distance of streams to agriculturally used fields for right and left site of the stream, at sampling site	First axis; -0.13	0.64	0.25
Corridor land use meadows	[%] land cover with meadows within a 200 m distance to the stream	First axis; -0.14	52.5	25.78
Catchment size	[km²] Size of catchment area	First axis; 0.30	76.03	67.16
Catchment land use type 1	[%] land cover with arable land and permanent crops in the catchment[¶]	First axis; -0.31	30.34	13.92
Maximal width stream	[m] widest part of the stream at sampling site	First axis; 0.39	3.57	1.35
Minimal width stream	[m] narrowest part of the stream at sampling site	First axis; 0.42	2.28	1.23
Riffles	[%]	First axis; 0.36	41.92	28.03
Sulphate[†]	[mg/L]	Second axis; 0.36	64.54	33.38
Riparian cover shrubs	[%] riparian land covered with shrubs within 5 m to stream along a 20 m transect	Second axis; 0.62	16.15	15.93
Riparian cover meadows	[%] riparian land covered with meadows within 5 m to stream along a 20 m transect	Second axis; -0.59	25.38	25.02
Catchment land use type 2	[%] Pastures and heterogenous agricultural areas in the catchment[¶]	Second axis; -0.37	39.07	7.54

† Compact-photometer PF-12, Machery-Nagel was used for measurement
$Multiparameter pH/ORP/EC/TDS/ Salinity /DO/ Pressure /Temperature Waterproof Meter - HI98194, Hanna Instruments was used for measurement
CORINE codes from Catchment land use 1: non-irrigated arable land 211, vineyards 221, fruit trees and berry plantations 222; CORINE codes from Catchment land use 2: pastures 231, complex cultivation patterns 242, land occupied by agriculture with significant areas of natural vegetation and pastures in the catchment area 243

Dry biomass of potential aquatic prey emerging at the study sites ranged between 48.9 and 588.2 mg per trap per day. Diptera made up 1 – 40 %, Ephemeroptera 5 – 94 % and Trichoptera 2 – 90 % of the total aquatic prey biomass. Aquatic prey contributed 40.8 – 55.4 % (mean = 48 %, standard deviation = 3.8%) to the diet of web weaving spider and 34.0 – 53.0 % (mean = 42%, standard deviation = 6.2%) to the diet of ground-hunting spiders (details supporting information, Table S9). The proportions of aquatic prey in the diets of both spiders were not correlated (Pearsons correlation coefficient r = 0.05).

The best-fit model for emerged biomass of potential aquatic prey biomass only contained the first SPCA axis, which exhibited a positive relation (AICc = 185.7, R^2 = 0.31, n = 14). Potential aquatic prey biomass increased with increasing pH, oxygen concentration in water, % riffles, stream width and catchment size. It decreased with increasing intensive agricultural land use. The diet of the web weaver was best explained by the potential aquatic prey biomass and the first SPCA axis (AICc = -41.36, D^2 = 0.2, n = 13). The contribution of aquatic prey in the diet of the web weaver increased with the potential aquatic prey biomass (Figure *4.1*A). Additionally, we found a negative relation of aquatic prey in the spider diet of the web weaver with the first SPCA axis (Figure *4.1*B). The aquatic diet contribution for the web weaver decreased with pH, oxygen concentration in water, % riffles, stream width and catchment size and increased with intensive agricultural land use. The aquatic diet of the ground hunting spider *P. amentata* was positively related with the toxicity gradient (Figure *4.1*C). In addition, it decreased along the second SPCA axis (Figure 4.1D) (AICc = -24.8, R^2 = 0.57, n = 10). The aquatic diet contribution increased with meadows within 5 m to the stream and extensive agricultural land use in the catchment. It was negatively related with sulphate in the streams and increasing shrubs within 5 m to the streams. The AICc of all models in stepwise selection can be found in the supporting information Table SI 6.6.
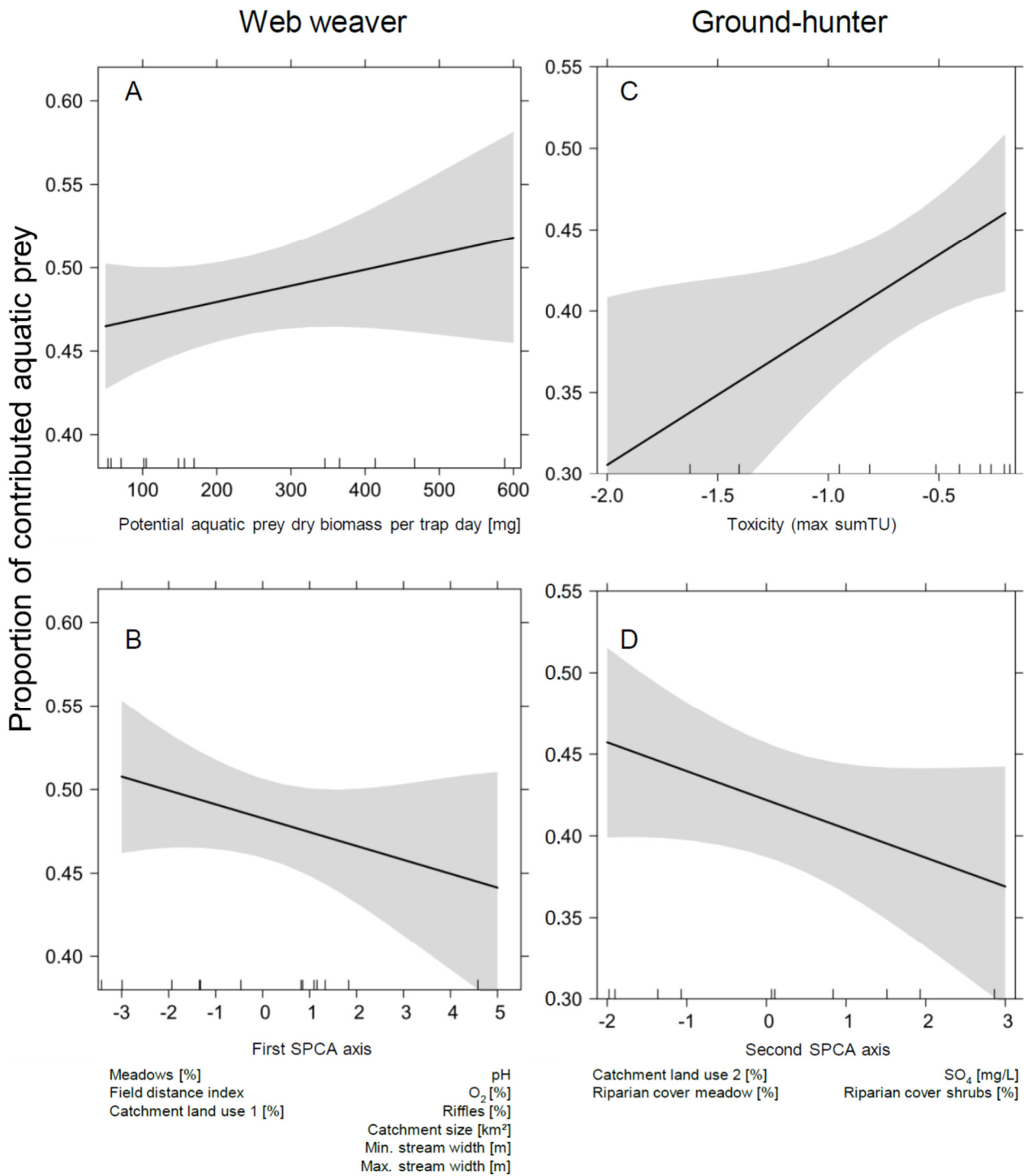
Figure 4.1: Predictor-effect plots (Fox and Weisberg, 2019) for the aquatic contribution to the diet of the web weaver with (A) aquatic prey dry biomass per trap day, (B) the first SPCA axis and the diet of the ground-hunter with (C) in-stream pesticide toxicity in terms of logarithmic sum of the toxic unit [max sumTU] and (D) the second SPCA axis based on the final models. See Table 2 for loadings of variables composing the SPCA axis, indicating their relevance for the axis. Grey areas indicate 95 % confidence bands for the explanatory variable. Rugs show the marginal distribution of an explanatory variable. Note that the values of the response are the sum of multiple predictors and therefore the original responses cannot be displayed in the figures of individual predictors. For details see (Fox and Weisberg, 2019).

4.5 Discussion

4.5.1 Changes in potential aquatic prey biomass in relation to environmental variables

The biomass of potential aquatic prey was related with a gradient (SPCA axis 1) that primarily represented stream characteristics such as water quality (pH, oxygen), in-stream habitat conditions (% riffles) and stream size as well as intense agricultural catchment land use. Hence, the gradient ranged from sites influenced by high intensity agriculture with lower stream oxygen concentrations, and slower flow to sites less influenced by high intensity agriculture with higher oxygen concentrations, pH and fast flow sections in terms of riffles. This gradient resembles an agricultural gradient that is known to influence stream macroinvertebrate communities (Burdon et al., 2019; Piggott et al., 2015). Moreover, oxygen and fast flow, as present in riffles, are important variables determining invertebrate community composition in stream ecosystems (Schindler and Smits, 2017). The higher potential aquatic prey biomass in sites less influenced by intense agricultural land use and with generally better water and habitat quality is in line with previous studies. Agricultural land use in the catchment resulted, relative to forested land use, in smaller and weaker flying aquatic prey (e.g. Diptera) (McKie et al., 2018). In terrestrial ecosystems, agriculture has also been related to the loss of flying insect biomass (Hallmann et al., 2017). Riffles correlated positively with the abundance of flying aquatic prey in a study on nine Swedish streams (McKie et al., 2018).

However, the drivers of changes in the potential aquatic prey biomass are not necessarily the drivers of the diet of riparian predators as shown in a meta-analysis where the aquatic diet contribution of spiders increased with agricultural land use (Lafage et al., 2019). This is because predators likely exhibit differential feeding preferences and therefore not only the amount but also the composition of the potential aquatic prey is relevant. Furthermore, the contribution of aquatic prey to the diet also depends on the availability of terrestrial prey organisms. The drivers of changes in the potential aquatic prey biomass may also influence the biomass and composition of terrestrial prey organisms. Hence, predicting the diet of riparian predators would require knowledge of the composition and biomass of both aquatic and terrestrial prey as well as of predator feeding preferences. More detailed studies with a longer duration would be required to produce such knowledge. Though we lacked most of this knowledge, we were able to identify drivers of the spider diet based on direct analyses, which we discuss below.

4.5.2   Contribution of aquatic prey to the diet of spiders with different foraging strategies

The maximum and mean contribution of aquatic prey taxa to the diet of the web weaving spider was higher and the variability and range were narrower compared to the ground-hunter, which matches our expectation. The higher contribution of aquatic prey to web weaving spiders is likely due to the greater reliance of *these types of spiders* on stream habitats (Krell et al., 2015). Most spiders are generalist and opportunist predators and consequently flexible concerning their prey, which is especially pronounced for wolf spiders, such as ground-hunting *P. amentata* (Foelix, 2011). Although hunting spiders such as *P. amentata* can obtain a substantial amount of resources from aquatic prey, they are less dependent on aquatic prey and less strictly associated with riparian habitats than web weaving spiders (Briers et al., 2005; Collier et al., 2002; Sanzone et al., 2003). The lower dependence of the ground hunter on riparian habitats was reflected in a lower mean and maximum dietary contribution, most likely as reductions in aquatic biomass can be compensated by contribution of terrestrial prey. The ground-hunter *P. amentata* is more mobile, and therefore the collected individuals might have fed further away from the stream (less aquatic), whereas web weaving spiders are less mobile during spring time (i.e. the season our study was conducted in (Pfister et al., 2015). Moreover, we found a wider range and higher variability of aquatic diet contribution for ground-hunters reflecting their stronger flexibility in the dietary contribution. This stronger flexibility translates to the expectation that, for ground-hunting spiders, the contribution of aquatic prey to diet responds more strongly to land use than for web weaving spiders, which have a lower capacity to compensate potential losses. However, in our study, we found that both spiders responded to gradients, in terms of SPCA axes, that among other variables represented land use (further discussed below).

The contribution of aquatic prey to the diet differed for both web weaving and ground-hunting spiders between different land uses. Overall, land use likely exhibits multiple direct and indirect effects on riparian predators such as spiders and manipulative field experiments would be more suitable to rigorously test the hypothesis that ground hunters respond stronger to land use than web weavers.

4.5.3   Changes in aquatic prey consumption in relation to environmental variables

The drivers of the contribution of aquatic prey to the diet of the two spiders differed and only the ground hunter responded to toxicity. The contribution of aquatic prey to the web weavers' diet was positively related with potential aquatic prey biomass (Figure 4.1A). This is in line with other studies showing that aquatic prey contribution increases when more aquatic prey is available (Kraus et al., 2014) and reiterates the dependence of these spiders on aquatic prey. The web weavers' diet was also related to the first SPCA axis, representing a gradient of stream conditions and land use. We suggest that this gradient affected the contribution of the aquatic diet through several direct and indirect paths. First, as discussed above, this gradient affected the potential aquatic prey biomass, representing an indirect effect because the web weavers' diet was directly related to the potential aquatic prey biomass. Second, given that the gradient exhibited independent explanatory power in addition to the potential aquatic prey biomass, the agricultural stressors represented by this gradient may have directly affected the spiders. Third, the agricultural stressors represented by this gradient may have directly affected terrestrial prey composition and biomass, thereby indirectly affecting the diet of the web weaver. Overall, although field studies are important to identify the main drivers of populations and communities in real world ecosystems, more controlled conditions would be required to test

specific hypotheses regarding the mechanisms (e.g. direct or indirect effects) of observed patterns.

The diet of ground-hunting spiders also responded to a complex gradient of environmental variables (SPCA axis 2). However, this gradient mainly represented riparian habitat conditions (Table 4.2), whereas the gradient to which the diet of the web weaver responded mainly represented stream conditions. The contribution of aquatic diet in the ground-hunter increased when the riparian habitat changed from shrubs to meadows.

Moreover, the proportion of aquatic diet consumed by ground-hunting spiders increased with in-stream toxicity. While the proportion of aquatic diet of the ground hunter increased, a follow-up analysis showed that the abundance of ground hunting *Pardosa* sp. responded strongly negatively to toxicity (GLM, $p = 0.002$, $D^2 = 0.48$, $n = 17$), whereas no statistically significant relationship between web weaving *Tetragnatha* sp. and toxicity was found (GLM, $p = 0.150$, $D^2 = 0.13$, $n = 17$). A study on insectivorous birds also found a negative response of their abundance to pesticides, which the authors attributed to a decrease in available prey (Poulin et al., 2010).It is well established that the levels of pesticide toxicity observed in our study can lead to compositional changes in aquatic invertebrate communities as shown in a meta-analysis (Schäfer et al., 2012; Wijngaarden et al., 2005) and review (Schäfer, 2019). This will in turn affect the composition of emerging insects and riparian predators, where the direction of the effect will depend on predator preferences. However, we are not aware of other studies demonstrating such a response (i.e. the decrease in abundance of a riparian predator but increase in aquatic prey consumption in response to in-stream toxicity) and, as emphasised above, controlled experiments would be required to rigorously test mechanisms underlying the observed pattern. We speculate that in-stream toxicity is a proxy for the general pesticide exposure and that this has directly reduced the abundance of the ground-hunting spiders. Indeed, we found that in-stream pesticide toxicity is a major driver of the riparian spider community composition in our sites (Graf et al., 2019). High in-stream toxicity might also have sublethal effects on emerging aquatic insects, making them easy prey for ground-hunting spiders and thus increasing their contribution to the diet of ground-hunter. At the same time, uptake of toxicants by preying on moribund aquatic insects could also explain the decline in ground-hunting *Pardosa* sp. densities with increasing stream toxicity (Graf et al., 2019).

As an alternative explanation for the increase of aquatic prey in ground-hunting spiders with increasing in-stream toxicity, the lower abundances of ground hunting spiders along streams with high toxicity may in turn reduce competition for aquatic prey and consequently increase the contribution of aquatic prey in their diets (i.e. "death frees up resources"). However, this explanation contrasts with the fact that a reduction in spiders would also decrease the competition for terrestrial prey. This apparent contradiction may be resolved when taking prey quality into account. Aquatic and terrestrial organisms differ in their quality and compositions (Twining et al., 2016). Studies of aquatic prey have mainly considered their quantity and not their quality (Moyo et al., 2017), although nutritional quality of aquatic prey is important for spiders (e.g. immune system, prey capture efficiency) (Fritz et al., 2017; Pekár, 2012). Indeed, the quality of aquatic prey organisms can vary (Martin-Creuzburg et al., 2017). A study suggested that qualitative rather than quantitative (e.g. biomass) differences in prey may have driven responses in terrestrial spiders related with land use in their study (Stenroth et al., 2015). A study in South Africa showed a general net flow of highly unsaturated fatty acids from river to land, even though land-to-river inputs were dominated by biomass (Moyo et al., 2017). Due to the accumulation of predators at streams (Henschel et al., 2001; Kato et al., 2004; Kraus et al., 2014) we assume that aquatic prey might be preferred by spiders due to a higher quality.

However, we can only speculate on the mechanisms underlying the observed patterns. To rigorously test hypotheses related to underlying mechanisms, we suggest that future studies on the response of spiders to agricultural land use and specifically pesticide gradients should consider i) potential direct effects on abundances and ii) potential indirect effects resulting from direct effects on aquatic and terrestrial prey availability. This will likely require different study designs, i.e. controlled field experiments or mesocosm studies.

4.5.4   Conclusions

The contribution of aquatic prey to the diet was related to different drivers in two riparian spiders. Partly matching our expectation, in-stream toxicity influenced the abundance of ground hunting *Pardosa* sp*.,* and the relative contribution of aquatic and terrestrial prey in its diet. However, the web weaver also responded to an agricultural gradient, to which pesticide toxicity may have contributed. The agricultural gradient reduced the biomass of potential aquatic prey, and their contribution to the diet of the web-building spider, but not abundance of the web builder. In general, a reduction in riparian spiders may in turn impact organisms that rely on them as prey such as birds (Hallmann et al., 2014; Poulin et al., 2010). Spiders can be affected by pesticides in streams via lower quantity of aquatic prey as well as lower quality, but also via an accumulation of the contaminants in spiders and their prey (Martin-Creuzburg et al., 2017; Richmond et al., 2018; Stenroth et al., 2015; Walters et al., 2008). Further field, mesocosm and laboratory studies on the relationships between prey organisms from a pesticide-contaminated habitat and their predators are needed to differentiate between qualitative and quantitative as well as direct and indirect aspects of cross-ecosystem effects. This seems particularly important given the worldwide increase in the use of agricultural pesticides (Bernhardt and Rosi, 2017).

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5. DISCUSSION, SYNTHESIS & OUTLOOK

5.1 Discussion

In this section, the results of the publications are discussed, followed by a synthesis for the thesis, concluding with a description of the limitations of this thesis and an outlook.

5.1.1 Impact of aquatic insects on a terrestrial model food web

Aquatic insects showed different effects on terrestrial herbivores and plants. Survival of plant sucking leafhoppers increased in the presence of aquatic subsidies. Similar results were found in an arctic system with wolf spiders as predators (Dreyer et al., 2016). Predation on weevils by spiders is increased in the presence of aquatic subsidies. To avoid predation, weevils use thanatosis and mechanical defence mechanisms (Reitze and Nentwig, 1991).

Aquatic subsidies might have enhanced the movement of hungry predators, possibly causing a higher encounter rate by spiders with some herbivores. Additionally, spiders require more energy and time to catch smaller bodied prey (e.g. leafhoppers and aquatic insects), which causes a preference for catching bigger, slower prey (weevils). Alternatively, a higher herbivory by leafhoppers in the treatment with aquatic insects increased the plant defence mechanisms, causing a higher movement in weevils, and enhancing the encounter rate with spiders (Bálint et al., 2016; Kaplan et al., 2007). Plant growth in our study is mainly affected by the reduction of the weevils than by enhanced plant sucking leafhopper densities.

5.1.2 Impact of in-stream toxicity on spider traits, spider community and prey consumption

In this section I focus the discussion on the relationship between pesticide toxicity and spiders as well as their diet. Other factors were relevant in the studies, see Chapters 3 and 4 for a detailed discussion of these habitat and physico-chemical variables.

Various statistical models in our studies showed the importance of toxicity for spider species richness, abundance and community composition and in parts for spider traits and spider diet. It is known that species composition can be changed by toxicity (Beketov et al., 2013). This is in agreement with previous studies, where contaminants in streams were identified to affect the terrestrial ecosystems (directly or indirectly) (Hallmann et al., 2014; Jakob and Poulin, 2016; Kraus et al., 2017; Paetzold et al., 2011; Poulin et al., 2010; Walters et al., 2008).

Toxicity was associated with a reduction in ballooning spiders (Entling et al., 2011; Mestre and Bonte, 2012). The lack of a correlation between toxicity and other traits, could indicate that the other chosen traits are not suitable to relate to pesticide toxicity. Additionally, species, which got reduced in abundance, were replaced by species (native or not) that are less sensitive towards toxicity and have a similar trait profile.

Web weaving *T. montana* consumed more aquatic prey than ground-hunting *P. amentata,* which can be explained by the higher reliance of these spiders on stream habitats (Krell et al., 2015), whereas free-living terrestrial spiders, are less dependent on aquatic prey. The aquatic prey consumption by web weaving *T. montana* correlated positively with the biomass of potential prey. Past studies could show positive relationships between the presence of aquatic prey and their consumption by riparian spiders (Burdon and Harding, 2008; Henschel et al.,

2001). The toxicity was positively associated with aquatic diet consumed by the ground-hunting spiders. In previous studies, stressors such as pesticides changed the composition of aquatic communities (Beketov et al., 2013) and in turn the biomass, which could have affected the contribution of aquatic prey. A negative association between the number of ground-hunting *Pardosa* sp. and toxicity was found. This may be attributed to an increasing toxicity and a decrease of ground-hunting spider *P. amentata.* This might lead to a reduction of competition for the aquatic prey, which is assumed to be more nutritious in comparison to terrestrial prey and in turn lead to a higher aquatic prey contribution (Twining et al., 2016). A lack in quality might have decreased the number of spiders additionally, since the quality of prey can affect consumers (e.g. reproduction success, immune system) (Pekár, 2012).

5.2 Synthesis

The aim of this thesis was to offer a better understanding of cross-ecosystem linkages in the riparian area and how they are affected by agricultural land use, including a specific focus on pesticide toxicity.

The indirect impact of aquatic insect subsidies on a terrestrial model food web in a mesocosm study with two herbivores, which have different predator avoidance strategies, including plants and spiders was examined. Not only predators are affected by aquatic insects, but the whole terrestrial food web. Densities of terrestrial herbivores can react to aquatic subsidies in contrasting ways. In experiments, herbivores increased in the presence of aquatic subsidies (Dreyer et al., 2016), whereas in field studies herbivore densities decreased (Henschel et al., 2001). In the field, predators accumulate, which is not possible in mesocosm studies. In this thesis, herbivores responded differently to subsidies, most likely due to different survival strategies. Even short-term effects of subsidies can affect in-situ prey in different ways, which highlights the complexity of effects of aquatic subsidies on riparian food webs (Bálint et al., 2016; Dreyer et al., 2016; Kaplan et al., 2007; Reitze and Nentwig, 1991). Due to the influence on terrestrial herbivores, aquatic insects can indirectly influence terrestrial plants, which was shown in the mesocosm study. In addition, aquatic subsidies can affect plants, when they serve as nutrients (Bultman et al., 2014; Dreyer et al., 2016).

The importance of aquatic insects in the mesocosm study could be confirmed in the field studies, where biomass of aquatic prey explained diet contribution of web weaving spiders, which conversely influenced terrestrial prey consumption and, according to our mesocosm study, might have also affected terrestrial plants. And here again subsidies affected spiders' prey in different ways. The aquatic prey biomass explained only the diet contribution of the web weaving spider and not the ground-hunting spider, which shows again the complexity of cross-ecosystem linkages.

The impact of agricultural land use and pesticides on the taxonomic composition of riparian spiders, their number, species richness, community composition, traits and diet of in-stream toxicity and other environmental variables in a field study was examined in a field study. Spiders are specialized to a high variety of habitats, the extent to which they are influenced by changes in prey availability and habitat conditions can differ (Henschel et al., 2001). It was shown that in-stream pesticide toxicity can explain species richness and abundance of riparian spiders. Arthropod traits allow to address land use intensity within and between land-use types (Birkhofer et al., 2017). With increasing toxicity, the community is more mobile.

The potential impact of agricultural land use on the potential aquatic prey biomass by in-stream pesticide toxicity and other agricultural stressors on the contribution of aquatic prey to riparian

spider diet was investigated. Changes in potential aquatic prey biomass were explained by habitat characteristics and not by in-stream toxicity. Habitat conditions such as oxygen and riffles (Schindler and Smits, 2017; Vannote et al., 1980) are important for aquatic insects (McKie et al., 2018).

Stressors in streams can decrease the consumption of aquatic prey by spiders (Paetzold et al., 2011). In our study it is increasing with increasing toxicity for one spider, which might be explained by a decreasing competition due to less spiders, potentially caused by toxicity.

The quality of prey organisms has received little attention in the past (Moyo et al., 2017), but nutritional quality of aquatic prey is important for spiders (e.g. immune system, prey capture efficiency) (Fritz et al., 2017; Pekár, 2012). A reduction in quality of prey might lead to a reduction of spiders in riparian areas. In the previous study on spider traits and community, it was shown that in-stream toxicity correlates with spider community and trait composition as well as abundance and individual numbers. This can be a direct or indirect effect. Direct via exposure to direct application, spray drift or contact via the surface water. Indirect by a reduction of prey, which can be excluded according to our results, or a reduction in quality.

Due to the relationships between agricultural land use and spiders found in the field studies, I conclude that these impacts will not only affect spiders directly but also, according to the mesocosm study, influence the whole adjacent ecosystem via the food web indirectly. In-stream toxicity affects prey consumption, abundance, species richness and composition of spiders. Even though prey biomass was not associated with toxicity, these effects will have an impact on the adjacent system, as demonstrated in the first study. The diet of spiders and the composition of consumers changed, which will probably affect the terrestrial prey in abundance, species richness and composition, which will then affect the terrestrial plants.

This thesis is one of the first to address the impact of aquatic insects on terrestrial herbivores and plants, and the first to show effects of in-stream pesticide toxicity on riparian spider communities as well as their aquatic prey consumption. Based on the results I conclude that cross-ecosystem linkages are highly complex. Stressors, such as agricultural land use and pesticides can have direct effects in the system, and indirect effects in adjacent systems, due to those linkages. This thesis confirms that agricultural land use and other anthropogenic stressors can directly and indirectly affect terrestrial ecosystems, adjacent to streams, via cross-ecosystem linkages (Hallmann et al., 2014; Jakob and Poulin, 2016; Kraus et al., 2017; Paetzold et al., 2011; Poulin et al., 2010; Walters et al., 2008). The findings have direct relevance for cross-ecosystem food webs.

It was shown in this thesis that in-stream pesticide toxicity has an impact on at least some riparian spiders. A reduction of aquatic prey organisms can decrease riparian spiders (Paetzold et al., 2011). This again can affect organisms that are depending on them as prey (e.g. birds, lizards, bats) (Baxter et al., 2005; Hallmann et al., 2014; Jakob and Poulin, 2016; Poulin et al., 2010). Consumers can not only be affected by the quantity of prey but also by the quality. They can accumulate contaminants from prey in their tissues (Walters et al., 2008). Moreover, a lower quality, in terms of nutrition and energy, of the prey can lower the fitness of the consumers (Martin-Creuzburg et al., 2017; Stenroth et al., 2015).

5.3 Limitations & Outlook

Information on spider sensitivity or tolerance towards toxicity are lacking. This type of data would help to evaluate effects on spiders, and as they are indicator organisms for habitat conditions and affected by pesticides, as I have shown, it would also help to understand potential responses across ecosystems. The observed relationships in this thesis can be used provisionally to classify into sensitive and tolerant species, even though it remains uncertain if the effects are direct or indirect.

Additional traits for spider communities that show correlations with toxicity could help to better and easier identify pesticide stressed habitats. For example, a trait representing the breeding season might show a relationship with pesticides, which are usually applied in spring time (Brinke et al., 2016). This may reveal sensitive life stages of spiders and provide insights about the habitat conditions.

Previous studies primarily examined effects of quantity of aquatic prey on consumers, as was done in this thesis. However, the effects of quality are largely unknown (Moyo et al., 2017; Schulz et al., 2015). The quantity cannot fully explain e.g. non-lethal effects and its consequences for organisms and ecosystems. Nutritional quality of prey is important for e.g. immune system and prey capture efficiency of spiders (Fritz et al., 2017; Pekár, 2012). Previous studies showed that the quality of prey organisms can vary with habitat conditions (Martin-Creuzburg et al., 2017). Furthermore, it has been suggested that qualitative differences of prey organisms caused impacts in spiders as opposed to quantitative differences, in association with agricultural land use (Stenroth et al., 2015). To only use the quantity or mass of prey material does not consider the quality or nutritional value of the resource (Bartels et al., 2012), which can be measured as energy (joules), proteins (amino acids), poly saturated fatty acids or just the size of the prey (Marcarelli et al., 2011). In the past, organic nutrients such as fatty acids, amino acids and vitamins, have largely been ignored by ecologists (Twining et al., 2016). A few studies have started to examine the relevance of poly unsaturated fatty acids (PUFAs) (Iverson, 2009; Martin-Creuzburg et al., 2017) in cross-ecosystem relationships. Fatty acid composition in combination with stable isotope analyses (or as a replacement) can be used for diet reconstructions (Iverson, 2009; Williams and Buck, 2010), because terrestrial and aquatic resources differ in their composition (Hixson et al., 2015).

The contribution of prey to consumers depends on their quantity, quality and composition but also on the availability of alternatives from adjacent ecosystems. Further information on prey from adjacent systems is needed to fully understand the effects of stressors on diet contribution from linked ecosystems. Additionally, data about the habitats such as toxicity can help to separate direct and indirect effects. The foraging strategy of the predator might explain, additionally, the contribution of consumed prey, as well as their success. Sampling different species with the same foraging strategies, and not just one per strategy, could confirm if the stressors affect the consumption of aquatic prey for specific foraging strategies.

Agricultural land use and specifically pesticides have direct effects and indirect effects via the food web and by that can alter one system and also coupled ones. The knowledge about these effects is important to enhance the knowledge of basic ecology and to and to the development of strategies for effective and efficient restoration and bioassessment programmes (Heino, 2013; Maloney and Weller, 2011; Tonkin, 2014).

It is suggested that habitat restoration coupled with an agricultural redesign are an effective solution to stop further declines in biodiversity. Ideas include buffer strips at riparian areas, grassland and flower strips (Blaauw and Isaacs, 2014; Hopwood, 2008), rotating crops (Ekroos

et al., 2014; Haaland and Bersier, 2011). Buffer strips next to land used areas can buffer the run-off of pesticides and fertilizers into surrounding habitats and ecosystems (Castelle et al., 1994). Wildflower strips can increase pollinators and predators of pest organisms in land used areas and increase crop yield (Ganser et al., 2019). The use of different agricultural land use methods such as reduced foliation cut (Pennington et al., 2017) can promote predatory organisms like spiders. Other authors suggest a general reduction of agro-chemicals. Since synthetic insecticides do not directly contribute to crop yield, but trigger resistances of crop pests and negatively affect food safety, it is suggested by many authors to reduce their use (Bredeson and Lundgren, 2015; Lechenet et al., 2017; Pennington et al., 2017).

Today it is known that ecosystem linkages are strong and complex. Knowledge about them is not only important for a better understanding of the consequences of anthropogenic stressors, e.g. agricultural land use and pesticides, but also to prevent further losses of ecosystems and their organisms. Controlled field and laboratory studies are necessary in cross-ecosystems to clarify underlying mechanisms and to distinguish between direct and indirect effects of subsidies on the adjacent system and of stressors, especially in the face of worldwide increasing use of agricultural pesticides (Bernhardt et al., 2017).

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6. APPENDIX

6.1 Supporting information
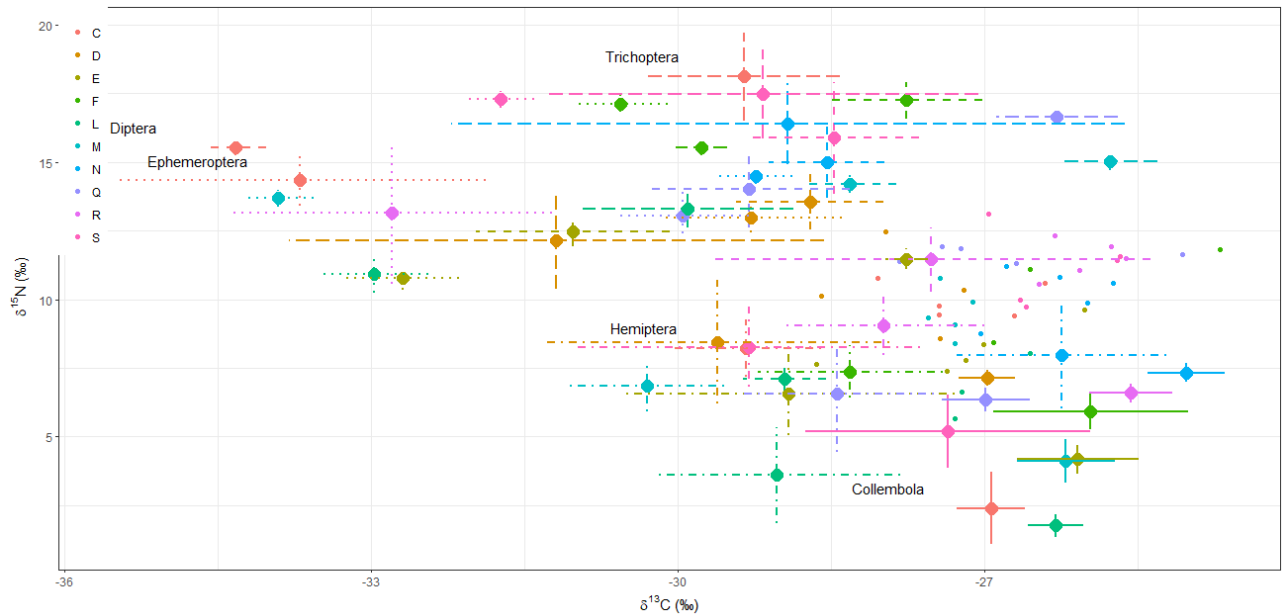
Figure SI 6.1: Isoplot for ground-hunting *Pardosa amentata*. Dots represent *P. amentata* isotope signals. Colour represents site coding. Prey organisms are represented with standard deviation (SD); dotted SD: Ephemeroptera, short dashed SD: Diptera, long dashed SD: Trichoptera, dashed and dotted SD: Hemiptera, continuous: Collembola. Amount consumer *P. amentata* within prey mixing polygon per site: C = 60%, D = 75%, E = 80%, F = 67%, L = 67%, M = 100%, N = 100%, Q = 80%, R = 100%, S = 60%
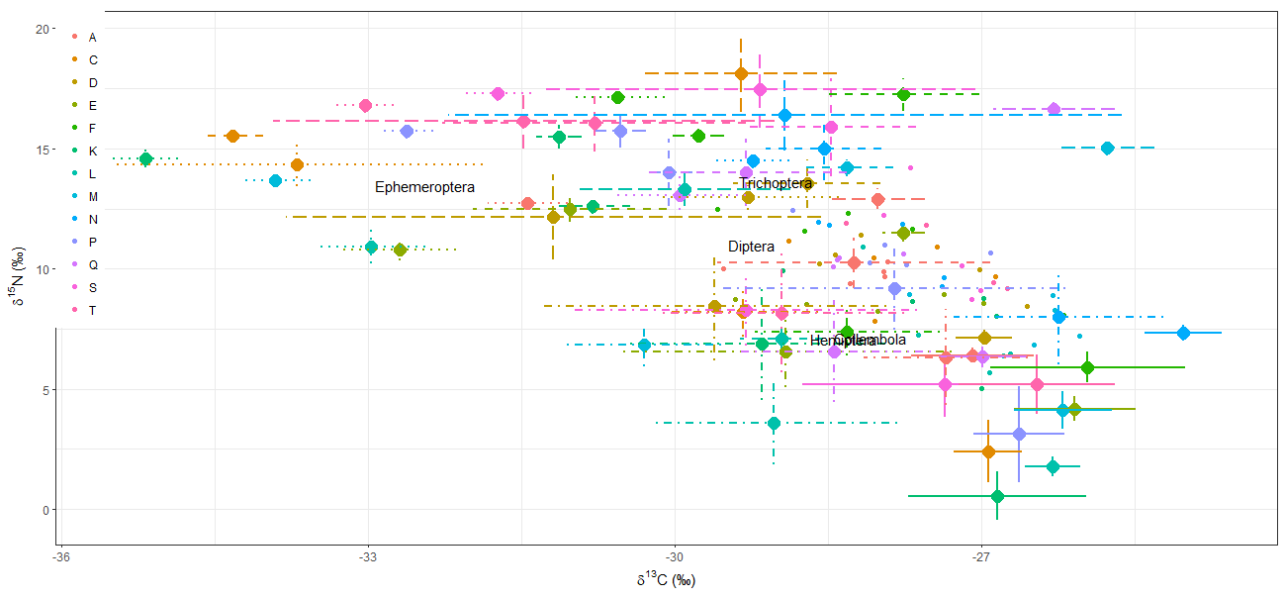
Figure SI 6.2: Isoplot for orb web weaving *Tetragnatha montana*. Dots represent *T. montana* isotope signals. Colour represents site coding. Prey organisms are represented with standard deviation (SD); dotted SD: Ephemeroptera, short dashed SD: Diptera, long dashed SD: Trichoptera, dashed and dotted SD: Hemiptera, continuous: Collembola. Amount consumer T. montana within prey mixing polygon per site: A = 100%, C = 80%, D = 60%, E = 100%, F = 100%, K = 60%, L = 60%, M = 100%, N = 100%, P = 100%, Q = 100%, S = 100%, T = 100%

Table SI 6.1: Coordinates of study sites

site	Latitude north	Longitude east	Altitude [m]
A	46.84921	23.07857	565
B	46.82658	22.99440	576
C	46.91014	23.0544	361
D	46.76527	23.36033	415
E	47.49242	23.22602	168
F	47.41272	23.27111	183
G	47.37907	23.14218	178
H	47.0970	23.17982	253
I	47.08473	23.18643	259
K	46.93456	23.11216	317
L	46.93583	22.94882	448
M	46.95094	23.05741	369
N	46.84914	23.02365	553
O	46.57814	23.6671	465
P	46.58483	23.64969	472
Q	46.63419	23.52278	527
R	46.66156	23.66503	527
S	46.95959	23.69000	304
T	46.94083	23.64793	316

Table SI 6.2: Stable Isotope signals, and characteristics for orb web weaving *Tetragnatha* sp. specimen

site	d13C	d15N	genus	species	adult/juvenile	sex	inside mixing polygon
A	-28.2897	9.3749	Tetragnatha	montana	adult	f	y
A	-27.9477	9.6789	Tetragnatha	montana	adult	f	y
A	-27.9617	9.8939	Tetragnatha	montana	adult	f	y
A	-29.5267	10.0059	Tetragnatha	montana	adult	m	y
A	-27.9263	10.2953	Tetragnatha	sp.	juvenile	f	y
C	-28.0467	7.8139	Tetragnatha	montana	adult	f	y
C	-26.8727	9.6939	Tetragnatha	montana	adult	f	n
C	-28.0597	10.4679	Tetragnatha	montana	adult	f	y
C	-27.4357	10.9299	Tetragnatha	montana	adult	f	y
C	-28.8867	11.1509	Tetragnatha	montana	adult	f	y
D	-26.5617	8.4529	Tetragnatha	montana	adult	f	n
D	-27.0217	9.9809	Tetragnatha	montana	adult	f	n
D	-28.5917	10.2079	Tetragnatha	montana	adult	f	y
D	-28.4407	10.6019	Tetragnatha	montana	adult	f	y
D	-28.1857	11.3959	Tetragnatha	montana	adult	f	y
E	-28.0163	8.2213	Tetragnatha	montana	adult	f	y
E	-28.7157	8.5239	Tetragnatha	montana	adult	f	y
E	-26.9897	8.5689	Tetragnatha	montana	adult	f	y
E	-29.4097	8.7149	Tetragnatha	montana	adult	f	y
E	-27.3747	8.9319	Tetragnatha	montana	adult	f	y
F	-26.1987	8.0879	Tetragnatha	montana	adult	f	y
F	-28.7347	11.5639	Tetragnatha	montana	adult	f	y
F	-27.6777	11.6569	Tetragnatha	montana	adult	f	y
F	-28.3127	12.3219	Tetragnatha	montana	adult	f	y
F	-29.5867	12.4929	Tetragnatha	montana	adult	f	y
K	-27.0017	5.0079	Tetragnatha	sp.	adult	f	y
K	-26.8597	8.0189	Tetragnatha	montana	adult	f	n
K	-27.6807	8.6649	Tetragnatha	montana	adult	m	y
K	-26.9897	8.7619	Tetragnatha	sp.	juvenile	f	n
K	-28.9497	9.9379	Tetragnatha	montana	adult	m	y
L	-26.9307	5.6709	Tetragnatha	montana	adult	m	y
L	-26.7907	6.3799	Tetragnatha	montana	adult	f	n

L	-26.7193	6.4853	Tetragnatha	montana	adult	f	n
L	-27.6237	7.2569	Tetragnatha	montana	adult	f	y
L	-28.1623	10.8963	Tetragnatha	montana	adult	f	y
M	-26.4963	6.8513	Tetragnatha	sp.	juvenile	m	y
M	-26.0443	7.2203	Tetragnatha	montana	adult	m	y
M	-26.2913	8.2853	Tetragnatha	montana	adult	m	y
M	-26.3083	8.8873	Tetragnatha	montana	adult	f	y
M	-27.7133	8.9353	Tetragnatha	montana	adult	f	y
N	-27.3903	9.2593	Tetragnatha	montana	adult	f	y
N	-27.3733	9.6293	Tetragnatha	montana	adult	f	y
N	-28.4983	11.8073	Tetragnatha	montana	adult	f	y
N	-27.7793	11.8663	Tetragnatha	montana	adult	f	y
N	-28.5993	11.9403	Tetragnatha	montana	adult	f	y
P	-27.7393	10.1593	Tetragnatha	montana	adult	f	y
P	-28.0973	10.2373	Tetragnatha	montana	adult	f	y
P	-26.9173	10.6503	Tetragnatha	montana	adult	f	y
P	-27.9553	11.0013	Tetragnatha	montana	adult	f	y
P	-28.8543	12.4303	Tetragnatha	montana	adult	f	y
Q	-28.4573	10.0963	Tetragnatha	montana	adult	m	y
Q	-28.4183	10.3853	Tetragnatha	sp.	juvenile	m	y
Q	-28.3933	10.4643	Tetragnatha	montana	adult	f	y
Q	-27.7663	10.6343	Tetragnatha	montana	adult	m	y
S	-27.1033	8.7313	Tetragnatha	montana	adult	f	y
S	-27.0163	9.1103	Tetragnatha	montana	adult	f	y
S	-27.1983	10.1293	Tetragnatha	montana	adult	f	y
S	-27.9643	12.2453	Tetragnatha	montana	adult	f	y
S	-27.6963	14.1963	Tetragnatha	montana	adult	f	y
T	-26.7483	9.1983	Tetragnatha	montana	adult	f	y
T	-26.8913	9.4423	Tetragnatha	montana	adult	f	y
T	-27.5443	11.8193	Tetragnatha	montana	adult	f	y
T	-28.3263	11.9093	Tetragnatha	montana	adult	f	y
T	-28.7053	13.7153	Tetragnatha	montana	adult	f	y

Table SI 6.3: Stable Isotope signals, and characteristics for ground-hunting *Pardosa* sp. specimen

d13C	d15N	site	genus	species	adult/juvenile	sex	inside mixing polygon
-26.7136666667	9.4134166667	C	Pardosa	amentata	adult	f	n
-27.4496666667	9.4324166667	C	Pardosa	prativaga	adult	f	y
-27.4486666667	9.7724166667	C	Pardosa	prativaga	adult	f	y
-26.4176666667	10.5904166667	C	Pardosa	amentata	adult	f	y
-28.0486666667	10.7844166667	C	Pardosa	prativaga	adult	f	y
-27.4406666667	8.5904166667	D	Pardosa	amentata	adult	f	n
-28.5976666667	10.1304166667	D	Pardosa	sp.	juvenile	f	y
-27.2036666667	10.3364166667	D	Pardosa	sp.	NA	f	y
-27.9706666667	12.4664166667	D	Pardosa	prativaga	adult	f	y
-27.3706666667	7.3964166667	E	Pardosa	sp.	NA	f	y
-28.6466666667	7.6304166667	E	Pardosa	sp.	NA	f	y
-27.1876666667	7.7744166667	E	Pardosa	sp.	NA	f	y
-27.0106666667	8.3614166667	E	Pardosa	amentata	adult	f	y
-26.0236666667	9.6304166667	E	Pardosa	sp.	NA	f	n
-26.91875	8.4350833333	F	Pardosa	lugubris	adult	f	y
-26.5576666667	11.1034166667	F	Pardosa	sp.	NA	f	y
-24.7006666667	11.8244166667	F	Pardosa	amentata	adult	f	n
-27.2916666667	5.6504166667	L	Pardosa	amentata	adult	f	y
-27.2316666667	6.6414166667	L	Pardosa	sp.	juvenile	m	y
-26.5586666667	8.0444166667	L	Pardosa	amentata	adult	f	n
-27.29175	8.3910833333	M	Pardosa	sp.	juvenile	f	y
-27.29875	9.0880833333	M	Pardosa	amentata	adult	f	y
-27.55575	9.3550833333	M	Pardosa	sp.	juvenile	f	y
-27.12275	9.9070833333	M	Pardosa	amentata	adult	f	y
-27.43575	10.7830833333	M	Pardosa	amentata	adult	f	y
-27.04275	8.7680833333	N	Pardosa	sp.	NA	f	y
-26.00275	9.8940833333	N	Pardosa	lugubris	adult	f	y
-25.74475	10.6110833333	N	Pardosa	lugubris	adult	f	y
-26.27075	10.8100833333	N	Pardosa	amentata	adult	f	y
-26.79475	11.1970833333	N	Pardosa	amentata	adult	f	y
-26.69175	11.3050833333	Q	Pardosa	amentata	adult	f	y

-27.83375	11.3990833333	Q	Pardosa	amentata	adult	f	y
-25.06975	11.6520833333	Q	Pardosa	amentata	adult	f	n
-27.24075	11.8500833333	Q	Pardosa	amentata	adult	f	y
-27.42275	11.9370833333	Q	Pardosa	amentata	adult	f	y
-26.322	12.33075	R	Pardosa	amentata	adult	f	y
-26.473	10.54875	R	Pardosa	amentata	adult	f	y
-25.764	11.93275	R	Pardosa	amentata	adult	f	y
-26.072	11.07275	R	Pardosa	amentata	adult	f	y
-25.62	11.49875	R	Pardosa	amentata	adult	f	y
-26.59775	9.7180833333	S	Pardosa	amentata	adult	f	y
-26.65375	9.9900833333	S	Pardosa	amentata	adult	f	y
-25.70775	11.4120833333	S	Pardosa	amentata	adult	f	n
-25.67775	11.5690833333	S	Pardosa	amentata	adult	f	n
-26.96775	13.1260833333	S	Pardosa	amentata	adult	f	y

Table SI 6.4: Estimated length of the organisms, parameter a and b describe the allometric relationship between the dry mass and length (Sabo et al.; 2002); 1: Müller & Bährmann, 2015 2: Schaefer et al., 2016.

Order	Suborder	Family	Genus	Species	a	b	Length [mm]	Reference for length
Diptera	Brachycera	Empididae			0.006	3.05	5.5	1
	Nematocera	Chironomidae			0.1	1.57	1.5	2
	Nematocera	Simuliidae			0.1	1.57	5.5	1
Ephemeroptera					0.014	2.49	10.56	2
		Arthropleidae	*Arthroplea*	*congener*	0.014	2.49	10	2
		Baetidae	*Baetis*		0.014	2.49	7	2
			Procloeon	*bifidum*	0.014	2.49	6	2
		Ephemerellidae			0.014	2.49	8	2
		Ephemeridae	*Ephemera*		0.014	2.49	17.5	2
		Heptageniidae	*Epeorus*	*sylvicola*	0.014	2.49	14.5	2
		Leptophlebiidae			0.014	2.49	9	2
		Siphlonuridae	*Siphlonurus*		0.014	2.49	12.5	2
Trichoptera					0.01	2.9	15.75	2
		Goeridae			0.01	2.9	15.75	2
		Hydropsychidae	*Hydropsyche*		0.01	2.9	15.75	2
		Hydroptilidae			0.01	2.9	3.25	2
		Lepidostomatidae			0.01	2.9	15.75	2
		Leptoceridae			0.01	2.9	15.75	2
		Leptoceridae	*Mystacides*	*nigra*	0.01	2.9	15.75	2
		Leptoceridae	*Triaenodes*		0.01	2.9	15.75	2
		Limnephilidae	*Limnephilus*		0.01	2.9	15.75	2
		Psychomyidae			0.01	2.9	15.75	2
		Rhyacophilidae			0.01	2.9	15.75	2
		Sericostomatidae	*Notidobia*	*ciliaris*	0.01	2.9	15.75	2

Table SI 6.5: Contribution of aquatic prey for *P. amentata* and *T. montana* per site

site	Pardosa	Tetragnatha
A	NA	0.554
B	NA	NA
C	0.388	0.433
D	0.432	0.468
E	0.466	0.535
F	0.345	0.493
G	NA	NA
H	NA	NA
I	NA	NA
K	NA	0.463
L	0.409	0.495
M	0.467	0.408
N	0.34	0.485
O	NA	NA
P	NA	0.47
Q	0.53	0.489
R	0.365	NA
S	0.46	0.502
T	NA	0.48

Table SI 6.6: AICc for tested models

Model for	Explanatory variables in model	AICc
Aquatic Biomass	Toxicity, first SPCA axis, second SPCA axis	190.97
	Toxicity, first SPCA axis	187.14
	First SPCA axis	185.72
Tetragnatha diet	Toxicity, first SPCA axis, second SPCA axis, aquatic biomass	-34.77
	Toxicity, first SPCA axis, aquatic biomass	-38.75
	first SPCA axis, aquatic biomass	-41.36
Pardosa diet	Toxicity, first SPCA axis, second SPCA axis, aquatic biomass	-10.61
	Toxicity, first SPCA axis, second SPCA axis	-19.58
	Toxicity, second SPCA axis	-24.78

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6.2 Overview of Publications

This cumulative dissertation is adapted from the following three scientific publications:

Graf, N., Bucher, R., Schäfer, R. B., Entling, M. H., 2017. Contrasting effects of aquatic subsidies on a terrestrial trophic cascade. Biology Letters 13, 20170129. https://doi.org/10.1098/rsbl.2017.0129

Graf, N., Battes, K. P., Cimpean, M., Dittrich, P., Entling, M. H., Link, M., Scharmüller, A., Schreiner, V. C., Szöcs, E., Schäfer, R. B., 2019. Do agricultural pesticides in streams influence riparian spiders? Science of the Total Environment 660, 126–135. https://doi.org/10.1016/j.scitotenv.2018.12.370

Graf, N., Battes, K. P., Cimpean, M., Entling, M. H., Frisch, K., Link, M., Scharmüller, A., Schreiner, V. C., Szöcs, E., Zubrod, J. P., Schäfer, R. B., 2019. Relationship between agricultural pesticides and the diet of riparian spiders. Submitted to Environmental Sciences Europe.

6.3 Author affiliations

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6.4 Status and author contributions of publications included in the thesis

Title: Contrasting effects of aquatic subsidies on a terrestrial trophic cascade

Authors: Nadin Graf, Roman Bucher, Ralf B. Schäfer and Martin H. Entling

Status: Published in 2017 in Biology Letters, Volume 13, pp. 20170129

Contribution: **Graf** designed the study, carried out the field work, conducted the statistical analysis and drafted the manuscript.

Bucher, Schäfer and Entling designed the study, provided feedback on the analysis, discussion of results and writing of the manuscript

Title: Do agricultural pesticides in streams influence riparian spiders?

Authors: Nadin Graf, Karina P. Battes, Mirela Cimpean, Pitt Dittrich, Martin H. Entling, Moritz Link, Andreas Scharmüller, Verena C. Schreiner, Eduard Szöcs, Ralf B. Schäfer

Status: Published in 2019 in Science of The Total Environment, Volume 660, pp. 126–135.

Contribution: **Graf** designed the study, selected the sites, sampled invertebrates, prepared samples and identified the spiders, conducted the statistical analysis and drafted the manuscript.

Battes and Cimpean assisted with site selection, logistics and commented the manuscript.

Dittrich prepared samples, identified the spiders and commented the manuscript.

Entling designed the study and provided feedback on the statistical analysis and the manuscript.

Link and Szöcs provided feedback on the statistical analysis and the manuscript.

Scharmüller provided data from toxicity tests and commented the manuscript.

Schreiner conducted the pesticide analysis, created the pesticide gradient, provided data from toxicity tests and commented the manuscript.

Schäfer designed the study, assisted in site selection and gave feedback on the statistical analysis and manuscript.

Title: Relationship between agricultural pesticides and the diet of riparian spiders

Authors: Nadin Graf, Karina P. Battes, Mirela Cimpean, Martin H. Entling, Katharina Frisch, Moritz Link, Andreas Scharmüller, Verena C. Schreiner, Eduard Szöcs, Jochen Zubrod, Ralf B. Schäfer

Status: submitted in 2019 to Environmental Sciences Europe

Contribution: **Graf** designed the study, selected the sites, sampled invertebrates, prepared samples, and identified the organisms, conducted the statistical analysis and drafted the manuscript.

Battes and Cimpean assisted with site selection, logistics and commented the manuscript.

Entling designed the study and provided feedback on the statistical analysis.

Frisch prepared samples, identified the organisms and commented the manuscript.

Link and Szöcs provided feedback on the statistical analysis and the manuscript.

Scharmüller provided data from toxicity tests and commented the manuscript.

Schreiner conducted the pesticide analysis, created the pesticide gradient and provided data from toxicity tests and commented the manuscript.

Zubrod performed stable isotope analysis and commented the manuscript.

Schäfer designed the study, assisted in site selection and gave feedback on the statistical analysis and manuscript.

6.5 Curriculum vitae

Work experience

Jun. 2013 – Jan. 2015	ECT Oekotoxikologie GmbH, Flörsheim/Main (full time)
	Quality Assurance
	Research Scientist
Nov. 2012 – Jun. 2013	ECT Oekotoxikologie GmbH, Flörsheim/Main (full time)
	Research Scientist

Education

Feb. 2015 – Jul. 2019	University Koblenz-Landau, Landau
	PhD Candidate, Dr. rer nat.
	Title: „ Aquatic-terrestrial linkages and how they are affected by land use related stressors: The case of riparian spiders", supervised by Prof. Dr. Ralf B. Schäfer und Prof. Dr. Martin H. Entling
Oct. 2010 – Oct. 2012	Johann Wolfgang Goethe Universität, Frankfurt/Main
	Master of Sciences, Environmental Sciences
	Thesis title "Entwicklung eines Konzeptes für die weiterführende Pflanzentestung und -bewertung bei der Umweltrisikoprüfung von Tierarzneimitteln"
Oct. 2007 – Feb. 2011	Johann Wolfgang Goethe Universität, Frankfurt/Main
	Bachelor of Sciences, Geosciences
	Thesis title: Untersuchungen zum Eintrag von 2,4,7,9-Tetramethyl-5-decin-4,7-diol (TMDD) und Terbutryn in Fließgewässern"

Scientific publications

Graf, N., Battes, K. P., Cimpean, M., Entling, M. H., Frisch, K., Link, M., Scharmüller, A., Schreiner, V. C., Szöcs, E., Zubrod, J. P., Schäfer, R. B.: Relationship between agricultural pesticides and the diet of riparian spiders, submitted 2019 Environmental Sciences Europe, submitted

Graf, N., Battes, K. P., Cimpean, M., Dittrich, P., Entling, M. H., Link, M., Scharmüller, A., Schreiner, V. C., Szöcs, E., Schäfer, R. B. (2019): Do agricultural pesticides in streams influence riparian spiders? Science of The Total Environment, 660, 126–135. https://doi.org/10.1016/j.scitotenv.2018.12.370

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from 28 ecotoxicological test laboratories. Applied Soil Ecology 03/2015) doi:10.1016/j.apsoil.2015.02.010

Conference presentations (first author is presenting author)

Graf, N., Dittrich, P., Entling, M. H., Frisch, K., Link, M., Schreiner, V. C., Szöcs, E., Schäfer, R. B.: "Influence of an agriculture-associated toxicity gradient on a riparian predator-prey relationship in Romania", 2018 SETAC Europe 28th meeting

Frisch, K., **Graf, N.**, Schreiner, V. C., Szöcs, E., Entling, M. H., Schäfer, R. B.: "Influence of agricultural land use related stressors on aquatic-terrestrial predator-prey relationships in Romania", 2017, 22. Jahrestagung Society of Environmental Toxicology and Chemistry German Language Branch e.V. (SETAC GLB)

Graf, N., Dittrich, P., Entling, M. H., Schreiner, V. C., Schäfer, R. B.: "Influence of agricultural land use on riparian spider communities in Romania", 2017, 22. Jahrestagung Society of Environmental Toxicology and Chemistry German Language Branch e.V. (SETAC GLB)

Bucher, R., **Graf, N.**, Dupré, J., Schäfer, R. B., Entling, M. H.: Effects of resource pulses on spider top-down control, 2017, 30th European Congress of Arachnology, Nottingham

Graf, N., Entling, M.H., Frisch, K., Schreiner, V. C., Szöcs, E., Schäfer, R. B.: „Influence of agricultural land-use on aquatic-terrestrial predator-prey relationships in Romania", 2017, 10th Symposium for European Freshwater Sciences

Graf, N., Bucher, R., Entling, M. H., Schäfer, R. B.: „Indirect linkages between terrestrial and aquatic food webs", 2016, 46th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland

Graf, N., Bucher, R., Entling, M. H., Schäfer, R. B: "Direct and indirect linkages between terrestrial and aquatic food webs" 2015, Young Academics Conference 2015

6.6 Declaration

I hereby declare that I autonomously conducted the work presented in this PhD thesis entitled "*Aquatic-terrestrial linkages and how they are affected by land use related stressors: The case of riparian spiders*". All used assistances and involved contributors are clearly declared. This thesis has never been submitted elsewhere for an exam, as thesis or for evaluation in a similar context; to any department of this university or any scientific institute. I am aware that a violation of the aforementioned conditions can have legal consequences.

Place, date   Signature