Aquatic-terrestrial predator-prey relationships across ecosystem boundaries: How does agricultural land use affect the quantity and quality of stream exports?

by

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Accepted Dissertation thesis for the partial fulfillment of the requirements for a Doctor of Natural Sciences

Fachbereich 7: Natur- und Umweltwissenschaften

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Date of oral examination: 18th December 2023

List of publications

This cumulative dissertation is based on the following scientific publications:

Ohler, K., Schreiner, V.C., Martin-Creuzburg, D., Schäfer, R.B., 2023b. Trophic transfer of polyunsaturated fatty acids across the aquatic–terrestrial interface: An experimental tritrophic food chain approach. Ecology and Evolution. 13, e9927. https://doi.org/10.1002/ece3.9927

Ohler, K., Schreiner, V.C., Link, M., Liess, M., Schäfer, R.B., 2023a. Land use changes biomass and temporal patterns of insect cross-ecosystem flows. Global Change Biology. 29, 81–96. https://doi.org/10.1111/gcb.16462

Ohler, K., Schreiner, V.C., Reinhard, L., Link, M., Liess, M., Brack, W., Schäfer, R.B., 2023. Land use alters cross-ecosystem transfer of high value fatty acids by aquatic insects. Environmental Sciences Europe. Submitted.

Danksagung

Hier möchte ich mich bei allen Menschen bedanken, die mich während meiner Promotion unterstützt haben.

Mein erster Dank geht an Ralf Schäfer, der mir ermöglicht hat, meine Promotion in seiner Arbeitsgruppe durchzuführen. Ich bin sehr dankbar für die allzeit schnelle Rückmeldung auf Fragen, gute Anmerkungen zum wissenschaftlichen Schreiben, die Möglichkeit, dass ich das Thema meiner Promotion frei wählen konnte und er dafür Finanzierungsquellen gefunden hat.

Verena Schreiner möchte ich ganz besonders für die gute Zusammenarbeit, ihre Expertise im Labor, ihre große Hilfsbereitschaft und ihren Blick für die kleinsten Details danken. Selbst die längsten Arbeitstage werden mit ihr kurzweilig und man kann ausgezeichnet über Gott und die Welt mit ihr reden.

Des Weiteren möchte ich der Arbeitsgruppe quantitative Landschaftsökologie danken. Fachliche Fragen konnten oft schnell bei einem gemeinsamen Mittagessen besprochen werden. Auch wenn es für manche schwer vorstellbar ist, ihr seid die Elite meiner Wahl ;-)

Ganz besonders möchte ich Nadin Graf für die Unterstützung bei der Bestimmung der Emergenz

und Anke Schneeweiß für den regelmäßigen Austausch über mehr oder weniger wissenschaftliche Themen danken.

Vielen Dank an Therese Bürgi, für ihren unermüdlichen Einsatz im Labor und ihre große Hilfe bei sämtlichen kleinen und großen Problemen im Labor.

Kilian Kenngott möchte ich für die Unterstützung bei den Fettsäure-Analysen danken.

Ein herzliches Dankeschön an alle Studierenden, insbesondere Tim Ostertag, Lukas Reinhard, Moritz Schäfer und Laura Kieffer für ihre Hilfe bei den Experimenten.

Ein ganz besonderer Dank geht an meine Familie, die immer für mich da war und ist. Vielen Dank an meine Eltern, die mich nie in Schubladen stecken wollten und immer an mich geglaubt haben, an meine Schwester, die mich immer motivieren konnte und vielerlei Desserts zaubert, an meinen Mann, für das Leben außerhalb der Uni, an Georg, der mir andere Dimensionen der Zeit gezeigt hat und was wirklich wichtig ist im Leben.

Zusammenfassung

Fließgewässerökosysteme und ihre angrenzenden terrestrischen Ökosysteme sind durch den Austausch von organischen und anorganischen Stoffen miteinander verbunden. Die Emergenz aquatischer Insekten dient im Uferbereich als Beute für Prädatoren wie Spinnen und Vögel. Informationen über die Qualität, Quantität und die Phänologie aquatischer Emergenz sind notwendig, um zu bestimmen wie Prädatoren von Emergenz als Nahrungsquelle profitieren können. Intensive Landwirtschaft ist weltweit stark vertreten, dennoch ist unklar, inwieweit sie sich auf die Qualität, Quantität und Phänologie aquatischer Emergenz auswirkt. Aquatische Emergenz enthält einen höheren Anteil langkettiger mehrfach ungesättigter Fettsäuren (PUFA, von engl. polyunsaturated fatty acids) als terrestrische Insekten. Insbesondere langkettige PUFA, können das Wachstum und die Immunantwort von Räubern verbessern. In Kapitel 2 wurde der PUFA-Transfer zu Spinnen und der Effekt von Nahrungsquellen mit unterschiedlichem PUFA-Gehalt auf Spinnen in Freiland-Mikrokosmen untersucht. Neben dem PUFA-Gehalt scheinen Umweltfaktoren, wie die Temperatur, wichtig für das Wachstum und die Körperkondition der Spinnen zu sein. Im dritten Kapitel wurde der Effekt von Landwirtschaft auf die Quantität, im Sinne von Biomasse, Phänologie und Zusammensetzung der aquatischen Emergenz untersucht. Bisher wurden Studien dazu nur zeitlich begrenzt durchgeführt, was phänologische Effekte verschleiert und eine genaue Abschätzung des Biomasse-Exports erschwert. Daher wurde aquatische Emergenz von März bis September kontinuierlich gesammelt und Umweltvariablen erfasst. An landwirtschaftlichen Standorten war die Biomasse der aquatischen Emergenz 61 – 68% höher als im Wald und deren Phänologie verändert. Insgesamt emergierten 71% der Familien der aquatischen Emergenz früher in landwirtschaftlichen Standorten als im Wald. Außerdem wurde in landwirtschaftlichen Standorten ein Austausch von Familien beobachtet. Die Toxizität von Pestiziden und elektrische Leitfähigkeit waren die wichtigsten Umweltvariablen, die mit der Änderung der Quantität in Verbindung standen. Im selben Experiment wurden zudem Spinnen gesammelt und sowohl der Fettsäuren (FA, von engl. fatty acids) Gehalt der Spinnen also auch aquatischer Emergenz bestimmt. Diese Ergebnisse sind in Kapitel 4 dargestellt. Der FA Export durch aquatische Emergenz war im Gegensatz zur Quantität im Wald 26 – 29% größer als in landwirtschaftlichen Standorten, was auf eine verringerte Qualität in landwirtschaftlichen Standorten hindeutet. Anders als bei Spinnen, unterschieden sich die FA Profile von Fliegen, Eintagsfliegen und Köcherfliegen zwischen Standorten in der Landwirtschaft und im Wald. Die Beschattung und der Anteil von Habitaten mit langsamer Fließgeschwindigkeit waren die wichtigsten Einflussgrößen für die FA Profile, wenn auch nur mit wenig Erklärungskraft. Insgesamt unterschieden sich die Quantität, Qualität und Phänologie aquatischer Emergenz zwischen Landnutzungsarten, was Populationsdynamiken im terrestrischen Ökosystem beeinflussen kann. Die Ergebnisse dieser Arbeit können zum Modellieren von Nahrungsnetzen oder Metaökosystemen genutzt werden, um das Verständnis der Kopplungen von Ökosystemen zu verbessern.

Summary

Streams and their adjacent terrestrial ecosystem are tightly linked via the flux of organisms and matter. Emergent aquatic insects can be an important food source for riparian predators like bats, birds, spiders, and lizards. Information about the quality, quantity and phenology of emergent aquatic insects is necessary to estimate how riparian predators can benefit from them as food source. Though intensive agriculture is a globally dominant land use, little is known about how agricultural land use affects the quantity, quality as well as phenology of emergent aquatic insects. Typically, emergent aquatic insects contain more long-chain polyunsaturated fatty acids (PUFA) than terrestrial insects. Especially long-chain PUFA, were shown to enhance growth and immune response of spiders and birds.

In chapter 2, the PUFA transfer to spiders and the effect of food sources differing in their PUFA profiles on spiders was examined in outdoor microcosms under environmentally realistic conditions (i.e., normal weather conditions, possibility to construct orb webs as in their natural habitat). The environmental context determined how PUFA can affect the spiders. For instance, besides PUFA profiles of food sources, environmental variables like the temperature were important for the growth and body condition of spiders.

In the third chapter, the effect of agricultural land use on the quantity in terms of biomass as well as abundance, phenology and composition of emergent aquatic insects was assessed. Previous studies were limited to seasons or single time points, which hampered determining annual biomass export and shifts in phenology. Therefore, emergent aquatic insects were sampled continuously over the primary emergence period of one year and environmental variables associated with agricultural land use were monitored. The biomass and abundance in total were higher (61 – 68 and 79 – 86%, respectively) in agricultural than forested sites. In addition to that, a turn-over of emergent aquatic insect assemblages and a shift in phenology of aquatic insects was identified. In agricultural sites, 71% families of aquatic insects emerged earlier than in forested sites. Pesticide toxicity was associated with different aquatic insect order biomass and abundances. During the same experiment spiders were sampled in spring, summer, and autumn. Additionally, the fatty acid (FA) content of the spiders and emergent aquatic insects was determined. These results are presented in chapter 4. The FA export via emergent aquatic insects was higher (26 – 29%) in forested than agricultural sites, which indicated a reduced quality of aquatic insects as food source for riparian predators in agricultural sites. The FA profiles of mayflies, flies and caddisflies differed between land-use types, but not for spiders. Shading and pool habitats were the most important environmental variables for the FA profiles, though environmental variables explained only little variation in FA profiles. Overall, the quantity, quality and phenology of emergent aquatic insects differed between land-use types, which can affect population dynamics in the adjacent terrestrial ecosystem. Our results can be used in modeling food-web dynamics or meta-ecosystems to improve understanding of linked ecosystems.

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List of abbreviations

ALA:	Alpha-Linolenic acid
ANOSIM:	Analysis of similarity
ANOVA:	Analysis of variance
ARA:	Arachidonic acid
CI:	Confidence interval
DHA:	Docosahexaenoic
EC:	Electrical conductivity
EC_{50}:	Effect concentration
EA:	Eicosanoic acid
ELA:	Elaidic acid
EPA:	Eicosapentaenoic acid
FA:	Fatty acids
FAME:	Fatty acid methyl esters
GLA:	Gamma-Linolenic acid
GLMM:	Generalized linear mixed-effects models
HGAM:	Hierarchical generalized additive models
JI:	Jaccard index
LIN:	Linoleic acid
LLA:	Linolelaidic acid
LM:	Linear models
LMM:	Linear mixed-effects models
MUFA:	Monounsaturated fatty acids
NMDS:	Non-metric multidimensional scaling
ODA:	Octadecanoic acid
PPDB:	Pesticide Property Data Base
PUFA:	Polyunsaturated fatty acids
SFA:	Saturated fatty acids
SIMPER:	Similarity percentage analyses
sumTU:	Logarithmic sum of toxic units
TMSH:	Trimethylsulfonium hydroxide
TU:	Toxic units

1. Introduction and Objectives

1.1 Aquatic-terrestrial linkages

Typically, different ecosystems are linked by spatial flows of energy, materials, and organisms (Loreau et al., 2003). Organisms connect ecosystems during foraging, dispersal, and seasonal migration (Gounand et al., 2018). These linkages are important for ecosystem functioning, i.e., “the joint effects of all processes that sustain an ecosystem” (Reiss et al., 2009), because they affect amongst others the composition of species assemblages and interactions (Bauer & Hoye, 2014). Ecosystem functioning is crucial for human well-being because it is the requirement for ecosystem services like provisioning fresh water, pollution control, and recreational opportunities (Harvey et al., 2017; MEA, 2005; Truchy et al., 2015).

Especially, stream ecosystems and adjacent terrestrial ecosystems are strongly linked via the flows of materials and organisms. Terrestrial insects and leaves falling into streams can subsidize the in-stream food web (Baxter et al., 2005). In streams, those leaves are colonized by microorganisms like aquatic hyphomycetes and bacteria. Aquatic hyphomycetes decompose hardly degradable leaf compounds like cellulose, enhance the protein as well as lipid content (Bärlocher, 1985) and alter the fatty acid (FA) content of leaves (Arce Funck et al., 2015; Zubrod et al., 2017). Together with algae, conditioned leaves are the basic food sources in stream ecosystems. The proportion of these basic food sources depends for example on the shading by riparian vegetation. In headwater streams, conditioned leaves usually contribute a higher proportion than algae at the base of the food web and downstream the proportion of algae increases (Vannote et al., 1980; Webster, 2007). In streams, those basic food sources are consumed by aquatic invertebrates, such as juvenile aquatic insects. Aquatic insects like Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies) and Diptera (flies) conduct life-cycle migration by emerging from streams to live as adults in terrestrial ecosystems. There, emergent aquatic insects subsidize the terrestrial food web, because they can be a food source for riparian predators like spiders, bats, birds, and lizards (Gray, 1993; Kato et al., 2004; Sabo & Power, 2002; Sullivan et al., 1993).

Most emergent aquatic insects are directly consumed in the riparian area, but at a mean distance of 550 m from the stream edge still 10% of the energy in the food web can originate from streams. Therefore, the influence of streams on terrestrial ecosystems is much wider than the actual stream width (Muehlbauer et al., 2014). The median flow of biomass from streams to the adjacent terrestrial ecosystem is one sixth of the reverse flow, although the contribution to the carbon of animals is similar, likely because of quality differences (Bartels et al., 2012). Furthermore, emergence of aquatic insects varies over time and shows temporal patterns, because of the phenology of aquatic insects (Nakano & Murakami, 2001), which can affect population dynamics in the adjacent terrestrial ecosystem (Sato et al., 2016; Uno, 2016). Thus, food quality along with food quantity and temporal patterns of food sources are substantial to understand the flow of energy between ecosystems and predict impacts on the subsidized food web (Gounand et al., 2018; Marcarelli et al., 2011).

While the influence of terrestrial ecosystems on stream ecosystems is well studied (Tank et al., 2010), fewer studies focused on the influence of the stream ecosystem on the adjacent terrestrial ecosystem (Schulz et al., 2015). Therefore, this thesis will focus on the linkage between stream ecosystems and the adjacent terrestrial ecosystem via emergent aquatic insects including their temporal patterns, composition of assemblages, quantity, and quality as food source for riparian predators. In the next section (1.2 Emergent aquatic insects as prey for riparian predators), these factors are explained in detail.

1.2 Emergent aquatic insects as prey for riparian predators

How riparian predators can utilize emergent aquatic insects as food source depends on the quantity, quality, temporal patterns due to phenology, and assemblage composition of aquatic insects (Gounand et al., 2018; Marcarelli et al., 2011; Stenroth et al., 2015). The biomass and abundance of emergent aquatic insects reflect their quantity as food source for riparian predators. Biomass is generally more suitable than abundance to assess energy flow, productivity, and food-web dynamics (Brown et al., 2004).

Emergent aquatic insects are a high-quality food source for riparian predators because they contain up to 10 times more long-chain (≥20 carbon atoms) polyunsaturated fatty acids (PUFA) than terrestrial insects (Hixson et al., 2015; Parmar et al., 2022). These difference in PUFA content originates from the base of the food web and the trophic transfer of PUFA: Aquatic primary producers, like diatoms or cryptophytes, can synthesize long-chain PUFA (Ahlgren et al., 1992; Kainz et al., 2004; Strandberg et al., 2015), while terrestrial primary producers, i.e., vascular plants, typically cannot (Sayanova & Napier, 2004). PUFA are transferred across trophic levels nearly twice as efficiently as bulk carbon, tend to bioaccumulate, and are stored in tissue without greater modifications, due to their important role in physiological processes as they are membrane components and precursor for bioactive molecules (Arts et al., 2001; Brett & Müller-Navarra, 1997; Gladyshev et al., 2013). Many consumers are not able to synthesize long-chain PUFA de novo and are therefore dependent on dietary intake of these compounds (Twining, Bernhardt, et al., 2021). However, some birds and spiders are capable to synthesize long-chain PUFA from precursor C18 PUFA, which is energetically costly and therefore competes with other energetically costly processes, like growth (Mathieu-Resuge et al., 2022; Sokolova et al., 2012; Twining, Bernhardt, et al., 2021). Hence, PUFA-rich food sources can promote growth and immune response of spiders and birds (Fritz et al., 2017; Mayntz & Toft, 2001; Twining et al., 2016) and riparian predators may benefit from consuming emergent aquatic insects. Though, most studies on effects of dietary PUFA intake on riparian predators were conducted under controlled laboratory conditions (Mayntz & Toft, 2001; Twining et al., 2016, 2019). How these results can be transferred to field conditions remains open, because environmental variables like temperature can affect growth (Brown et al., 2004) and immune response (Wojda, 2017).

The emergence of aquatic insects can be highly variable in time. Typically, emergence of aquatic insects peaks in spring in temperate regions, when terrestrial food sources are scarce (Nakano & Murakami, 2001; Sweeney & Vannote, 1982; Wesner, 2010). Therefore, riparian predators can benefit from aquatic insects as additional food source (Nakano & Murakami, 2001; Wesner, 2010). The abundance of riparian predators can increase during emergence peaks due to higher food availability (Baxter et al., 2005). Particularly, matching life-cycles of riparian predators and the emergence of aquatic insects are favorable. For instance, bats may cover their enhanced energy demand during reproduction in spring with emergent aquatic insects (Encarnação & Dietz, 2006) and the condition, as well as survival of nestlings increases, when fed with emergent aquatic insects (Berzins et al., 2021; Dodson et al., 2016; Shipley et al., 2022; Twining et al., 2018). In addition to that, the timing of emergence affects the growth rate (Sato et al., 2016; Uno, 2016), population biomass, and maturity rate of predators (Sato et al., 2016).

The composition of emergent aquatic insect assemblages can affect riparian predators via different pathways. First of all, the size of aquatic insect orders usually differs, e.g., flies are typically smaller bodied than caddisflies (Nilsson, 1996b, 1996a). Riparian predators chose food sources amongst others based on taxon-specific traits, such as the size of aquatic insect species (Davis et al., 2011; Stenroth et al., 2015). Most predators chose food sources of four to one orders of magnitude smaller than themselves (Brown et al., 2004; Cohen et al., 2003; Jonsson et al., 2005; Woodward & Hildrew, 2002). Therefore, in contrast to the total biomass, the biomass of single aquatic insect orders indicates which predators may benefit most from their emergence. Second, the temporal patterns of emergence of aquatic insects are dependent on the composition of aquatic insect assemblages, as individual species show differences in phenology. Species, exhibiting complementary phenology, contribute to temporal stability of emergence (Uno & Pneh, 2020). This can improve foraging opportunities for predators (Armstrong et al., 2016). Finally, the quality in terms of PUFA of aquatic insects also depends on the composition of aquatic insect assemblages, because the PUFA profiles of aquatic insect orders (Martin-Creuzburg et al., 2017) and families (Scharnweber et al., 2020) differ.

Related aquatic-terrestrial studies have mostly been conducted in sites without anthropogenic influence or under controlled conditions. Studies on the effect of agricultural land use on the aquatic-terrestrial linkages via emergent aquatic insects are lacking (Schulz et al., 2015). Thus, this thesis will assess associations of agricultural land use on emergent aquatic insects and in turn on riparian predators. How agricultural land use may affect aquatic insects and riparian predators is the subject of the next section (1.3 Effect of agricultural land-use on emergent aquatic insects and riparian predators).

1.3 Effect of agricultural land use on emergent aquatic insects and riparian predators

Worldwide, intensive agriculture is a dominant land-use type (Václavík et al., 2013). Intensive agriculture implies increasing habitat degradation, use of pesticides and fertilizers (Fischer et al., 2012; Stoate et al., 2001), which threatens stream ecosystems by channelization, the loss of riparian vegetation, and a reduction of water quality due to excessive nutrient inputs and the toxicity of pesticides. These environmental variables associated with agricultural land use jeopardize biodiversity, ecosystem functioning and in turn ecosystem services like provisioning of fresh water (Collen et al., 2014; Dudgeon et al., 2006; MEA, 2005; Reid et al., 2019; Vörösmarty et al., 2010). The effect of these environmental variables can propagate across ecosystem boundaries via aquatic emergent insects from the stream into the adjacent terrestrial ecosystem. For instance, the loss of stream biodiversity can affect the composition of aquatic insects, and in turn the temporal patterns of emergence and quality of emergent aquatic insects as food sources (Collen et al., 2014; Martin-Creuzburg et al., 2017; Uno & Pneh, 2020). Notwithstanding, studies investigating how agricultural land use affects biomass, composition, phenology, and the quality (in terms of FA) of emergent aquatic insects are scarce.

Generally, mayflies, stoneflies, and caddisflies are among the least studied insect taxa, while flies, mostly Culicidae (mosquitos), have been studied more frequently (InsectGapMap, 2020). Changes in aquatic insect assemblages have been linked to agriculture. An increasing trend in total biomass and abundance of aquatic insects over the last decades in agricultural regions was suggested in a recent meta-analysis (van Klink et al., 2020), but decreasing temporal trends have been reported for mayflies, stoneflies, and caddisflies (Baranov et al., 2020; Stepanian et al., 2020). Total biomass and abundance, however, can be poor indicators for changes in species assemblages (Desquilbet et al., 2020; Jähnig et al., 2021).

Agriculture can increase the biomass of aquatic insects, since it is accompanied with nutrient elevation and a reduction of shading. Both may enhance primary production, which increases food sources for aquatic insects in agricultural streams (Carlson et al., 2016; Griffiths et al., 2013; Stenroth et al., 2015; Terui et al., 2018). However, the biomass of single aquatic insect orders can respond differently to elevated nutrient concentrations. Also, other environmental variables associated with agricultural land use, like increased electrical conductivity and oxygen depletion can cause different responses of the biomass of single aquatic insect orders (Raitif et al., 2018). In addition, channelization usually increases the proportion of pool habitats (Petersen Jr, 1992). Within those, the total abundance of aquatic insects can be smaller than in riffle habitats, though single aquatic insect orders can react differently (Carlson et al., 2013; McKie et al., 2018).

Aquatic insect assemblages are altered by agriculture, for example by promoting small-bodied insects like flies (Carlson et al., 2016; Krell et al., 2015; Stenroth et al., 2015) and aquatic insects with a shorter generation time, because their high reproduction rate makes them less vulnerable to environmental variables associated with agricultural land use (Larsen & Ormerod, 2010; Liess & von der Ohe, 2005). Generally, the distribution of functional traits, such as generation time, in insect assemblages can indicate responses to land use (Berger et al., 2018; Mondy et al., 2012). Better insights into the connection between land use like agriculture and the distribution of functional traits in aquatic insect assemblages can enhance mechanistic understanding of species-environment relationships (Kearney & Porter, 2009). Furthermore, the use of pesticides has been linked to the loss of up to 42% of aquatic invertebrates, including aquatic insects (Beketov et al., 2013).

Agricultural land use has the potential to affect the phenology of aquatic insects directly. For instance, pesticides can cause earlier emergence of aquatic insects (Cavallaro et al., 2018) and agricultural land use can enhance water temperature, which in turn is associated with earlier emergence of aquatic insects (Anderson et al., 2019). Furthermore, the temporal patterns of emergence can be altered indirectly by agricultural land use, due to changes in the composition of aquatic insect assemblages (Uno & Pneh, 2020). The alteration of temporal patterns of emergence can in turn affect riparian predators, for example by a mismatch of their life-cycle and the time point of emergence (Sato et al., 2016; Uno, 2016). Additionally, recent studies on the impact of agricultural land use on aquatic emergent insects were limited to random snap-shot samples in different seasons (Carlson et al., 2016; Raitif et al., 2018) or to a few weeks (Graf et al., 2020; Krell et al., 2015; Stenroth et al., 2015). Due to the high temporal variability in emergence, this hampered the estimation of complete balances of biomass export from different land uses to the riparian ecosystem.

The effect of agriculture on the FA profiles of aquatic insects and riparian predators is largely unknown. Most studies assessed FA profiles of aquatic insects and riparian predators without including potential effects of environmental variables associated with agricultural land use (e.g. Martin-Creuzburg et al., 2017; Moyo et al., 2017; Parmar et al., 2022; Twining, Parmar, et al., 2021). However, in a food chain, where larval stages of non-biting midges were exposed to toxicants under laboratory conditions, no effect on FA profiles of adult non-biting midges and a tendency to reduce FA content in spiders was observed (Pietz et al., 2023). Furthermore, FA export via Chironomidae (non-biting midges) was highest at intermediate nutrient concentrations in a mesocosm study (Scharnweber et al., 2020). Though, no associations of stream-bed characteristics and physicochemical variables, such as nutrients, on FA profiles of aquatic insects were found in one field study conducted in two streams (Kowarik et al., 2022). In addition, the composition of spider assemblages at family level was the best predictor for FA content of spiders in a field study performed in agricultural and forested streams focusing on the type of vegetation (Ramberg et al., 2020).

Particularly, in agricultural areas, riparian predators may benefit from consuming emergent aquatic insects as additional food source (Graf et al., 2020; Krell et al., 2015; Murakami & Nakano, 2002; Nakano & Murakami, 2001; Stenroth et al., 2015), because agriculture is linked to the loss of terrestrial insects (Ewald et al., 2015; Hallmann et al., 2017; Seibold et al., 2019; Shortall et al., 2009). Higher food source availability can increase predator abundance. Indeed, the abundance of spiders increased from the center of agricultural fields towards riparian areas, presumably driven by availability of emergent aquatic insects (Pfister et al., 2015). This points to a better food availability outside of agricultural fields, because it was shown that terrestrial predators like spiders, birds, and lizards aggregate in riparian areas due to high availability of aquatic emergent insects (Gray, 1993; Henschel et al., 2001; Paetzold Achim et al., 2011; Sabo & Power, 2002). Based on the previously mentioned research gaps and the high potential of agricultural land use to affect emergent aquatic insects and riparian predators, this thesis will focus on the transfer of PUFA to riparian predators under realistic environmental conditions and the impact of agriculture on emergent aquatic insects as well as riparian predators. In the following section (1.4 Objectives and outline of the thesis) the specific research objectives are described.

1.4 Objectives and outline of the thesis

The aim of this thesis was to study the effect of agricultural land use on emergent aquatic insects, including their quantity, quality, phenology, and composition as well as on the FA profiles of spiders. Moreover, the influence of the quality of emergent aquatic insects in terms of PUFA on riparian predators was examined (Figure 1.1).

The second chapter of this thesis comprises experiments examining how PUFA are transferred along a tritrophic food chain, comprising basic food sources, the non-biting midge *Chironomus riparius* (Diptera, Chironomidae) and the spider of the genus *Tetragnatha*. Further, the response of the spider on the PUFA transfer was examined in outdoor microcosms under environmentally realistic conditions, namely: normal weather conditions and the possibility to construct orb webs as in their natural habitat. The research questions were in detail:

- Will the differences in the PUFA profiles of the basic food sources propagate along the food chain and result in different PUFA profiles between treatments at all trophic levels of the food chain?
- How quickly will the PUFA be assimilated into the tissue of spiders? Can we identify temporal changes in the PUFA profiles of spiders?
- Do the spiders respond differently to differences in PUFA profiles of the non-biting midges consumed by the spiders?

In the third chapter, the biomass as well as abundance of emergent aquatic insects was quantified over the primary emergence period of a year in agricultural and forested sites. In addition, the composition and phenology of emergent aquatic insects were examined. Finally, environmental variables associated with agricultural land use explaining changes in biomass, abundance and composition of emergent aquatic insects were identified. Specific research questions were:

- How do the biomass, abundance and phenology of emergent aquatic insects differ between agricultural and forested sites in total as well as on order and family level?
- Do the taxonomic and trait composition of emergent aquatic insects differ between agricultural and forested sites?
- Which environmental variables are associated with changes in the biomass and abundance in total as well as on family level?

The fourth chapter deals with the influence of agricultural land use and associated environmental variables on FA profiles of emergent aquatic insects and riparian predators in forested and agricultural sites. Moreover, the FA, saturated FA (SFA), monounsaturated FA (MUFA), and PUFA exports via emergent aquatic insects were quantified over the primary emergence period within one year. The following research questions were answered:

- Do the FA profiles of emergent aquatic insects and riparian predators differ between forested and agricultural sites?
- How do the FA, SFA, MUFA, and PUFA exports differ between agricultural and forested sites?
- Which environmental variables associated with agricultural land use explain the FA profiles of emergent aquatic insects and spiders?

In the fifth chapter the results of the second, third, and fourth chapter are connected and discussed. Furthermore, a general conclusion is given.

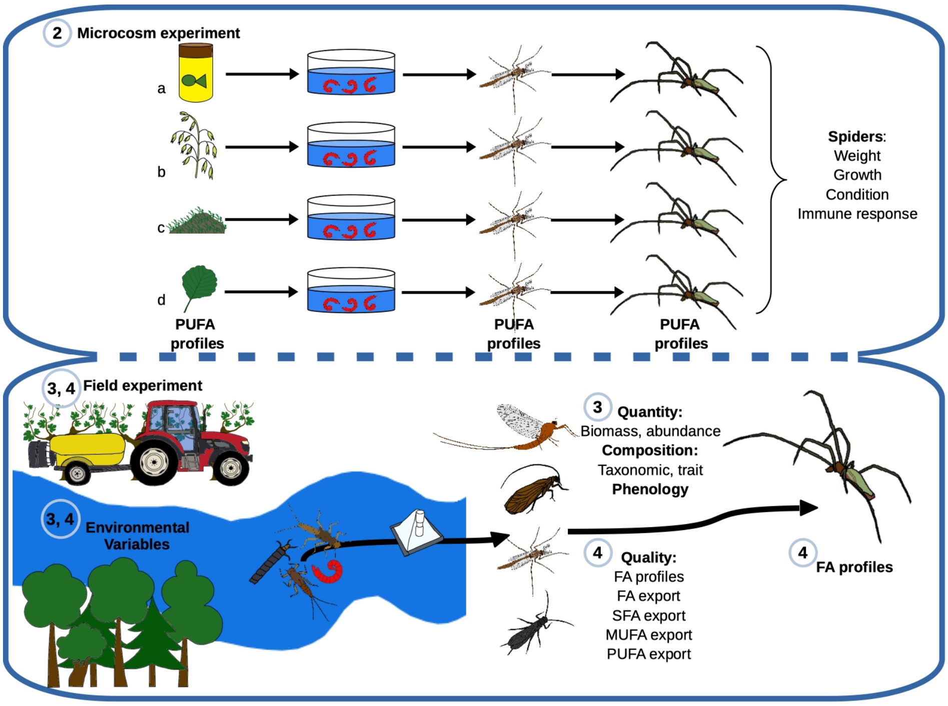

Figure 1.1: Overview of the objectives of this thesis. The thesis included two microcosm experiments and one field experiment conducted in agricultural and forested sites. Numbers in circles indicate the chapters of this thesis. 2: Trophic transfer of polyunsaturated fatty acids across the aquatic-terrestrial interface: an experimental tritrophic food chain approach, 3: Land use changes biomass and temporal patterns of insect cross-ecosystem flows, 4: Land use alters cross-ecosystem transfer of high value fatty acids by aquatic insects. The letters a-d represent the basic food sources used as different treatments of the microcosm experiment (a: fish food, b: oatmeal, c: algae, d: conditioned leaves). FA is the abbreviation for fatty acids, SFA for saturated fatty acids, MUFA for monounsaturated fatty acids and PUFA for polyunsaturated fatty acids.

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2. Trophic transfer of polyunsaturated fatty acids across the aquatic-terrestrial interface: an experimental tritrophic food chain approach

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This chapter is adapted from the article published in 2023 in Ecology and Evolution, 13, e9927, https://doi.org/10.1002/ece3.9927

2.1 Abstract

Aquatic and their adjacent terrestrial ecosystems are linked via the flux of organic and inorganic matter. Emergent aquatic insects are recognized as high quality food for terrestrial predators, because they provide more physiologically relevant long-chain polyunsaturated fatty acids (PUFA) than terrestrial insects. Effects of dietary PUFA on terrestrial predators have been explored mainly in feeding trials conducted under controlled laboratory conditions, hampering the assessment of the ecological relevance of dietary PUFA deficiencies under field conditions.

We assessed the PUFA transfer across the aquatic-terrestrial interface and the consequences for terrestrial riparian predators in two outdoor microcosm experiments. We established simplified tritrophic food chains, consisting of one out of four basic food sources, an intermediary collector gatherer (*Chironomus riparius*, Chironomidae), and a riparian web-building spider (*Tetragnatha* sp.). The four basic food sources (algae, conditioned leaves, oatmeal, fish food) differed in PUFA profiles and were used to track the trophic transfer of single PUFA along the food chain and to assess their potential effects on spiders, i.e., on fresh weight, body condition (size-controlled measurement of nutritional status) and immune response.

The PUFA profiles of the basic food sources, *C. riparius* and spiders differed between treatments, except for spiders in the second experiment. The PUFA α-linolenic acid (ALA, 18:3n-3) and ɣ-linolenic acid (GLA, 18:3n-6) were major contributors to the differences between treatments. PUFA profiles of the basic food sources influenced the fresh weight and body condition of spiders in the first experiment, but not in the second experiment, and did not affect the immune response, growth rate and dry weight in both experiments. Furthermore, our results indicate that the examined responses are dependent on temperature. Future studies including anthropogenic stressors would deepen our understanding of the transfer and role of PUFA in ecosystems.

2.2 Introduction

Natural ecosystems are in exchange with neighboring ecosystems. Aquatic and adjacent terrestrial ecosystems are linked via the exchange of organic and inorganic matter (Baxter et al., 2005; Schindler & Smits, 2017). Leaves and terrestrial invertebrates falling into streams can be an important subsidy for stream food webs (Baxter et al., 2005; Wallace et al., 1997). In turn, emergent aquatic insects can be a food source for terrestrial predators, like bats, lizards, and spiders (Kato et al., 2004; Sabo & Power, 2002; Sullivan et al., 1993). Especially, when terrestrial food sources are scarce, e.g., in spring, terrestrial predators can benefit from feeding on emergent aquatic insects (Nakano & Murakami, 2001; Wesner, 2010). Besides food quantity, food quality is key for understanding energy fluxes between ecosystems and for predicting effects on subsidized food webs (Marcarelli et al., 2011).

Emergent aquatic insects are considered high-quality food for terrestrial predators, because they contain up to ten times more long-chain (≥ 20 carbon atoms) polyunsaturated fatty acids (PUFA) than terrestrial insects (Hixson et al., 2015; Parmar et al., 2022). Aquatic primary producers, such as diatoms or cryptophytes, can synthesize long-chain PUFA that are subsequently available to aquatic consumers and transferred across trophic levels (Ahlgren et al., 1992; Kainz et al., 2004; Strandberg et al., 2015). In contrast, terrestrial primary producers typically do not produce long-chain PUFA (Sayanova & Napier, 2004). Most consumers are incapable of synthesizing long-chain PUFA de novo and thus rely on an adequate dietary supply with these essential compounds (Twining, Bernhardt, et al., 2021). Long-chain PUFA, such as eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6), are important membrane components and serve as precursors for a plethora of other bioactive molecules. However, some animals, e.g. some spiders and birds, are able to synthesize C20 PUFA from dietary C18 PUFA precursors, i.e., α-linolenic acid (ALA, 18:3n-3) and linoleic acid (LIN, 18:2n-6c), which is energetically costly and thus may be performed only if required (Mathieu-Resuge et al., 2022; Twining, Bernhardt, et al., 2021; Twining, Parmar, et al., 2021). Riparian predators may thus benefit from the consumption of emergent aquatic insects (Fritz et al., 2017; Mayntz & Toft, 2001; Twining et al., 2016). PUFA-rich food was shown to promote growth, immune responses, and good body condition of tree swallow chicks (Twining et al., 2016), as well as growth (Mayntz & Toft, 2001) and immune responses of spiders (Fritz et al., 2017).

The transfer of PUFA from aquatic into adjacent terrestrial ecosystems via emergent aquatic insects may thus strongly influence riparian food webs. Due to their important role in physiological processes, long-chain PUFA are typically stored in tissues without greater modifications. Thus, PUFA tend to bioaccumulate and are nearly twice as efficiently transferred to the next trophic level as bulk carbon (Arts et al., 2001; Brett & Müller-Navarra, 1997; Gladyshev et al., 2013).

Previous studies examined the PUFA profiles of aquatic insects (Martin-Creuzburg et al., 2017; Moyo et al., 2017; Scharnweber et al., 2020), the PUFA transfer via aquatic insects to terrestrial predators (Kowarik et al., 2021; Twining et al., 2019; Twining, Parmar, et al., 2021) and effects of dietary PUFA on terrestrial predators in the field (Fritz et al., 2017) as well as under controlled laboratory conditions (Mayntz & Toft, 2001; Twining et al., 2016, 2019). For the latter, it remains open to which extent the results can be transferred to the field because environmental factors like temperature can also affect the performance of predators, e.g., regarding growth (Brown et al., 2004) and immune function (Wojda, 2017).

Our aim was to examine how PUFA are transferred along experimental tritrophic food chains and to explore potential effects on a terrestrial predator. The latter was studied in outdoor microcosms under virtually realistic environmental conditions, i.e., the spiders were exposed to normal weather conditions and had the possibility to build orb webs on nettles like in their natural habitat. Overall, we conducted two outdoor microcosm experiments each with basic food sources, the *Chironomus riparius* (Diptera, Chironomidae) and the spider *Tetragnatha* sp. in a food chain. *Tetragnatha* sp. are suitable model organisms to study direct and indirect effects of PUFA in aquatic-terrestrial food webs because they commonly occur in riparian areas, consume aquatic emergent insects (Kato et al., 2004) and capture their prey with orb webs (Reitze & Nentwig, 1991). Furthermore, it was shown that spiders aggregate in riparian areas during peak emergence of aquatic insects (Henschel et al., 2001; Paetzold et al., 2011) and potentially serve as prey for other organisms like birds (Poulin et al., 2010), which may result in a PUFA transfer via spiders to higher trophic levels.

The food chains varied in the basic food source, which were selected to represent different PUFA profiles enabling us to determine potential differences in their transfer and effects on spiders. Therefore, PUFA profiles of the basic food sources, *C. riparius* and spiders were analyzed. Additionally, we recorded fresh as well as dry weight, growth rate, body condition (size-controlled measurement of nutritional status) and immune response of spiders to assess potential effects of PUFA on spider performance. We expected (I) different PUFA profiles between treatments at all trophic levels of the food chain, because differences in PUFA profiles of the basic food sources can propagate along the food chain; (II) temporal changes in PUFA profiles of the spiders during the experiment, due to the fact that PUFAs are assimilated after a certain time into the tissue of organisms; and (III) treatment differences in the spiders' fresh as well as dry weight, growth rate, body condition and immune response due to the differences in PUFA profiles of the chironomids consumed by the spiders.

2.3 Material and Methods

2.3.1 Spider and nettle collection

For the outdoor microcosm experiments, spiders of the genus *Tetragnatha* were collected at pristine streams (49°16'13''N, 8°2'54''E and 49°15'43''N, 7°57'36''E) in the Palatinate Forest Nature Park, a forested low mountain range in Germany. The collection for the first experiment was conducted on the 17th, 23rd, 24th and 25th of April 2019 and for the second experiment on the 17th, 19th and 24th of June 2019. Until the start of the experiment, the spiders were kept in climate chambers at 20 °C and were fed once a week with one adult *C. riparius* raised in standard cultures (OECD, 2011).

Feeding frequency varies between and within spider species (Foelix, 2011) and the immune response of male as well as pregnant female spiders can be highly variable (Ahtiainen et al., 2004, 2005). Therefore, whenever possible, only female, adult, and non-pregnant (checked visually) spiders of the species *T. montana* were used in the experiment to minimize variability in their feeding and immune response (Table A.1).

Nettles, *Urtica dioica*, were used in the microcosms because they are common along streams (Davis, 1989) and are frequently used by spiders in their natural habitat to build their orb webs. Nettles were collected at two locations (49°16'57''N, 8°5'18''E and 49°12'15''N, 8°6'27''E) on the 10th, 11th, 16th, 24th and 29th of April 2019. After collection, nettles were planted in fertilized soil (nitrogen 150 – 450 mg L^{-1}) in 10 x 10 x 10 cm pots and kept in 60 x 60 x 90 cm aerarium (Matthäus Hahn e.K.) until the experiment started to prevent insect infestation. Further, the microcosms were regularly checked for invading insects, which were removed by hand.

2.3.2 Chironomidae and basic food sources

Adult *C. riparius*, hereafter chironomids, were used as food for the spiders. Chironomids were gathered from laboratory cultures that were maintained based on the OECD-guideline 235 (OECD, 2011); they were cultured in glass vessels (30 cm x 20 cm x 10 cm) with a layer of silica sand (height ~ 0.1 cm) in a climate chamber (20 ± 2 °C) with a 16 h light (~ 1000 lux) and 8 h darkness light cycle.

Chironomid cultures differing in the food source for chironomid larvae, termed as basic food sources below, were set up at the end of February 2019 to gain organisms with specific PUFA profiles. The early set-up of the cultures was done, to ensure that at the beginning of the experiment the chironomids had only consumed the specific basic food sources during their larval stages. Overall, four basic food sources were used and later the treatments of the experiments are named after the basic food sources.

The basic food sources were provided ad libitum and were algae (Liquizell, Hobby), standard fish food (TetraMin, Tetra), oatmeal (Avena sativa, dmBio) and conditioned alder leaves (*Alnus glutinosa*). The latter three were ground before being fed to the chironomids. Fish food is used in the OECD-guideline 235 (OECD, 2011) to feed chironomids as it comprises a suitable nutrient composition for chironomids. Furthermore, fish food includes ingredients with aquatic as well as terrestrial origin amongst others fish and cereals and hence should have a broad PUFA profile. Algae was used as aquatic food source and contained phytoplankton and minerals, which usually is enriched in n-3 PUFA in comparison to n-6 PUFA (Strandberg et al., 2015). In contrast, oatmeal represented a typical terrestrial food source, because it contains high levels of LIN and only small to no amounts of ALA and EPA (Torres-Ruiz et al., 2010). Conditioned leaves were considered as semiaquatic food, because they originate from terrestrial plants, but are modified by aquatic organisms. Conditioned leaves can be an important food source for aquatic invertebrates, especially in small order streams (Graça & Canhoto, 2006; Vannote et al., 1980). Conditioned leaves are colonized by microorganisms like aquatic fungi and bacteria as well as eukaryotic microalgae. Particularly aquatic fungi can enhance the protein and lipid content of leaves and decompose otherwise hardly degradable leaf compounds, like cellulose, rendering leaves more nutritious and digestible (Bärlocher, 1985). Additionally, aquatic fungi change the PUFA content of leaves (Zubrod et al., 2017). The leaves were collected directly from the tree in autumn 2017 from a biosphere reserve (49°14'24''N, 7°53'24''E) at the time of abscission, air-dried and stored in the dark at room temperature until usage. Approximately 8 g of leaves were weighed with a precision of 0.01 g in leaf bags (0.5 mm mesh size, 15 x 15 cm). Starting at the end of January 2019, every two weeks two leaf bags were conditioned in the Sulzbach in Eußerthal, Germany (49°15'43''N, 7°57'36''E).

2.3.3 Experimental design

We conducted two experiments on Campus Landau (49°12'15''N, 8°6'27''E) in southwest Germany. The first experiment took place from 29th of April to the 12th of June 2019 and the second experiment was run from the 8th to 29th of July 2019. The duration of the two experiments differed, because during the second experiment the mortality of spiders was elevated, probably due to very hot temperatures inside the microcosms of up to 32 ± 8 °C (Figure A.1).

Every treatment consisted of one food chain with a basic food source at the lowest trophic level, followed by chironomids as the second level and spiders as the highest trophic level. The treatments differed only in the basic food source supplied to the chironomid larvae (Figure 2.1). The first experiment included algae, fish food and oatmeal as basic food treatment, whereas the second experiment included leaves, fish food and oatmeal. The basic food treatments differed between experiments due to limited labor capacity preventing us to run all treatments simultaneously. Per treatment 20 replicates were used. Every replicate consisted of one spider and one nettle in a microcosm (60 x 60 x 90 cm aerarium). The spiders and nettles were assigned randomly to the microcosms.

Spiders were fed on two days per week with a tweezer to ensure the spiders consumed the chironomids. In the first experiment, spiders were fed two chironomids per week and in the second experiment four chironomids per week. The number of chironomids differed because the algae-based chironomid cultures were less productive during the first experiment and therefore prohibited feeding the spiders with four chironomids, which presumably better matches the energy needs of spiders. For the first two weeks, dead spiders were replaced in both experiments. Before the replacement spiders were put in the microcosms, they were being fed with the total amount of chironomids the dead spider had consumed and over the same period the dead spider was fed to safeguard that the results are not influenced by different food quantities.

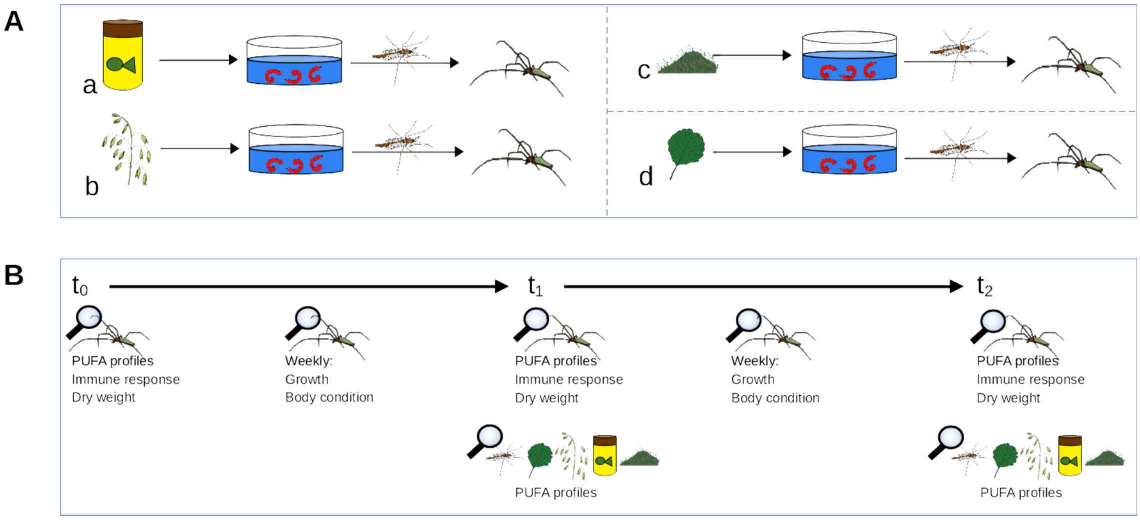


Figure 2.1: Experimental design. A: Treatments of the experiments. Every treatment consisted of one food chain with a basic food source at the lowest trophic level, followed by chironomids at the second level and spiders on the highest trophic level. Basically, the treatments differed in the basic food source. a: Fish food; b: Oatmeal; c: Algae; d: Leaves. The first experiment included algae, fish food and oatmeal as basic food treatment, whereas the second experiment included leaves, fish food and oatmeal. B: Time course of the microcosm experiments. t_0: Start of experiment (day = 0); t_1: 23 and 14 days in first and second experiment. t_2: 44 and 21 days in first and second experiment. Between the time points t_0, t_1, t_2, fresh weight and body condition of spiders were assessed weekly.

2.3.4 Weekly measurement of fresh weight, growth, and body condition

Once per week, the spiders were weighed. This fresh weight was used to calculate the growth rate (g week^{-1}) of the spiders, where t_x is the number of weeks of the experiment and t_0 the start of the experiment:

$$\text{growth rate}=\frac{\ln(\text{fresh weight}_{tx})-\ln(\text{fresh weight}_{t0})}{t_x} \quad (2.1)$$

Furthermore, a picture of every spider was taken on top of millimeter paper to estimate their body condition using their thorax and abdomen width. The thorax grows only by molting, while the abdomen is more dynamic than the thorax and changes its size during food uptake. As the spiders were fed the same amount during the experiment, the size change due to food uptake is supposed to be similar. The body condition is a better indicator of the nutritional status of a spider than body weight alone because the abdomen is the main food storage, and its proportion of the total weight increases with increasing relative width to the thorax width (Anderson, 1974). Furthermore, the body condition controls for the size of spiders, which is important when organisms of different life stages like in our study are compared (Jakob et al., 1996). The program ImageJ version 1.53k (Rasband, 2018) was used to measure the width.

Subsequently, the body condition was calculated as follows (Anderson, 1974):

$$\text{body condition}=\frac{\text{thorax width}}{\text{abdomen width}} \quad (2.2)$$

2.3.5 Measurement of immune response, dry mass and PUFA profiles

In the beginning, during and at the end of the experiment, spiders were taken randomly out of the experiment to analyze the immune response, dry mass and PUFA profiles of spiders. We planned to analyze ten spiders per treatment and time point, which was hampered by a higher than expected mortality. Nevertheless, at least three spiders were taken per treatment and time point (for exact numbers see Table A.2). Additionally, adult chironomid and basic food source samples (Table A.2) were taken to facilitate the comparison of PUFA profiles along the food chain. We sampled chironomids approximately one week before the spiders to allow spiders to digest and assimilate PUFA from the consumed chironomids.

To estimate the immune response of the spiders their ventral abdomen was punctuated with a sterile needle (gauge = 0.45 mm) and subsequently, a nylon monofilament (length ≈ 2 mm, diameter ≈ 0.2 mm) was inserted (Fritz et al., 2017; Rantala et al., 2002; Siva-Jothy & Thompson, 2002). In spiders, the monofilaments cause the same encapsulation reaction as parasites (Ratcliffe & Rowley, 1979). By quantifying the hemocytes adhering to the monofilament, the level of encapsulation can be determined. 24 hours after insertion the spiders were euthanized in liquid nitrogen. The monofilament was recovered from the thawed spiders and stored in ethanol (70%). The spiders were stored at – 80 °C until further processing. Each monofilament was photographed under a binocular with millimeter paper in three random orientations. In the pictures, the encapsulated area ($A_{\text{encapsulated}}$) and the area of the non-encapsulated monofilaments ($A_{\text{not encapsulated}}$) were measured using ImageJ (Rasband, 2018). The ratio of these two areas was reported as proportional encapsulation area (Re/ne, Fritz et al., 2017):

$$R_{e/ne}=\frac{A_{\text{encapsulated}}}{A_{\text{not encapsulated}}} \quad (2.3)$$

Furthermore, the area (mm^2) of the encapsulation (A_E) was determined (Fritz et al., 2017):

$$A_E=A_{\text{encapsulated}}-A_{\text{not encapsulated}} \quad (2.4)$$

To determine the dry weight of the spiders and subsequently the PUFA profile of the individual spiders, they were lyophilized to complete dryness and weighed to the nearest 0.1 µg. Additionally, the basic food sources and chironomids were lyophilized to complete dryness. Individual chironomids were pooled, and the pooled samples were weighed to the nearest 0.1 µg. For the basic food sources approximately 40 mg with a precision of 0.1 µg were weighed. The PUFA profiles of adult chironomids and basic food sources were also analyzed. During the experiment no samples were touched directly to avoid PUFA cross contamination from fingers to the samples. This was ensured using tweezers, suction samplers, or gloves.

The PUFA of all samples were extracted based on Folch et al. (1957) with chloroform/methanol (5 mL, v:v; 2:1) over night at – 20 °C and the addition of an internal standard (C17:00 200 µg mL-1; C23:0 250 µg mL-1, Sigma-Aldrich). After extraction, the samples were filtered through a syringe filter (PTFE, 13 mm, 0.45 µm, BGB) and evaporated until dryness under nitrogen at 40 °C. Then the samples were redissolved in methanol and stored until derivatization under nitrogen at -20 °C. The PUFA in the samples were derivatized to fatty acid methyl esters (FAME) with methanolic trimethylsulfonium hydroxide (TMSH, 0.2 M, Macherey-Nagel) at room temperature for 60 minutes. The FAMEs were analyzed using a gas chromatograph with a flame ionization detector (Varian CP-3800, Varian Inc) equipped with a DB-225 capillary column (30 m x 0.25 mm x 0.25 µm, Agilent J&W). External standards (Supelco 37 component FAME mix, 18:1n-7 FAME, ALA FAME, Sigma-Aldrich) were used to identify and quantify the FAMEs. The identification was done with OpenChrom version 1.4x (Wenig & Odermatt, 2010). Quantification was conducted in R version 4.2.0 (R Core Team, 2022). Details on the analytical procedure are given in the section “A Supplementary material: Trophic transfer of polyunsaturated fatty acids across the aquatic-terrestrial interface: an experimental tritrophic food chain approach” under “Analytical procedure PUFA” and on the quantification in the same section under “Calculation PUFA content”.

2.3.6 Data analysis

The spiders used at the beginning of the experiment for determining PUFA profiles, dry weight and immune response were assigned randomly to the treatments to calculate the start values of the experiment. For the analysis, 11 PUFA (Figure A.3, A.4), commonly found in organisms, with ≥ 18 carbon atoms were used, including the physiological important PUFA ALA, LIN, EPA, and ARA. The content of a single PUFA was calculated as the proportion of total PUFA.

The PUFA profiles of spiders, chironomids and basic food sources were visualized with non-metric multidimensional scaling (NMDS) with Euclidean distances in two dimensions, which resulted in stress-values < 0.1. With analysis of similarity (ANOSIM; 999 permutations, Euclidean distance, R-package vegan version 2.5-7, Oksanen et al., 2020) PUFA profiles of spiders were compared between time points within the same treatment and between treatments within the same time point. The latter was also done for chironomids and the basic food sources. To decrease the false discovery rate in multiple testing, p-values were adjusted with the Benjamini-Hochberg method (Benjamini & Hochberg, 1995).

Whenever ANOSIM resulted in significant differences among treatments, similarity percentage (SIMPER) analyses (R-package vegan version 2.5-7, Oksanen et al. 2020) were performed to identify PUFA contributing to the differences among treatments.

Fresh weight and body condition were analyzed with linear mixed models (LMM) including the explanatory variables time point and treatment as well as their interaction as fixed effects and replicate as random factor because the spider of a replicate was measured repeatedly. The LMM were fitted with the R-package glmmTMB version 1.1.3 (Brooks et al., 2017). Linear models (LM) with time point, treatment and their interaction as explanatory variables were used to analyze the dry weight and immune response, with the mean area of the encapsulation and proportional encapsulation area as response variables for the latter. No random factor was required for this analysis because spiders were measured only once for these responses. The effects of treatment, time point and their interaction on the responses were tested by type II analysis of variance (ANOVA) using $\chi2$-test for the LMM and F-tests for the LM. We removed the interaction term from the model when it was not statistically significant. All analyses were done with R version 4.2.0 (R Core Team, 2022). Including time point and treatment in the analysis covered the temporal dependence of the response variables. By testing for the treatment and time point effect in the same model we were able to disentangle their effects. Whenever only time point was significant, the temporal dependence was stronger than the treatment effect. The data and R-Scripts are openly available at https://doi.org/10.5281/zenodo.7692685.

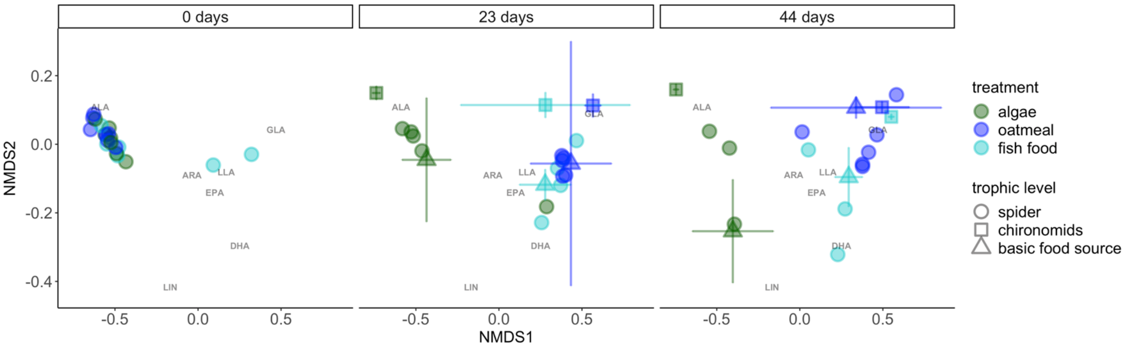
2.4 Results

2.4.1 Change in PUFA profiles along the food chain

We found different PUFA profiles between treatments at all trophic levels of the food chain, except for spiders in the second experiment: In the first microcosm experiment, the PUFA profiles of spiders differed significantly across treatments for both treatment time points (ANOSIM: 23 days: R = 0.63, p = 0.010; 44 days: R = 0.69, p = 0.012). The spiders of the algae treatment were most strongly separated from the other treatments (Table 2.1, Figure 2.2). In contrast, the spiders' PUFA profiles were similar for all treatments (ANOSIM: 14 days; R = -0.03, p = 0.693; 21 days: R = 0.21, p = 0.075) in the second experiment. The spiders had variable PUFA profiles at the beginning of each of the two experiments (Figure 2.2).

In both experiments, the PUFA profiles of chironomids and the basic food sources differed statistically significant between the treatments at all time points (Table 2.1, Figure 2.2). The treatments oatmeal and fish food were more similar to each other in both experiments then to the algae treatment in the first experiment and the leaves treatment in the second treatment (Figure 2.2).

(a) Experiment 1



(b) Experiment 2

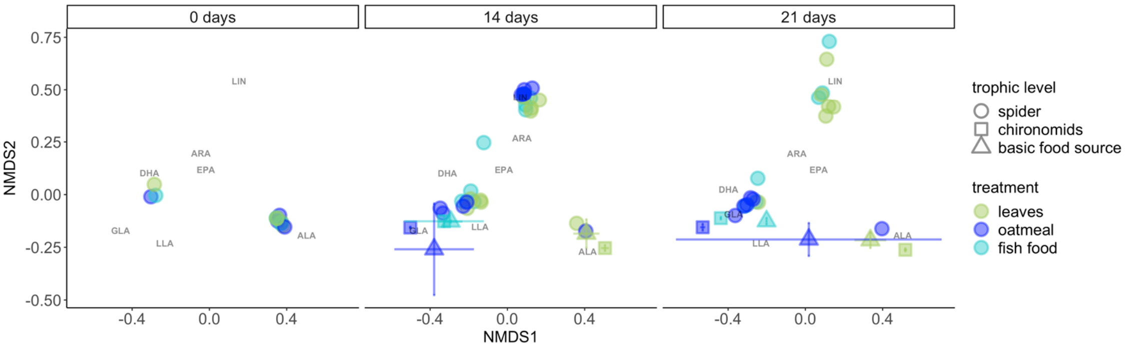


Figure 2.2: Non-metric multidimensional scaling (NMDS) of the polyunsaturated fatty acid (PUFA) profiles in the first (A) and second (B) experiment. Colors indicate treatment, shape indicates trophic level. The first experiment included algae, fish food and oatmeal as food treatment, whereas the second experiment included leaves, fish food and oatmeal. For the chironomids and chironomid food sources mean and standard deviation are presented. On day 0, only PUFA profiles of spiders were analyzed to gain their starting values before they were fed with chironomids of the different treatments and the spiders were assigned randomly to the treatments. Chironomids and chironomid food sources were sampled and analyzed as soon as the spiders were fed with them. For a better overview only eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6), α-linolenic acid (ALA, 18:3n-3), ɣ-linolenic acid (GLA, 18:3n-6), linolelaidic acid (LLA, 18:2n-6t) and linoleic acid (LIN, 18:2n-6) are displayed. The NMDS plot with all PUFA can be found in the supplementary data (Figure A.2).

Table 2.1: Results of the analysis of similarities (ANOSIM) for the polyunsaturated fatty acids (PUFA) profiles. Treatments were compared for spiders, chironomids and basic food sources within the same time point and time points for spiders within the same treatment. P-values were adjusted using the Benjamini-Hochberg method. R values indicate the differences between groups: R < 0.25 barely separated, R < 0.5 clearly separated with some overlap, R > 0.75 well separated (Jaschinski et al., 2011).

Experiment	**Compared groups**			**R**	**p-value**
1	treatments	spiders	time point 23 days	0.63	0.010
			time point 44 days	0.69	0.012
		chironomids	time point 23 days	0.50	0.012
			time point 44 days	0.83	0.006
		basic food sources	time point 23 days	0.71	0.006
			time point 44 days	0.68	0.006
	time points	spiders	treatment algae	0.11	0.194
			treatment oatmeal	0.80	0.006
			treatment fish food	0.38	0.052
2	treatments	spiders	time point 14 days	-0.03	0.693
			time point 21 days	0.21	0.075
		chironomids	time point 14 days	0.94	0.008
			time point 21 days	1.00	0.028
		basic food sources	time point 14 days	0.68	0.008
			time point 21 days	0.83	0.030
	time points	spiders	treatment leaves	0.26	0.036
			treatment oatmeal	0.14	0.170
			treatment fish food	0.30	0.030

2.4.2 Contribution of individual fatty acids to treatment differences

SIMPER analysis identified ALA, LIN, EPA, DHA, ɣ-linolenic acid (GLA, 18:3n-6) and linolelaidic acid (LLA, 18:2n-6t) as main contributors to the differences between treatments (SIMPER, Table 2.2, Figure 2.2). In both experiments ALA and GLA contributed in most cases to the differences between treatments, while LIN, EPA, DHA as well as LLA explained the differences in only a few cases. In the first experiment, the entire algae treatment (algae, chironomids and spiders) tended to contain higher levels of ALA than the other treatments (SIMPER, Table 2.2, Figure A.3). For spiders between 39 and 43% of the differences in the PUFA profiles were explained by ALA, for chironomids between 49 and 50% and for the basic food sources between 29 and 38%. The oatmeal treatment differed markedly from the algae and fish food treatments by the GLA content, except once the oatmeal treatments tended to contain more GLA than the other treatments (SIMPER, Table 2.2). In general, GLA explained between 27 and 45% of the differences in the PUFA profiles between the oatmeal and the other treatments. In the second experiment the ALA content tended to be higher in chironomids of the leaves treatment than in the chironomids of the fish food and oatmeal treatment. Furthermore, leaves also tended to contain more ALA than fish food and oatmeal (SIMPER, Table 2.2, Figure A.4). Overall, ALA explained between 23 and 49% of the differences between the treatments. The GLA content of the chironomids and base food sources differed markedly between the oatmeal and leaves as well as fish food treatments and explained between 31 and 49% of the differences in the PUFA profiles (SIMPER, Table 2.2).

Table 2.2: Results of the similarity percentage (SIMPER) analyses performed whenever analysis of similarity (ANOSIM) identified significant differences between treatments. Average is the contribution of polyunsaturated fatty acids (PUFA) to the average between-group dissimilarity, ratio the average to standard deviation ratio, average a the average abundance per first group in contrast and average b the average abundance per second group. Only PUFA with the closest higher cumulative contribution to 0.7 are displayed. EPA: eicosapentaenoic acid (20:5n-3), ALA: α-linolenic acid (18:3n-3), GLA ɣ-linolenic acid (18:3n-6), LLA: linolelaidic acid (18:2n-6t) and LIN: linoleic acid (18:2n-6).

Experiment	Trophic level	Time point	Contrast	PUFA	Average	Standard deviation	Ratio	Average a	Average b	Cumulative contribution
1	spiders	23 days	algae and fish food	ALA	0.27	0.14	1.91	0.54	0	0.42
				GLA	0.24	0.11	2.28	0.09	0.57	0.79
			algae and oatmeal	ALA	0.27	0.14	1.92	0.54	0	0.43
				GLA	0.26	0.09	2.69	0.09	0.6	0.83
			fish food and oatmeal	GLA	0.05	0.03	1.48	0.57	0.6	0.27
				LLA	0.03	0.02	1.55	0.06	0.1	0.46
				EPA	0.03	0.02	1.33	0.14	0.16	0.63
				LIN	0.02	0.02	1.47	0.09	0.05	0.75
		44 days	algae and fish food	ALA	0.24	0.09	2.69	0.58	0.1	0.39
				GLA	0.2	0.02	12.47	0	0.41	0.72
			algae and oatmeal	GLA	0.33	0.07	4.38	0	0.65	0.45
				ALA	0.26	0.09	3.03	0.58	0.06	0.81
			fish food and oatmeal	GLA	0.12	0.08	1.59	0.41	0.65	0.36
				ALA	0.06	0.08	0.81	0.1	0.06	0.55
				LLA	0.06	0.04	1.36	0.2	0.13	0.73
	chironomids	23 days	algae and fish food	ALA	0.38	0.17	2.18	0.97	0.22	0.49
				GLA	0.34	0.16	2.16	0	0.68	0.94
			algae and oatmeal	ALA	0.48	0.02	27.31	0.97	0.01	0.50
				GLA	0.44	0.03	15.61	0	0.89	0.95
			fish food and oatmeal	ALA	0.11	0.17	0.64	0.22	0.01	0.43
				GLA	0.11	0.16	0.68	0.68	0.89	0.83

Table 2.2: continued.

Experiment	Trophic level	Time point	Contrast	PUFA	Average	Standard deviation	Ratio	Average a	Average b	Cumulative contribution
1	chironomids	44 days	algae and fish food	ALA	0.49	0.01	48.61	0.98	0	0.50
				GLA	0.43	0.01	42.46	0	0.86	0.93
			algae and oatmeal	ALA	0.46	0.07	7.06	0.98	0.06	0.50
				GLA	0.42	0.05	7.98	0	0.83	0.94
			fish food and oatmeal	GLA	0.03	0.04	0.76	0.86	0.83	0.31
				ALA	0.03	0.07	0.44	0	0.06	0.58
				EPA	0.02	0	4.23	0.04	0	0.77
	basic food sources	23 days	algae and fish food	ALA	0.29	0.06	4.44	0.64	0.06	0.37
				GLA	0.19	0.08	2.35	0.12	0.5	0.61
				LIN	0.1	0.07	1.35	0.2	0.02	0.73
			algae and oatmeal	GLA	0.31	0.13	2.31	0.12	0.72	0.38
				ALA	0.31	0.03	11.49	0.64	0.02	0.76
			fish food and oatmeal	GLA	0.17	0.05	3.88	0.5	0.72	0.39
				DHA	0.08	0.01	6.23	0.17	0	0.57
				LIN	0.07	0.13	0.57	0.02	0.15	0.74
		44 days	algae and fish food	ALA	0.24	0.11	2.22	0.52	0.07	0.29
				GLA	0.23	0.05	4.86	0.06	0.53	0.58
				LIN	0.15	0.05	3.05	0.35	0.05	0.77
			algae and oatmeal	GLA	0.34	0.15	2.3	0.06	0.72	0.43
				ALA	0.24	0.11	2.19	0.52	0.17	0.73
			fish food and oatmeal	GLA	0.18	0.04	4.54	0.53	0.72	0.39
				ALA	0.1	0.16	0.63	0.07	0.17	0.61
				DHA	0.06	0.03	2.15	0.13	0	0.74

Table 2.2: continued.

Experiment	Trophic level	Time point	Contrast	PUFA	Average	Standard deviation	Ratio	Average a	Average b	Cumulative contribution
2	chironomids	14 days	fish food and leaves	ALA	0.4	0.09	4.57	0.1	0.9	0.49
				GLA	0.35	0.08	4.44	0.71	0	0.92
			fish food and oatmeal	GLA	0.1	0.08	1.22	0.71	0.9	0.45
				EPA	0.05	0.01	8.79	0.1	0	0.67
				ALA	0.05	0.09	0.56	0.1	0	0.90
			leaves and oatmeal	GLA	0.45	0.01	83.97	0	0.9	0.48
				ALA	0.45	0.02	28.95	0.9	0	0.95
		21 days	fish food and leaves	ALA	0.46	0.01	77.11	0	0.92	0.49
				GLA	0.4	0	910.85	0.8	0	0.93
			fish food and oatmeal	GLA	0.07	0.01	7.77	0.8	0.94	0.43
				EPA	0.06	0	24.74	0.12	0	0.83
			leaves and oatmeal	GLA	0.47	0.01	56.68	0	0.94	0.49
				ALA	0.46	0.01	78.92	0.92	0	0.96
	basic food sources	14 days	oatmeal and fish food	GLA	0.17	0.07	2.38	0.69	0.6	0.46
				LLA	0.11	0.09	1.24	0.26	0.25	0.77
			oatmeal and leaves	ALA	0.36	0.03	11.52	0.04	0.77	0.42
				GLA	0.32	0.15	2.13	0.69	0.08	0.78
			fish food and leaves	ALA	0.36	0.03	10.53	0.04	0.77	0.44
				GLA	0.26	0.05	5.6	0.6	0.08	0.75
		21 days	fish food and leaves	ALA	0.28	0.03	8.58	0.16	0.72	0.46
				GLA	0.18	0.04	4.94	0.52	0.16	0.75
			fish food and oatmeal	ALA	0.23	0.17	1.34	0.16	0.45	0.41
				GLA	0.21	0.05	3.98	0.52	0.43	0.81
			leaves and oatmeal	ALA	0.23	0.14	1.56	0.72	0.45	0.43
				GLA	0.21	0.15	1.46	0.16	0.43	0.84

2.4.3 Change in PUFA profiles over time

In the first experiment between time points the PUFA profiles of spiders differed only in the oatmeal treatment (ANOSIM: R = 0.80, p = 0.006). In the second experiment, the spiders' PUFA profiles were similar for all treatments (ANOSIM: 14 days; R = -0.03, p = 0.693; 21 days: R = 0.21, p = 0.075), but differed significantly between time points of the leaves and fish food treatment (Table 2.1, Figure 2.2).

2.4.4 Response of fresh and dry weight, growth rate, body condition, and immune response

We aimed to identify a potential influence of dietary PUFA on the physiology and immune response of spiders. We found an effect of treatment only on fresh weight and body condition in the first experiment. The interaction of treatment and time point was significant (LMM, Table 2.3, Figure 2.3), whereas during the second experiment only time point affected the fresh weight of spiders significantly ($\chi2 = 12.44$, $p = 0.006$).

No significant effects of treatment on the immune response and dry weight in the first and second experiment were found. During both experiments, the time point was significant for growth rate, but no directional trend was visible (LMM: first experiment: $\chi2 = 30.74$, $p < 0.001$; second experiment: $\chi2 = 6.83$, $p = 0.033$, Figure 2.3). Both, the proportional encapsulation area (LM: F = 8.86, $p < 0.001$) and the area of encapsulation (F = 7.33, p = 0.002) were reduced significantly with time in the first experiment (Figure 2.4), but not in the second experiment (Table 2.4). Conversely, dry weight was similar across treatment and time in the first experiment, but was reduced significantly with the time (LM: F = 5.71, p = 0.006) in the second experiment (Table 2.4).

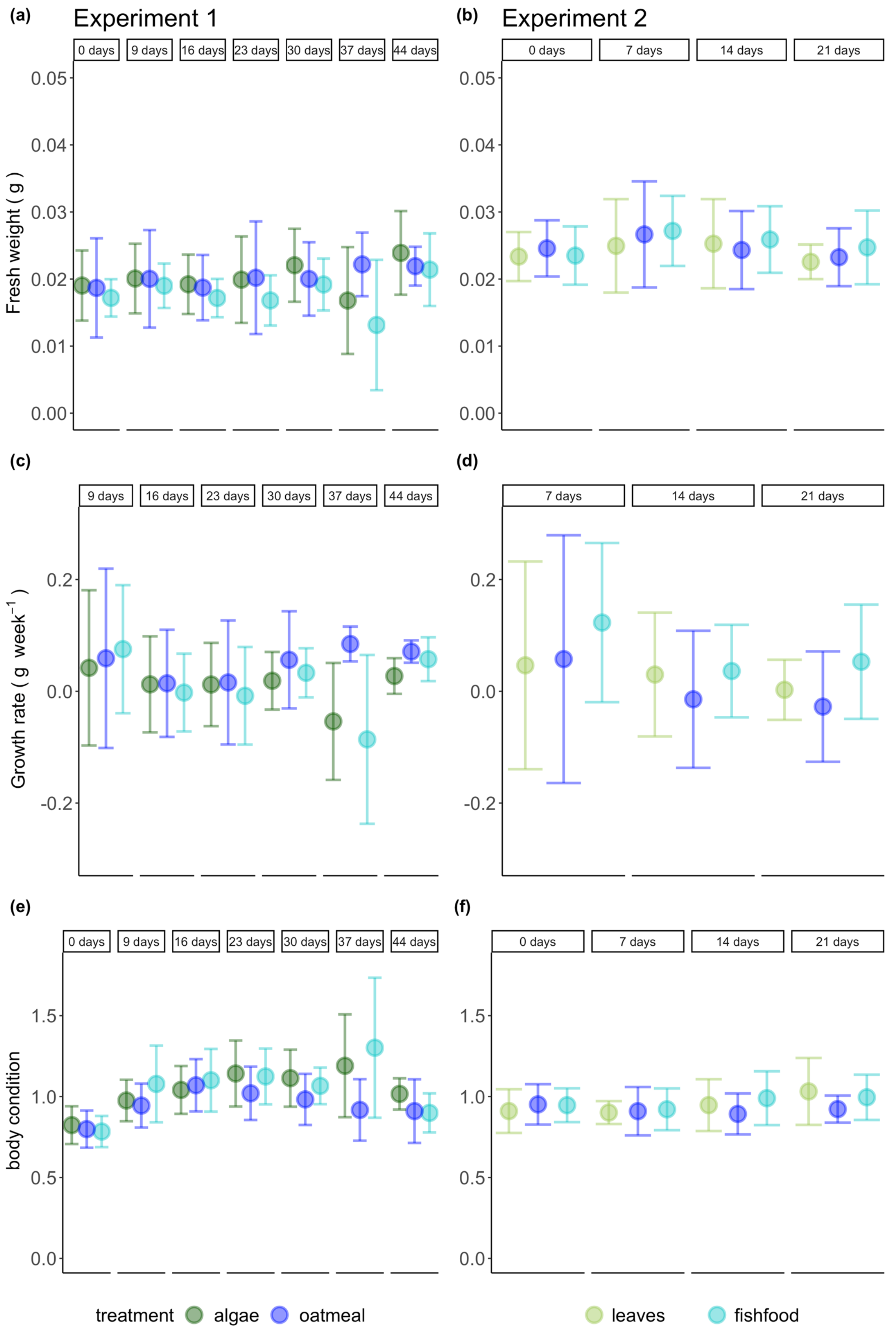


Figure 2.3: Mean and standard deviation of fresh weight, growth rate and body condition of the spiders. (a), (c), (e): First experiment. (b), (d), (f): Second experiment. Colors indicate treatments: The first experiment included algae, fish food and oatmeal as food treatment, whereas the second experiment included leaves, fish food and oatmeal. In the first mesocosm experiment time point was significant for growth rate, time point, and the interaction of treatment and time point for fresh weight and time point, treatment, and their interaction for body condition. In the second experiment time point was significant for fresh weight and growth rate.

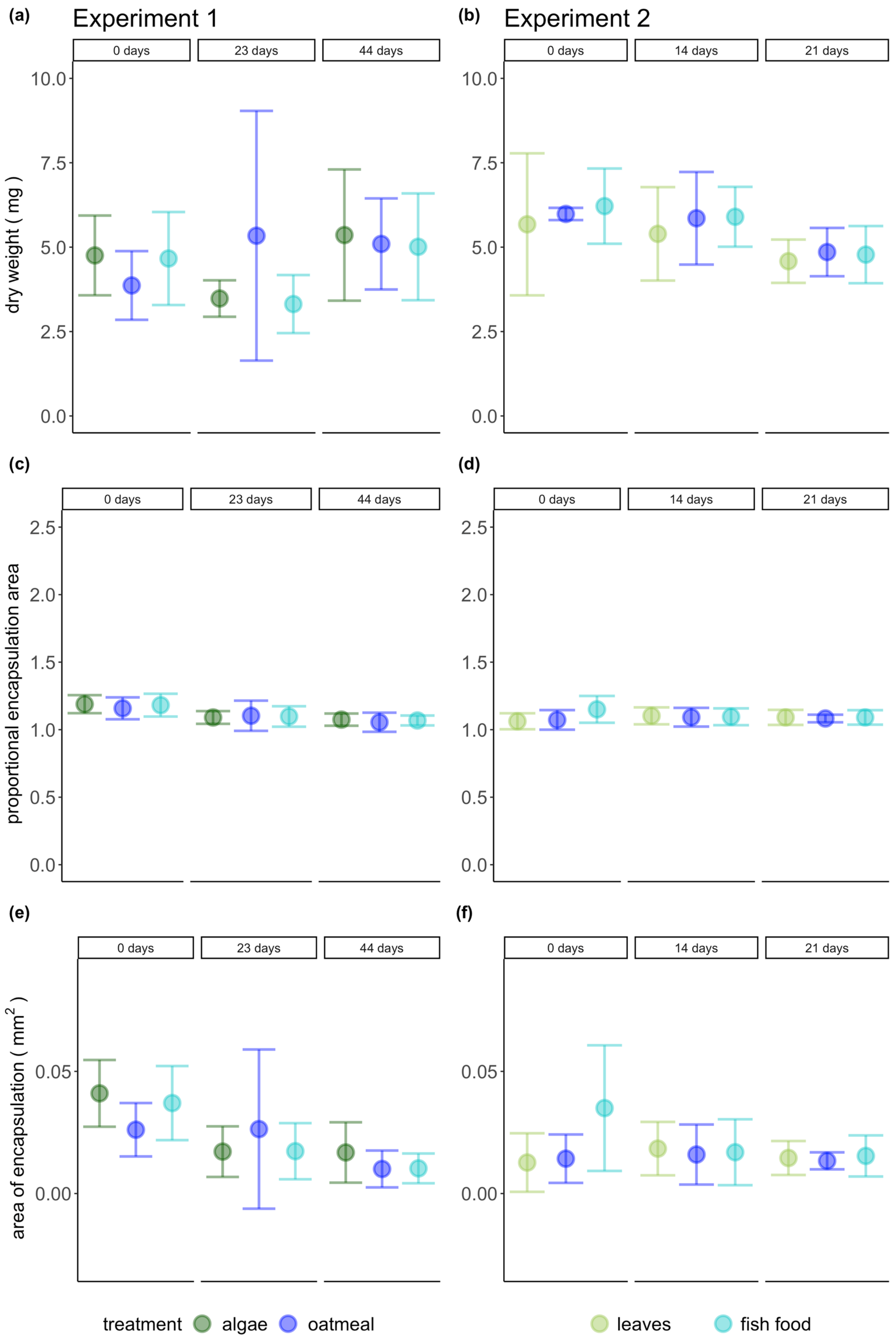


Figure 2.4: Mean and standard deviation of dry weight, proportional encapsulation area and area of encapsulation of the spiders. (a), (c), (e): First experiment. (b), (d), (f): Second experiment. Colors indicate treatments: The first experiment included algae, fish food and oatmeal as food treatment, whereas the second experiment included leaves, fish food and oatmeal. In the first experiment time point was significant for proportional encapsulation area and area of encapsulation. In the second experiment time point was significant for dry weight.

Table 2.3: Effects of the explanatory variables treatment and time point as well as their interaction on the fresh weight, growth rate and body condition of spiders tested with type II analysis of variance (ANOVA) with χ^2-test for the linear mixed models (LMM). Degrees of freedom (df), χ^2 and p-value of the ANOVA for fresh weight, growth rate and body condition of the spiders. Statistical significance is indicated in bold. When the interaction of treatment and time point was not significant, the interaction was not included in the final model.

Experiment	**Response**	**Explanatory variable**	**df**	**χ^2**	**p-value**
1	fresh weight	time point	6	27.66	0.0001
		treatment	2	3.07	0.2157
		treatment:time point	12	28.24	0.0051
	growth rate	time point	5	30.74	<0.0001
		treatment	2	0.38	0.8272
	body condition	time point	6	147.01	<0.0001
		treatment	2	6.95	0.0310
		treatment:time point	12	24.30	0.0185
2	fresh weight	time point	3	12.44	0.0060
		treatment	2	0.50	0.7780
	growth rate	time point	2	6.83	0.0329
		treatment	2	3.84	0.1467
	body condition	time point	3	4.79	0.1878
		treatment	2	1.51	0.4698

Table 2.4: Effects of the explanatory variables treatment and time point on the dry weight, proportional encapsulation area and area of encapsulation of spiders tested with type II analysis of variance (ANOVA) with F-test for the linear models (LM). Degrees of freedom (df), F and p-value of the ANOVA for dry weight and immune response of the spiders. Statistical significance is indicated in bold. The interaction of treatment and time point was not significant. Therefore, the interaction was not included in the final models.

Experiment	**Response**	**Explanatory variable**	**df**	**F**	**p-value**
1	dry weight	time point	2	1.19	0.3159
		treatment	2	0.10	0.9074
	proportional encapsulation area	time point	2	8.86	0.0008
		treatment	2	0.16	0.8547
	area of encapsulation	time point	2	7.33	0.0023
		treatment	2	0.37	0.6906
2	dry weight	time point	2	5.71	0.0056
		treatment	2	0.86	0.4285
	proportional encapsulation area	time point	2	0.11	0.8929
		treatment	2	0.55	0.5778
	area of encapsulation	time point	2	0.78	0.4614
		treatment	2	1.04	0.3614

2.5 Discussion

2.5.1 Change in PUFA profiles along the food chain

We aimed to identify the influence of dietary PUFA on PUFA profiles along a tritrophic food chain with different basic food sources. As expected, we found differences in PUFA profiles between treatments at all trophic levels of the food chain, except for the spiders in the second experiment. Temperature in the second experiment was higher than in the first experiment (Figure A.1) and may explain the absence of the treatment effect. Temperature is known to reduce the PUFA content of aquatic organisms as a response to decrease the fluidity of their cell membranes (Arts & Kohler, 2009; Fuschino et al., 2011).

Furthermore, spiders showed high mortality across the experiments, which suggests that the spiders including those surviving were stressed. The stress may have been caused by differences such as the absence of a forested stream in the microcosms compared to their natural habitats resulting in less humidity and shading as well as higher temperatures. Stress requires to invest more energy for maintenance (Calow & Forbes, 1998; Sokolova et al., 2012), thereby reducing the energy available for energetically costly biosynthesis of PUFA (Mathieu-Resuge et al., 2022; Twining, Bernhardt, et al., 2021; Twining, Parmar, et al., 2021) and other physiological processes like growth (Calow & Forbes, 1998; Sokolova et al., 2012).

In the first experiment, the PUFA profiles of spiders were more similar to the basic food sources than to the chironomids (Figure 2.2). Other factors than dietary PUFA uptake can influence the PUFA profiles of spiders. One example is GLA, which was not found in chironomids fed with algae, but in spiders in the algae-based treatment (Figure A.3). The spiders may have stored GLA in their tissue or synthesized it from its precursor LIN (Horrobin, 1992). The latter has not been shown in spiders, but a recent study suggests that spiders are capable of synthesizing EPA from dietary C18 PUFA precursors (Mathieu-Resuge et al., 2022). Further studies with compound-specific stable carbon and hydrogen isotopes could help to track the trophic transfer of GLA and thereby improve understanding the effects of PUFA on organisms and in turn on food webs (Mathieu-Resuge et al., 2022).

2.5.2 Contribution of individual fatty acids to treatment differences

The PUFA ALA and GLA and the PUFA LIN, EPA, DHA and LLA were major and minor contributors to the differences between treatments, respectively. In contrast, to another study, which found no GLA in oats (Goedkoop et al., 2007), we found on trend higher GLA levels in oatmeal than in fish food, algae, and leaves. Additionally, the chironomids of the oatmeal-based treatment tended also to contain higher GLA levels then the chironomids of the fish food-, algae- and leaves- based treatment. Therefore, GLA levels in chironomids probably reflect the GLA levels of their diet, which is in line with Strandberg et al. (2020). In the first experiment spiders of the oatmeal-based treatment also exhibited higher GLA levels then the algae- and fish food-based treatments. This suggests that the spiders GLA levels reflect their diet, though biosynthesis may also play a role as discussed above for the algae-based treatment.

The algae and conditioned leaves had higher levels of ALA than the basic food sources fish food and oatmeal. This is in line with other studies that found ALA in higher amounts in algae than in fish food and oatmeal (Strandberg et al., 2020; Torres-Ruiz et al., 2010). Furthermore, chironomids that consumed algae and leaves had higher levels of ALA than chironomids fed with oatmeal and fish food. Thus, ALA levels are likely driven by uptake from food sources, which is supported by studies showing that chironomid larvae (Strandberg et al., 2020) and caddisfly larvae (Torres-Ruiz et al., 2010) rely on dietary ALA supply.

Similarly, spiders in the algae-based food treatment displayed higher ALA levels than spiders of the oatmeal- and fish food-based treatments, but the leaves-based food treatment did not show different ALA levels. Therefore, non-dietary factors may affect the ALA levels in spiders. One factor can be the synthesis of EPA from ALA in spiders (Mathieu-Resuge et al., 2022). Another factor can be temperature, which was higher during the second experiment with the leaves treatment (Figure A.1). Temperature is known to affect PUFA profiles of invertebrates, as mentioned above. Specifically, ALA levels were shown to decrease with increasing temperature in *Daphnia magna* (Martin-Creuzburg et al., 2019; Zeis et al., 2019) and terrestrial vertebrates (Hagve et al., 1998; Lund et al., 1999), whereas studies on terrestrial invertebrates, including spiders, are lacking. Additionally, higher temperatures increase the metabolic rate and in turn energy demand of organisms (Brown et al., 2004). As ALA is an energy source for organisms (Tocher, 2003), the spiders may have used ALA to meet their energy demand, thereby decreasing ALA levels.

Anthropogenic stressors such increasing temperatures can have direct effects on the PUFA profiles of organisms, e.g., temperature was shown to reduce long-chain PUFA in algae (Hixson & Arts, 2016) and to cause indirect effects by altering species assemblages (Hixson et al., 2015; Martin-Creuzburg et al., 2017) and in turn the availability of PUFA for consumers. Given the wide occurrence of multiple anthropogenic stressors, a realistic assessment for most environmental conditions requires studies that consider the effects of anthropogenic stressors on PUFA transfer and organisms (Kowarik et al., 2022). This is also important in light of vertebrates with a high conservation status, such as birds and bats, potentially relying on long-chain PUFA in spiders. These aspects need also to be taken into account to estimate PUFA transfer from aquatic to terrestrial ecosystems.

2.5.3 Change in PUFA profiles over time

As expected, we found temporal changes in PUFA profiles of the spiders during the experiments. In both experiments, the spiders showed a high variability in initial PUFA profiles, for example in the second experiment two distinct groups were present (Figure 2.2). This variability may be due to different food sources consumed by the spiders in the field prior to collection. The initial PUFA profiles of the spiders could have affected the PUFA profiles of the spiders during the experiment and thereby the response to treatments (Galloway & Budge, 2020). For example, the initial EPA levels of spiders were similar to the EPA levels later in the experiment. The spiders were collected near streams, so their body may have stored EPA originating from aquatic emergent insects that contain EPA in relatively high amounts (Hixson et al., 2015; Martin-Creuzburg et al., 2017; Moyo et al., 2017). Furthermore, spiders are theoretically capable of synthesizing EPA (Mathieu-Resuge et al., 2022), which may not occur when the spiders retained EPA from their diet prior to collection (Galloway & Budge, 2020). Hence, a sufficiently long experimental duration is required to minimize the effect of the initial PUFA profiles and to detect treatment effects. We addressed this issue by analyzing the PUFA profiles at different timepoints, but the absence of treatment effects on the PUFA profiles of spiders in the second experiment may be explained by an insufficiently long experimental duration owing to the higher mortality.

2.5.4 Response of spiders fresh and dry weight, growth rate and body condition to the basic food treatments

We aimed to identify a potential influence of dietary PUFA on the physiology of spiders. We found a treatment effect only in the first experiment, where the interaction of treatment and time point was significant for fresh weight and body condition of spiders, while no effect on dry weight was found. The significant interaction shows that the effect of the treatment on body condition and fresh weight depends on the time point. The PUFA profiles of spiders differed also significantly between treatments. This is in line with Mayntz and Toft (2001), who showed that PUFA enhanced fresh weight of spiders.

By contrast, in the second experiment, fresh weight as well as dry weight differed between time points but not between treatments, while body condition was similar across treatments and time points. Furthermore, the PUFA profiles of the spiders were similar across treatments in the second experiment. The different response of spiders across the experiments may be explained by the study durations and conditions such as temperature. The first experiment lasted for approximately six weeks, whereas the second microcosm experiment was terminated after three weeks. A longer study duration may have resulted in detectable treatment effects also in the second experiment. Furthermore, the temperature during the second experiment was higher (Figure A.1) and it was shown that with increasing temperature the requirements of organisms for PUFA can be reduced (Masclaux et al., 2009): Growth and reproduction rates decreased in a study on zooplankton with decreasing PUFA quality, though the PUFA effect was reduced at higher temperature and became negligible. Similar results were found for *D. magna*, whose growth generally increased with increasing temperature, but a significant positive effect of PUFA-rich diets on growth was only observed at the lowest temperature of 10 °C (Martin-Creuzburg et al., 2012). In contrast, at higher temperatures a greater PUFA content in *D. magna* may even enhance the vulnerability to heat induced oxidative stress (Zeis et al., 2019), because the double bonds in PUFA are prone to oxidation by reactive oxygen species. Furthermore, the heat tolerance of *D. magna* can be reduced with increasing PUFA uptake (Martin-Creuzburg et al., 2019). Therefore, beneficial effects of dietary PUFA intake for spiders may be counterbalanced by increased oxidative stress and reduced heat tolerance at higher temperatures. Thus, other factors like temperature may have been more important than dietary PUFA intake for growth rate, dry weight, and body condition.

2.5.5 Immune response of spiders to the basic food treatments

Contrary to our expectation, the immune response of spiders was only affected significantly by time point and not by treatment. This is in contrast to a field study, in which the long-chain PUFA EPA and DHA were linked to enhanced immune responses of spiders (Fritz et al., 2017). In our study, food sources of spiders contained only low levels of these long-chain PUFA, which may explain the absence of a treatment effect. Nonetheless, the immune response is affected by many factors not considered in our experiments, which may explain our finding. For instance, the immune response can decrease with reduced food intake (Siva-Jothy & Thompson, 2002) and changes in the dietary composition (Srygley et al., 2009). In our study, spiders received only one food source. In real-world ecosystems, spiders consume and benefit from multiple prey types (Nyffeler, 1999; Uetz et al., 1992). That is because a balanced nutrient composition of prey is more important for the performance, e.g., survival of spiders, than single nutrients (Mayntz & Toft, 2001). Additionally, depending on their hunting strategy, spiders are capable to obtain an optimal nutrient composition through adjusting foraging strategies. For example, mobile spiders like ground hunters are capable to choose prey actively (Mayntz et al., 2005). Furthermore, population density (Schmid-Hempel, 2005; Wilson & Cotter, 2008), environmental factors (Adamo, 2012; Wojda, 2017) and anthropogenic stressors (Mangahas et al., 2019) can affect the immune response. Future studies that also consider other factors like nutrient availability and anthropogenic stressors can help to estimate the importance of PUFA for immune response in relation to other factors.

2.6 2.6 Conclusions

PUFA can affect the weight and body condition of spiders, where this depends on the environmental context. This context includes, amongst others, diverse food sources, i.e., several prey types, food chains with interactions between taxa, long duration for PUFA assimilation, a range of environmental factors (e.g., temperature) and anthropogenic stressors.

Furthermore, the PUFA profiles can differ across trophic levels for multiple food sources. Aquatic and semi-aquatic food sources may result in more distinct PUFA profiles of chironomids and spiders than terrestrial food sources. ALA and GLA are among the major contributors to these differences in PUFA between food sources. However, environmental factors like temperature also influence PUFA profiles. Future studies under more realistic conditions are needed to improve our understanding of the effect of PUFA in ecosystems and to evaluate the transferability of our results.

2.7 2.7 Acknowledgments

We thank Tim Ostertag, Moritz Schäfer, Lukas Reinhard and Laura Kieffer for assistance during the experiments and Kilian Kenngott for technical support during the PUFA analysis. This study was supported by the German Research Foundation (DFG, Project Number 326210499/GRK 2360). Katharina Ohler was funded by the German Academic Scholarship Foundation and the Interdisziplinäres Promotions- und Postdoczentrum (IPZ) completion scholarship.

2.8 2.8 References

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3. Land use changes biomass and temporal patterns of insect cross-ecosystem flows

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This chapter is adapted from the article published in 2023 in Global Change Biology, 29, 81–96. https://doi.org/10.1111/gcb.16462

3.1 Abstract

Emergent aquatic insects constitute an important food source for higher trophic levels, linking aquatic to terrestrial ecosystems. Little is known about how land use affects the biomass or composition of insect emergence. Previous studies are limited to individual time points or seasons, hampering understanding of annual biomass export patterns and detection of phenological changes. Over one year's primary emergence period, we continuously determined the biomass, abundance, and identity of >45,000 aquatic insects and recorded land-use-related environmental variables in 20 stream sites using a paired design with upstream forested sites and downstream agricultural sites. Total insect biomass and abundance were 2 – 7 mg d^{-1} m^{-2} and 7 – 36 ind d^{-1} m^{-2} higher in agricultural than forested sites. However, we found turnover of families between forested and agricultural sites, with more insects with shorter generation time in agriculture, indicating lower sensitivity to land-use-related stress because of higher recovery potential. Except for stoneflies, biomass and abundance of major orders were higher in agriculture, but their phenology differed. For different orders, emergence peaked 30 days earlier to 51 days later in agriculture than forest, whereas total abundance and biomass both peaked earlier in agriculture: 3 – 5 days and 3 – 19 days, respectively. The most important land-use-related drivers were pesticide toxicity and electrical conductivity, which were differentially associated with different aquatic insect order abundances and biomass. Overall, we found that land use was related to changes in composition and phenology of aquatic insect emergence, which is likely to affect food-web dynamics in a cross-ecosystem context.

3.2 Introduction

Intensive agriculture is a globally dominant land use (Václavík et al., 2013). The intensification of agriculture is accompanied by increased pesticide and nutrient use, as well as habitat degradation (Fischer et al., 2012; Stoate et al., 2001). Stream ecosystems in agricultural areas are affected by toxicants, excessive nutrient inputs, and habitat degradation, e.g., the loss of riparian vegetation and channelization, which threatens biodiversity and human water security (Collen et al., 2014; Dudgeon et al., 2006; Vörösmarty et al., 2010).

Stream ecosystems and their adjacent terrestrial ecosystems are tightly linked via the flux of matter and organisms (Baxter et al., 2005). Aquatic insects like Diptera (flies), Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) emerge from water bodies to live as adults in terrestrial ecosystems, where they serve as prey for riparian predators, such as spiders, bats, and birds (Gray, 1993; Kato et al., 2004; Sullivan et al., 1993). Only < 1 – 3% of these insects return to streams (Gray, 1989; Jackson & Fisher, 1986). Especially in agricultural regions, riparian predators consuming a broad range of prey including terrestrial insects can benefit from aquatic insects (Graf et al., 2020; Krell et al., 2015; Murakami & Nakano, 2002; Nakano & Murakami, 2001; Stenroth et al., 2015) because agriculture is associated with the loss of terrestrial insects (Ewald et al., 2015; Hallmann et al., 2017; Seibold et al., 2019; Shortall et al., 2009). Insect decline can adversely affect ecosystems and their services because insects play an important role in food webs and provide ecosystem services like pollination and pest control Kawahara et al., 2021; Raitif et al., 2019; Wagner, 2020).

Riparian predators are dependent on the nutritional quality, biomass, and composition of aquatic insects. Aquatic insects constitute a high-quality food because they contain up to ten times higher levels of long-chain polyunsaturated fatty acids (PUFA) than terrestrial insects (Hixson et al., 2015), which promote growth and immune function (Fritz et al., 2017; Twining et al., 2016). The choice of prey depends amongst others on taxon-specific traits, including aquatic insect species size (Davis et al., 2011; Stenroth et al., 2015). Typically, predators consume prey of one to four orders of magnitude smaller than themselves (Cohen et al., 2003; Jonsson et al., 2005; Woodward & Hildrew, 2002). Thus, the biomass of major insect orders indicates which predators may benefit most from their emergence.

Emergence of different aquatic insect species varies over time and exhibits seasonal patterns (Nakano & Murakami, 2001), which affects population dynamics in the linked terrestrial system. The timing of emergence controls the growth rate (Sato et al., 2016;

Uno, 2016), population biomass, and maturity rate of predators (Sato et al., 2016). In turn, emergence timing depends on the composition of aquatic insects because individual species exhibit differences in phenology. Complementary phenology between species contributes to temporal stability of emergence (Uno & Pneh, 2020), improving foraging opportunities for predators (Armstrong et al., 2016).

Furthermore, the trait distribution in insect assemblages can indicate responses to land use (Berger et al., 2018; Mondy et al., 2012). For instance, a short generation time translates to faster recovery and, therefore, insect assemblages with a high fraction of taxa with a short generation time may be associated with recent stress events in a habitat patch (Sherratt et al., 1999; Stark et al., 2004) also in agricultural streams (Liess & von der Ohe, 2005). Better understanding the links between land use, such as intensive agriculture, and distributions of functional traits in aquatic insect assemblages may improve mechanistic understanding of species-environment relationships (Kearney & Porter, 2009).

Studies investigating how agriculture influences the phenology, composition, and biomass of emergent aquatic insects are scarce. The major orders of emergent aquatic insects, mayflies, stoneflies, and caddisflies (EPT) are among the least studied taxa, whereas flies, mostly mosquitos, have been studied more often (InsectGapMap, 2020). Limited research, however, has shown changes in aquatic insect assemblages linked to agriculture. A recent meta-analysis suggested an increasing trend in total biomass and abundance of aquatic insects over the last decades, particularly in agricultural regions (van Klink et al., 2020), though contrasting temporal trends have been reported for several aquatic insect orders (Baranov et al., 2020; Outhwaite et al., 2020; Stepanian et al., 2020), and total abundance and biomass are poor indicators of compositional changes (Desquilbet et al., 2020; Jähnig et al., 2021). However, agriculture can alter aquatic insect assemblage composition, for instance, by favoring small-bodied insects like flies (Carlson et al., 2016; Krell et al., 2015; Stenroth et al., 2015) and shorter life cycle because their high reproduction rate can make them less prone to agricultural stress (Larsen & Ormerod, 2010; Liess & von der Ohe, 2005). In addition, agricultural pesticide use has been associated with the loss of up to 42% of aquatic invertebrate richness (including insects; Beketov et al., 2013).

Notwithstanding, agriculture can increase the biomass of aquatic insects because it is associated with elevated nutrient concentrations and reduced shading. Both can increase primary production and, in turn, food sources for aquatic insects in agricultural streams (Carlson et al., 2016; Griffiths et al., 2013; Stenroth et al., 2015; Terui et al., 2018). Furthermore, channelization typically results in increased proportion of pool habitats (Petersen Jr, 1992), where the total abundance of aquatic insects can be lower than in riffle habitats, while the abundance of single insect orders can respond differently (Carlson et al., 2013; McKie et al., 2018). Recent studies on the effects of land use on aquatic insects were limited to few weeks (Graf et al., 2020; Krell et al., 2015; Stenroth et al., 2015) and random snap-shot samples in different seasons (Carlson et al., 2016; Raitif et al., 2018). Given the high temporal variability in emergence, this limited data hampered the estimation of complete balances of biomass transfer to riparian ecosystems.

In this study we therefore continuously collected data over the primary emergence period within one year (from March until September 2018; Corbet, 1964) to assess differences in the biomass, abundance, and composition of aquatic insects between paired forested and agricultural sites, as well as phenological patterns in each of these factors. In addition, we monitored hydromorphological structure and water quality to assess the associations of specific environmental variables with aquatic insect assemblages.

With this data we (I) compared total biomass (IA) and abundance (IB), as well as aquatic insect phenology, between agricultural and forested sites, (II) studied the taxonomic and trait composition of aquatic insect assemblages, as well as their turnover, associated with land-use types, (III) identified land-use-related drivers associated with biomass, abundance, and composition of assemblages.

3.3 Material and Methods

3.3.1 Study sites

We studied 20 sites in ten parallel streams, which were a maximum of 50 km apart, in southwestern Germany from March to September 2018. The study period covered the primary emergence period of one year (Corbet, 1964). We used a paired design, where each stream had an upstream forested site and a downstream site where agricultural land use dominated (Figure B1). The mean (range) distance between sites within a stream was 5.5 (1.6 – 14) km. Sites were preselected with GIS analysis to ensure similar catchment size, discharge, and proportion of agriculture at downstream sites. Moreover, all sites were selected to be largely free from the influence of point-source pollution from wastewater treatment plants and industrial facilities. The preselected sites were then surveyed in person to ensure that all sampling devices could be deployed in the streams.

Although agricultural land use mainly consisted of viticulture, a previous study in this region showed that environmental parameters and ecosystem function (i.e., organic matter decomposition) were similar between viticultural sites and sites with other agricultural crops (e.g., corn, wheat; Voß et al., 2015).

All study sites were similar in stream size. The mean (range) depth and maximum width of the streams in forested sites were 0.11 (0.03 – 26) and 1.83 (0.68 – 4.00) m, respectively. In agricultural sites, the mean depth was 0.20 (0.04 – 0.49) m and the maximum mean width 1.98 (0.60 – 4.50) m. All streams were mostly small and fine substrate-dominated siliceous highland streams originating in the Palatinate Forest Nature Park, a forested low mountain range. Therefore, climate and geology were similar across streams. Forested streams exhibited fewer pool habitats (27 [10 – 80]%) than agricultural streams (44 [10 – 100]%) and more shading (73 [30 – 100]%) than streams in agriculture (62 [5 – 95]%)

3.3.2 Land-use-related drivers of aquatic insects

To identify land-use-related drivers associated with total abundance, total biomass, as well as abundance and biomass of major orders of emergent aquatic insects and for the number of EPT families, we recorded physicochemical variables as single measurements every three weeks over the entire study period (22nd March – 13th September 2018). These variables included water temperature, electrical conductivity (EC), oxygen saturation, phosphate, and nitrate concentration (Table 3.1). We recorded hydromorphological structure, including percentage of shading and percentage of pool habitat, in March, July, and August to capture changes throughout the study period (Table 3.1). These variables were chosen *a priori* because they are known to affect aquatic insect biomass and abundance (Graf et al., 2020; Raitif et al., 2018; Stenroth et al., 2015).

We also collected data on pesticide toxicity. In streams, pesticides were sampled every three weeks (grab samples) and during heavy rainfall events (event samples). Event samples were taken by automated samplers (MAXX TP5, Rangendingen, Germany and glass bottle samplers (Liess & von der Ohe, 2005), when the water level rose more than 5 cm. Further details on pesticide sampling, analytics, and exposure are provided in Halbach et al. (2021) and Liess et al. (2021). Additionally, the pesticide concentration on suspended particles was analyzed in event samples because pyrethroids regularly enter streams bound on particles (Gan et al., 2005). Furthermore, pyrethroids exhibit high relative toxicity for aquatic insects (Rico & Brink, 2015; Rubach et al., 2010).

The particle-associated concentration was converted into an estimate of the bioavailable concentration in water c_d following Schäfer et al. (2011) and Toro et al. (1991) using the equation:

$$c_d = \frac{c_{tot}}{f_{oc} \cdot k_{oc} + 1} \quad (3.1)$$

where c_{tot} is the total concentration on the suspended particles, f_{oc} is the fraction of organic carbon in the sample, and k_{oc} is the soil organic carbon-water partitioning coefficient. The k_{oc} values were taken from the Pesticide Property Data Base (PPDB; Lewis et al., 2016) or the PubChem database (Table B.1, National Center for Biotechnology Information, 2021).

The toxicity of single pesticides at different concentrations was calculated using toxic units (TU):

$$TU = \frac{c_i}{EC_{50i}} \quad (3.2)$$

where c_i is the concentration of the single pesticide, and EC_{50i} is the acute effect concentration of the single pesticide towards the most sensitive freshwater invertebrate species. The toxicity of the detected pesticide mixtures was estimated using the logarithmic sum of the TU (sumTU, Schäfer et al., 2013):

$$sumTU = \log \sum_{i=1}^{n} TU_i \quad (3.3)$$

where n is the number of pesticides, and TU_i is the TU of the single pesticide. This measure has proven successful in linking toxicity and community response (Schäfer et al., 2013). The EC_{50} values were compiled from the ECOTOX database (US EPA, 2021) using the R package Standartox (version 0.0.1, Scharmüller et al., 2020). In case of missing EC_{50} values in Standartox, the values were retrieved from the PPDB (Lewis et al., 2016) or Malaj et al. (2014) (Table B.2). To estimate the maximum pesticide pollution, which may be responsible for the strongest ecological response, we used the maximum sumTU (hereafter, pesticide toxicity) of all samplings per site and season in the analysis. MaximumTU, which is the TU of the most toxic substance in a pesticide mixture, was similar to maximum sumTU (Figure B.2).

Table 3.1: Mean and range of land-use-related drivers of aquatic insects used in the data analysis. All drivers recorded during the experiment are reported in Table B.12.

Land-use-related driver (unit)	Forest: mean (range)	Agriculture: mean (range)
Electrical conductivity (µS cm^{-1})[1]	191.8 (92.6–381)	462.2 (178.8–1899)
Nitrate (mg L^{-1})[2]	1.8 (0.5–5.1)	2.1 (1–5.3)
Oxygen saturation (%)[1]	96.6 (65.6–126.8)	91.9 (63.9–108.5)
Pesticide toxicity (maximum sumTU)[3]	-5.4 (-7.0–(-0.5))	-1.1 (-3.4–0.6)
Phosphate (mg L^{-1})[2]	0.7 (0.2–1.8)	0.7 (0.2–1.5)
Water temperature(°C)[1]	12.6 (9.4–16.6)	14.6 (11.4–18.2)
Pools (%)	27 (10–80)	44.2 (10–100)
Shading (%)	73 (30–100)	61.8 (5–95)

[1] Multi 340i, WTW Germany, was used for measurement.

[2] Compact-photometer PF-12 with visocolor, Macherey-Nagel, was used for measurement.

[3] Maximum of logarithmic sum of toxic units for the most sensitive freshwater invertebrate species

3.3.3 Insect sampling

Aquatic insects were sampled continuously with emergence traps (basal area 0.25 m^2) as described in Cadmus et al. (2016). At every site, two traps, each with a bottle trap without any solution, were installed in the middle of the stream, covering riffle and pool habitats. The traps were emptied twice per week by exchanging the bottle trap, which was transported on ice until the insects were euthanized in liquid nitrogen and identified in the laboratory. Insects were identified to at least family level under a stereo microscope using the following keys: Bährmann & Müller, (2015), Nilsson, (1996b, 1996a), Schäfer & Brohmer, (2010). Then they were lyophilized to complete dryness and weighed to the nearest 0.1 µg. Heavy rainfall events and vandalism led to 51 traps lost in forest and 112 traps lost in agriculture (Table B.3). On the whole, 45,831 specimens were identified from 1847 samples collected, 943 in forested sites and 904 in agricultural sites, which corresponds to a difference of 4%. The loss of traps resulted in unequal numbers of samples per site and sampling date. Furthermore, the number of collection days differed occasionally between samples. Thus, the abundance and biomass of every sample were standardized per area of the trap and the number of collection days.

3.3.4 Data analysis

To identify seasonal patterns of biomass and abundance of aquatic insects, hierarchical generalized additive models (HGAM) were used following the approach of Pedersen et al., (2019). The data per sample were used for total biomass as well as abundance as response, the data per sample and order were used for the biomass and abundance at order level as response and the data per sample and family were used for the biomass and abundance at family level as response. If samples were missing, the mean per month and site was used to correct for missing samples. Briefly, the data were modelled with group-level smoothers for land use, land use crossed with order, as well as land use crossed with family, but without a global smoother (i.e., each group can be differently shaped without restriction). HGAMs fitted with group-level smoothers having the same wiggliness (model S) yielded lower Bayesian Information Criterion (BIC, Table B.4) than HGAMs with different wiggliness for each group-level (model I). The latter incorporated one smoothing parameter for every group level, whereas model S included one smoothing parameter for all group levels. Stream was included as a random effect smoother. The R-package mgcv (version 1.8-36, Wood, 2011) was used to fit HGAMs. To quantify the effect of land use, the difference between the mean fit (mean at one time point) for agriculture and forest was calculated, along with the 95% confidence interval. Non-overlapping confidence intervals were considered statistically significant at alpha = 0.05. The average export per area in both land-use types of total biomass and abundance over the whole sampling period was estimated from the mean fits of total abundance and total biomass HGAMs.

To identify the environmental variables that explained the most variation between land-use types in the biomass and abundance of aquatic insects in total and per order, and for the number of EPT families, (generalized) linear mixed-effects models ((G)LMM) were used. LMMs were used for the biomass as well as abundance data, while a GLMM with Poisson distribution was applied for the number of EPT families. EPT families are sensitive towards land-use-related drivers (Raitif et al., 2018; Stenroth et al., 2015), therefore the number of EPT families was used as an indicator of ecological quality. Given that environmental variables had lower temporal resolution than aquatic insect data, the sample size was too low to fit HGAMs. To account for the mismatch in temporal resolution, the mean per season and site was calculated for abundance, biomass, and potential land-use-related drivers. Additionally, biomass and abundance data were log-transformed to improve normality, which was checked visually using Q-Q plots, by (ln [x + a]), where a represents the minimum value > 0 detectable for this variable. The number of EPT families was summed per site and season. Stream and season were incorporated as random effects. The land-use-related drivers toxicity, pools, EC, shading, oxygen saturation, and concentrations of nitrate and phosphate were standardized and used as fixed factors in the GLMMs. Variable selection was performed using the L1-penalty to identify the best-fit model with the R-package glmmLasso (version 1.5.1; Groll & Tutz, 2014). The L1-penalty conducts variable selection and shrinkage concurrently. This method is appropriate for our data because environmental variables were not collinear and the shrinkage reduces variance in predictions. The variance explained by the fixed factors (R^2marginal), the complete model (R^2conditional), and the random factors (adjusted intra-class correlation; ICC stream and season) were estimated (Nakagawa et al., 2017; Nakagawa & Schielzeth, 2013). To compare the number of all families and EPT families between forested and agricultural sites, a GLMM using land-use type as a fixed factor and stream and season as random factors was fitted with the R-package glmmTMB (version 1.1.2.3; Brooks et al., 2017).

To estimate the turnover of aquatic insects, we used the Jaccard index (JI) on the presence-absence data of families per site, which is commonly used to assess compositional similarities of assemblages (Chao et al., 2005). JI calculates the proportion of taxa two sites share:

$$\mathrm{JI}=\frac{j}{a+b-j} \quad (3.4)$$

where j is the number of taxa found in both sites A and B, and a and b are the total number of taxa in site A and B, respectively. If both sites do not share any taxon, JI = 0, and if both sites share identical taxa, JI = 1. The differences in JI between forested and agricultural sites were analyzed with analysis of similarity (ANOSIM; 999 of permutations), which is a fully non-parametric test and robust regarding paired samples (Clarke, 1993). The results of the ANOSIM were visualized with non-metric multidimensional scaling (JI, four dimensions which resulted in optimal stress values).

Finally, the traits generation time and size of aquatic insects were compared between forested and agricultural sites with a paired t-test. The normal distribution of differences between paired samples was checked visually with Q-Q plots and for outliers with violin plots. Families of aquatic insects with a generation time ≥ 0.5 year^{-1} were classified as sensitive because of their slow recovery potential (Sherratt et al., 1999; Stark et al., 2004). Generation time was extracted from the Indicate trait database (Department System Ecotoxicology-Helmholtz Centre for Environmental Research, UFZ, 2021). The size of aquatic insects, retrieved from Tachet et al. (2010), is an indicator of which predators may benefit most from aquatic insects present for additional food source because of their preference for prey of up to three magnitudes smaller than themselves

(Cohen et al., 2003). Families were assigned to three size classes: small (0.25 cm < size ≤ 1.0 cm), medium (1.0 cm < size ≤ 2.0 cm) and large (2.0 cm < size ≤ 8.0 cm). For both traits, the ratio of biomass per size or generation time was calculated because biomass is important to understand energy flow, productivity, and food-web dynamics (Brown et al., 2004). All statistical analyses were performed with the statistical software R (version 4.0.2; R Core Team, 2020) and the code and all data are available on a Github repository: https://doi.org/10.5281/zenodo.7123464 (Ohler et al., 2022).

3.4 Results

3.4.1 Higher total biomass in agriculture than forest

During our study period, more biomass was exported per area in agricultural sites than in forest sites (95% confidence interval: 0.106 – 0.124 and 0.066 – 0.074 kg m^{-2}, respectively). The temporal pattern in total emerging biomass also differed between agricultural and forest sites (hierarchical generalized additive model: HGAM, Table B.4, B.5). For example, the highest biomass in summer (17th May – 26th July) was reached 20 days earlier in agriculture than in forest. At the beginning of spring (22nd March – 16th May), total biomass in agricultural sites was 1 mg d^{-1} m^{-2} higher than in the forest sites (Figure 3.1a, B.3a, b). This difference was significant (i.e., non-overlapping 95% confidence intervals at alpha = 0.05) and increased to 7 mg d^{-1} m^{-2} until mid-spring where the maximum in both land-use types occurred: 13 (± 6) mg d^{-1} m^{-2} in agricultural sites (mean fit HGAM ± 2 standard errors) and 6 (± 3) mg d^{-1} m^{-2} in forest sites. Total biomass was similar at the beginning of summer (~5 mg d^{-1} m^{-2}) and in autumn (27th July – 13th September, ~3 mg d^{-1} m^{-2}), with a period in between where at agricultural sites biomass was significantly higher (difference from 3 – 4 mg d^{-1} m^{-2}).

3.4.2 Higher total abundance in agriculture than forest

Nearly twice as many individuals emerged in agricultural than in forest sites (95% confidence interval: 431,383 – 510,017 and 241,499 – 274,701 ind m^{-2}, respectively). The temporal patterns of total abundance differed between land-use types (HGAM, Table B.4, B.5, Figure 3.1b, B.3c, d), but were more similar than for total biomass. The mean fit of total abundance was significantly different between agriculture and forest from spring to the end of summer (range of difference: 7 to 36 ind d^{-1} m^{-2}) and, as with total biomass, converged to a similar pattern in autumn. During spring, the peak in forest total abundance was more pronounced than in forest total biomass, but at 24 (± 9) ind^{-1} m^{-2} it was only approximately one third as high as in agriculture

(59 [± 21] ind d^{-1} m^{-2}). During summer, the difference between agriculture and forest was smaller, ranging from 9 to 17 ind d^{-1} m^{-2}.

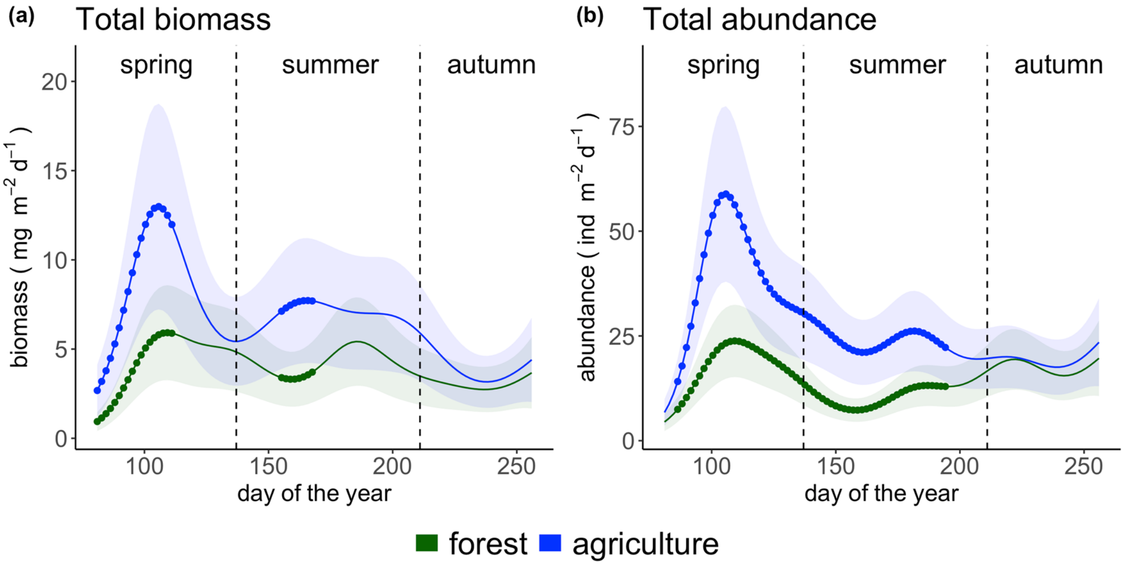

Figure 3.1: Predicted seasonal patterns (spring: 22nd March – 16th May, summer: 17th May – 26th July, autumn: 27th July–13 September) of aquatic insects derived with hierarchical generalized additive models (HGAM) fitted with 1911 observations. The solid line shows the predicted mean fit values of the HGAM, and the ribbon indicates ± 2 standard errors around the mean fit. Dots mark significant differences with non-overlapping 95% confidence intervals at alpha = 0.05 between agriculture and forest. Green represents the pattern in forest and blue in agriculture for (a) total biomass and (b) total abundance.

3.4.3 Turnover and temporal patterns in composition

The number of all families (generalized linear mixed-effects models; GLMM, p = 0.12) and EPT families (GLMM, p = 0.14) were similar between forested and agricultural sites (Table B.6, Figure B.4), but a turnover in the composition of insect families from forest to agriculture was found (analysis of similarity: ANOSIM, *R* = 0.31, p = 0.001, Figure B.5). A moderate increase close to statistical significance of families with a shorter generation time (paired *t*-test, p = 0.08, Cohen's *d* = 0.62, Table B.7, Figure B.6) was observed in agricultural sites, but there were no size differences observed between land-use types (Table B.7, Figure B.7).

The biomass and abundance of all major orders of emergent aquatic insects, i.e., flies, mayflies, stoneflies, and caddisflies, exhibited significant differences between land-use types in spring and summer. Stonefly and mayfly biomass and abundance, as well as caddisfly abundance, also differed between land-use types in autumn (Figure 3.2, 3.3), though the difference for stoneflies was much smaller than for the other orders. Except for stoneflies, biomass and abundance were both higher in agricultural sites.

The temporal patterns of fly abundance were similar in both land-use types (Figure 3.3a) but differed for biomass (Figure 3.2a). Fly biomass peaked during spring at 8 (± 2) mg d^{-1} m^{-2} in agriculture before declining drastically for the remainder of the study, whereas biomass reached a maximum at 2 (± 1) mg d^{-1} m^{-2} in forest sites, then declined slightly until mid-summer. During summer, fly biomass peaked 30 days earlier in agriculture than in forest (Table B.8). Over the whole sampling period, the same number of fly families was found in forested and agricultural sites, but their phenology differed between land-use types (Figure B.8, B.9; Table B.9). For instance, the biomass maximum of drain flies (Psychodidae) occurred 76 days earlier in agriculture than in forest (Table B.9).

The biomass of mayflies peaked in spring at the same time between land uses, with 5 (± 2) and 2 (± 1) mg d^{-1} m^{-2} in agriculture and forest, respectively (Figure 3.2b). A second peak of mayfly biomass occurred 20 days after the first peak in forest (Table B.8), whereas mayfly biomass in agriculture declined over the rest of the study period. In contrast, mayfly abundance showed a maximum plateau at 1 (±1) ind d^{-1} m^{-2} from spring to early summer in forest, whereas in agriculture biomass and abundance patterns were similar (Figure 3.3b). Flatheaded mayflies (Arthropleidae) and primitive minnow mayflies (Siphlonuridae) were absent from agricultural sites. The phenology of mayfly families between land-use types was otherwise similar except for the biomass of spiny crawler mayflies (Ephemerellidae; Figure B10, B.11).

Stonefly biomass and abundance peaked during spring in forest, whereas nearly no stoneflies emerged in agriculture (Figure 3.2c, 3.3c). All stonefly families peaked in spring. Needle flies (Leuctridae) and green stoneflies (Chloroperlidae) were absent from agricultural sites (Figure B.12, B.13).

The biomass pattern of caddisflies was similar over time in both land-use types (Figure 3.2d). The caddisfly biomass reached a maximum of 6 (± 2) mg d^{-1} m^{-2} in agriculture and peaked ten days later at 4 (± 2) mg d^{-1} m^{-2} in forest (Table B.8). By contrast, caddisfly abundance peaks exhibited a greater temporal shift with earlier emergence in forest. During summer, forest abundance peaked at 1 (± 1) ind d^{-1} m^{-2} and peaked 51 days later in agriculture at 1 (± 1) ind d^{-1} m^{-2} (Figure 3.3d, Table B.8). In forested sites no lepidostomatid case makers (Lepidostomatidae) were sampled, whereas in agricultural sites five caddisfly families were lacking (Figure B.14, B.15). Abundance patterns were similar for caddisfly families between land-use types, although biomass patterns differed. For example, net-spinning caddisflies (Hydropsychidae) biomass peaked during summer in forest, whereas they exhibited three overlapping peaks in agriculture (Figure B.14a, Table B.4, B.9). More information on families can be found in the Supplementary text: Results family biomass and abundance.

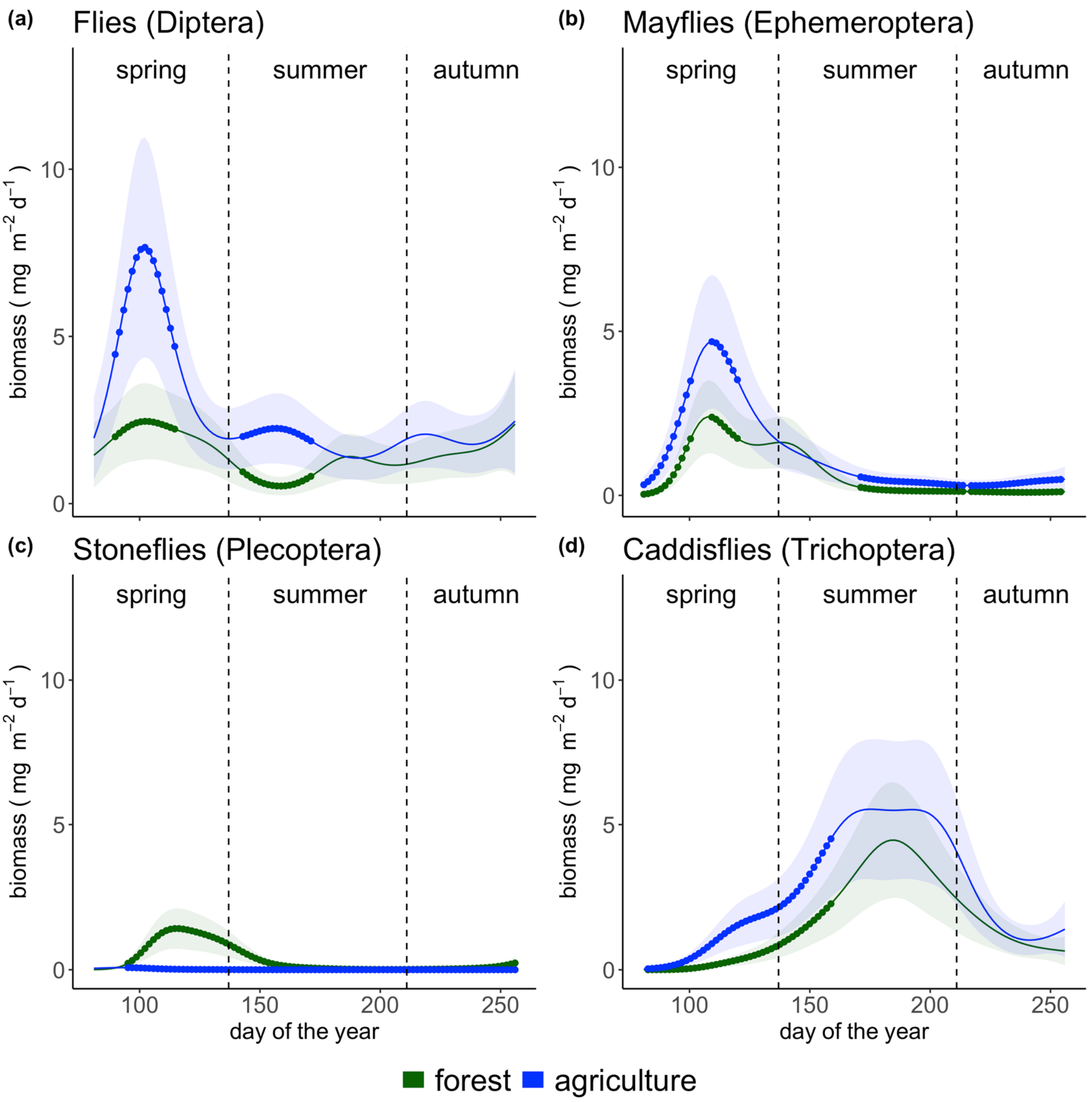

Figure 3.2: Predicted seasonal patterns (spring: 22nd March – 16th May, summer: 17th May – 26th July, autumn: 27th July – 13th September) of aquatic insect order biomass derived with hierarchical generalized additive models (HGAM) fitted with 7644 observations. The solid line shows the predicted mean fit values of the HGAM, and the ribbon indicates ± 2 standard errors around the mean fit. Dots mark significant differences with non-overlapping 95% confidence intervals at alpha = 0.05 between agriculture and forest. Green represents the biomass pattern in forest and blue in agriculture for (a) flies, (b) mayflies, (c) stoneflies, and (d) caddisflies.

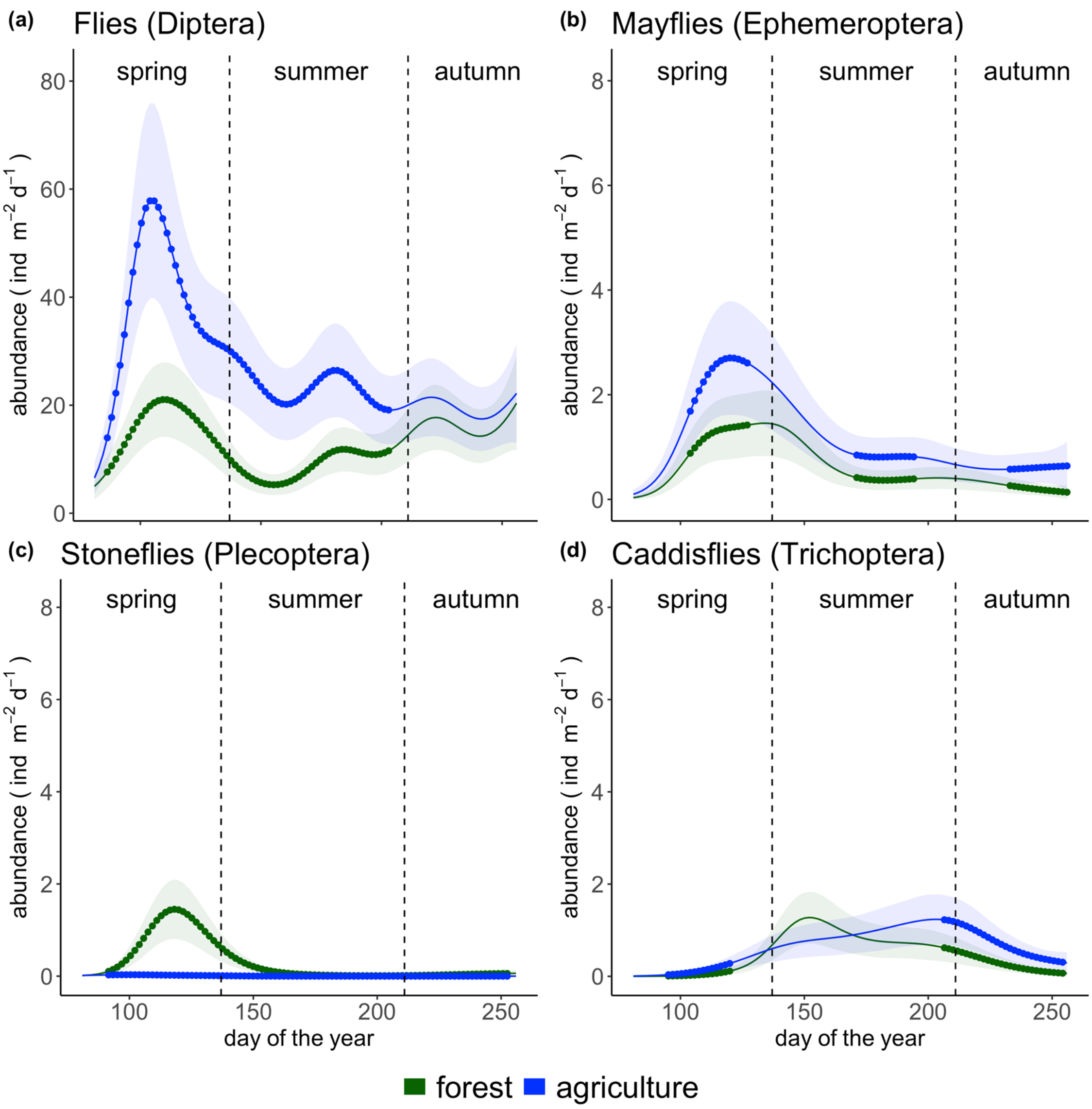

Figure 3.3: Predicted seasonal patterns (spring: 22 March – 16 May, summer: 17 May – 26 July, autumn: 27 July – 13 September) of aquatic insect order abundances derived with hierarchical generalized additive models (HGAM) fitted with 7644 observations. The solid line shows the predicted mean fit values of the HGAM, and the ribbon indicates ± 2 standard errors around the mean fit. Dots mark significant differences with non-overlapping 95% confidence intervals at alpha = 0.05 between agriculture and forest. Green represents the abundance pattern in forest and blue in agriculture for (a) flies, (b) mayflies, (c) stoneflies, and (d) caddisflies. Note that the y-axis scales vary by a factor of ten: (a) 0 – 80 ind d^{-1} m^{-2}, (b) – (d) 0 – 8 ind d^{-1} m^{-2}.

3.4.4 Land-use-related drivers of aquatic insect emergence

Pesticide toxicity and electrical conductivity (EC) exhibited the most pronounced differences among the selected land-use-related drivers between forested and agricultural sites, and both variables were higher in agriculture. On average, shading was higher in forested than agricultural sites, and temperature, pool habitats, and nitrate and phosphate concentrations were higher in agricultural sites (Figure B.16, Table 3.1). Oxygen saturation was higher in agriculture in spring but higher in forest in summer and autumn.

We fit 11 GLMMs to identify the main drivers of total biomass and total abundance of aquatic insects, as well as biomass and abundance of the major emergent aquatic insect orders. One GLMM was fit for every response variable e.g., total biomass, biomass of caddisflies and number of EPT families. The models for mayfly biomass and number of EPT families did not contain explanatory variables, but only random effects and the intercept. The explained variance of these models (R^2conditional) ranged between 33% for the number of EPT families and 69% for mayfly biomass. The variance explained by stream (random factor) varied between 16% for the number of EPT families and 41% for mayfly biomass, and variance explained by season (random factor) ranged from 23% for the number of EPT families to 61% for mayfly biomass (GLMM, Table 3.2).

The models containing explanatory variables explained between 30% and 79% of variance, when considering random effects (R^2conditional, GLMM, Table 3.2). Specifically, pesticide toxicity and EC were included in five models, shading in three, and pools as well as oxygen saturation in two models (Table 3.2). Pesticide toxicity was negatively associated with stonefly biomass and abundance, but it was positively associated with mayfly abundance, total biomass as well as caddisfly biomass. By contrast, EC showed a positive association with total biomass, total abundance, fly abundance, caddisfly abundance and biomass. Shading was negatively associated with total abundance, fly biomass as well as abundance. Finally, fly and total abundance increased with the percentage of pool habitats, but decreased with decreasing oxygen saturation.

Table 3.2: Estimated regression parameters, standard errors (SE), Z values for the best-fit generalized linear mixed-effects models (GLMM) obtained with L1-penalty and 60 observations. σ_{stream} is the standard deviation of the random effect stream, σ_{season} is the standard deviation of the random effect season, $R^2_{marginal}$ is the variance explained by the fixed factors, $R^2_{conditional}$ is the variance explained by fixed and random factors, $ICC_{adjusted}$ is the variance explained by the random factors stream and season. EPT is the abbreviation for stonefly, mayfly, and caddisfly families and EC for electrical conductivity.

Model	Parameter	Estimate	SE	Z value	σ_{stream}	σ_{season}	$R^2_{marginal}$ (%)	$R^2_{conditional}$ (%)	$ICC_{adjusted}$ stream (%)	$ICC_{adjusted}$ season (%)
Total biomass	Intercept	1.45	0.32	4.6	0.59	0.38	7	58	46	26
	Toxicity	0.13	0.15	0.89						26
	EC	0.17	0.17	1.03						26
Fly biomass	Intercept	0.45	0.21	2.12	0.27	0.25	13	30	12	10
	Shading	-0.32	0.14	-2.25						10
Mayfly biomass	Intercept	-1.05	0.89	-1.18	0.95	1.43	0	69	41	61
Stonefly biomass	Intercept	-4.16	0.7	-5.94	0.73	1.12	19	57	20	38
	Toxicity	-0.95	0.14	-6.9						38
Caddisfly biomass	Intercept	-0.65	0.82	-0.8	1.49	1.14	11	78	66	53
	Toxicity	0.3	0.16	1.9						53
	EC	0.62	0.19	3.34						53
Total abundance	Intercept	3.02	0.2	15.23	0.41	0.13	24	55	39	6
	Percentage pool habitats	0.08	0.17	0.48						6
	EC	0.07	0.16	0.45						6
	Shading	-0.28	0.15	-1.88						6
	Oxygen saturation	-0.18	0.15	-1.25						6
Fly abundance	Intercept	2.87	0.18	15.71	0.39	0.06	26	50	32	1
	Percentage pool habitats	0.1	0.17	0.61						1
	EC	0.06	0.16	0.39						1
	Shading	-0.31	0.15	-2.1						1
	Oxygen saturation	-0.21	0.15	-1.4						1
Mayfly abundance	Intercept	-0.75	0.52	-1.44	0.73	0.78	3	61	41	44
	Toxicity	0.25	0.14	1.85						44
Stonefly abundance	Intercept	-2.96	0.39	-7.55	0.4	0.6	23	55	63	58
	Toxicity	-0.61	0.13	-4.51						58
Caddisfly abundance	Intercept	-1.56	0.59	-2.63	0.95	0.85	14	79	18	33
	EC	0.6	0.16	3.71						33
Number EPT families	Intercept	1.26	0.19	6.62	0.22	0.28	0	33	16	23

3.5 Discussion

In this study, we assessed the association between land use and the export of aquatic insects to terrestrial ecosystems by continuously monitoring insects and physicochemical variables over the primary emergence period in paired agricultural and forested stream sites. An overview of the results is given in Table B.10. We (I) compared total biomass as well as abundance between agricultural and forested sites and found that total aquatic insect biomass and abundance were higher in agricultural than forested sites.

Additionally, we (II) analyzed the taxonomic and trait composition of aquatic insects, as well as their turnover, associated with land-use. Except for stoneflies, biomass, and abundance of major orders of aquatic insects were higher in agriculture, but their phenology differed. Differences in emergence timing among different taxa may affect the foraging opportunities of riparian predators (Armstrong et al., 2016). Furthermore, we identified turnover in the composition of insect families between forested and agricultural sites, with a tendency of families exhibiting a longer generation time in forest and shorter generation time in agriculture, which matches the results of Liess & von der Ohe (2005) for in-stream invertebrate communities in agricultural streams.

Moreover, we (III) determined land-use-related drivers associated with biomass, abundance, and composition of aquatic insects. Pesticide toxicity and EC were the most important land-use-related drivers associated with biomass and abundance for selected aquatic insect orders, whereas the aggregated parameters of total abundance and total biomass of aquatic insects were partly associated with different land-use-related drivers than aquatic insect orders. In the following we discuss the differences between land-use types as well as seasons and how the land-use-related drivers may have affected the aquatic insect assemblages.

3.5.1 Higher total biomass and abundance in agriculture than forest

Our finding that total biomass and abundance of aquatic insects were higher in agricultural than in forested streams is in line with previous studies (e.g., Carlson et al., 2016; Krell et al., 2015; for corresponding values see Table B.11). Higher total biomass and abundance in agricultural sites may be caused by elevated nutrient concentrations and reduced shading, both which have been shown to increase primary production in agricultural streams and, in turn, food availability for aquatic insects (Carlson et al., 2016; Griffiths et al., 2013; Stenroth et al., 2015; Terui et al., 2018). However, neither nutrients nor shading were selected as explanatory variables in the models for total biomass or abundance in our study, implying that other unmeasured stream-level variables may have contributed more to their differences. Furthermore, total abundance and biomass were affected differently. Total abundance was mainly constituted from small-bodied insects, whereas total biomass was frequently constituted by less abundant but large-bodied insects. Nonetheless, aquatic insects are part of the riparian food web, and changes in their total biomass and abundance can affect other trophic levels. For example, their presence can enhance predation on terrestrial herbivorous insects and, in turn, reduce herbivory (Graf et al., 2017; Henschel et al., 2001), although the underlying mechanism is unknown. Thus, increased total biomass and abundance of aquatic insects in agricultural areas have implications for both stream and terrestrial ecosystem structure and function.

3.5.2 Turnover and temporal patterns in composition

We identified seasonal patterns and shifted phenology between land-use types within the primary emergence period. Raitif et al. (2018) and Yuen & Dudgeon (2016) found similar seasonal patterns for mayflies and caddisflies, but different patterns for flies, in agricultural and forested sites, respectively. The shifted phenology between land-use types may be beneficial for mobile predators, which can exploit aquatic insects as food source in the landscape and thus prolong the use of this food source. By contrast, for sessile predators a shift in phenology may be disadvantageous, if the shift leads to a mismatch of life cycles (Schindler & Smits, 2017).

In addition, we found turnover of families between forested and agricultural sites, which is in line with other studies reporting turnover of species rather than an overall decline in total insect biomass (Baranov et al., 2020; Dornelas et al., 2019; Kaelin & Altermatt, 2016). The tendency for higher occurrence of aquatic insects with shorter generation time in agriculture indicates a turnover towards assemblages with lower vulnerability to land-use-related stress because they have a higher recovery potential (Sherratt et al., 1999; Stark et al., 2004). The fact that the site position was associated with the land use effect (i.e., downstream site always affected by land use) raises the question whether position itself contributed to the observed difference (Vannote et al., 1980). The upstream and downstream sites exhibited only small distances to each other. Aquatic insect assemblages of close sites reveal spatial autocorrelation, which means closer sites show higher similarities in insect assemblages. Spatial autocorrelation of aquatic insect communities occurs for example, because juvenile aquatic insects drift downstream (Bailey, 1966; Elliott, 1971). Further, it was shown, that upstream forested sites reduce effects of agricultural stressors on aquatic insect assemblages in downstream agricultural sites, probably by recolonization of aquatic insects from upstream sites (Orlinskiy et al., 2015). Therefore, the position itself is unlikely to cause the observed differences alone. Only experiments and modelling can answer this question finally, because under field conditions an upstream agricultural site would affect the downstream forested site as well. Better understanding turnover and trait composition helps to identify at-risk taxa in agricultural areas and, in turn, to inform implementation of mitigation measures preventing their loss, e.g., by reducing the amount of pesticide runoff into streams.

Patterns in insect abundance and biomass, as well as order-specific phenology, could have cascading effects on predator growth and immune function (Fritz et al., 2017; Twining et al., 2016). We found that in both land-use types, total abundance was driven by the abundance of flies, whereas total biomass was dominated by different orders across seasons (e.g., caddisflies in summer [Figure 3.1-3.3]). Other studies have shown similar results (e.g., Carlson et al., 2013; Krell et al., 2015; Raitif et al., 2018; Stenroth et al., 2015). These seasonal biomass patterns are accompanied by differences in traits like size, the export of PUFA, and, in turn, prey quality for riparian predators. For instance, of the major orders of aquatic insects, mayflies have the highest, and caddisflies the lowest, PUFA concentrations (Martin-Creuzburg et al., 2017). In our study, mayflies emerged mainly in spring and caddisflies in summer in both land use types; therefore, more PUFA may have been available during spring for riparian predators, thereby affecting predator dynamics.

Additionally, taxon-specific traits (e.g., size) determine which predators can benefit most from the presence of aquatic insects as an additional food source (Davis et al., 2011; Stenroth et al., 2015). We found a similar size distribution in forested and agricultural sites, though seasonal differences occurred. For example, in agriculture, small-bodied flies dominated biomass during spring, whereas larger-bodied caddisflies dominated biomass in summer. Thus, in spring, smaller predators like spiders may have benefited most from emerging insects, whereas larger predators like birds received a higher amount of suitable prey in summer. By contrast, during spring in forest, biomass of flies, mayflies and stoneflies was similar. So smaller as well as larger predators may have benefited equally. Overall, the changes in prey quality and phenology can have far-reaching consequences to the riparian food web by altering predator population dynamics (Sato et al., 2016) and, in turn, biomass at other trophic levels like terrestrial insects and plants (Graf et al., 2017; Henschel et al., 2001).

3.5.3 Land-use-related drivers of aquatic insect emergence

Identifying the factors that influence shifts in aquatic insect assemblages and phenology is important for understanding the cascading effects of human actions on stream ecosystems. We found that pesticide toxicity and EC were associated with differences in abundance and biomass of aquatic insects. These, and additional land-use-related drivers considered in this study (i.e., oxygen saturation, percentage of pool habitat, percentage of shading, water temperature, and nitrate and phosphate concentrations), are known to determine aquatic insect habitat quality in terms of hydromorphological structure and water quality (Graf et al., 2020; Raitif et al., 2018; Stenroth et al., 2015). Land-use-related drivers associated with reduced habitat quality like pesticide toxicity were worse in agricultural than forested sites. Toxicity and EC were selected in statistical models to explain total biomass and EC, pools as well shading total abundance of aquatic emergent insects. Total abundance and biomass represent aggregated features of the community that may amalgamate taxa with different responses to stressors, including land use *(Jähnig et al., 2021)*. Indeed, it is well known that the major orders of aquatic insects respond differently to land-use-related drivers (Carlson et al., 2016; Raitif et al., 2018; Stenroth et al., 2015). For instance, pesticide toxicity was associated with reduced biomass as well as abundance of stoneflies and increasing total biomass, while it was not included in the model for total abundance. Stoneflies are more vulnerable to pesticides than other aquatic insects (Rico & Brink, 2015; Rubach et al., 2010). Overall, this questions the usefulness of aggregated community features to study insect trends and drivers of community assembly as well as for management.

Changes in water salinity, and by extension in EC (a proxy for total dissolved ions regularly used to express salinity in water) may affect taxa through osmoregulatory stress, toxic effects of ions, or both (Cañedo-Argüelles et al., 2013). In Australia, the aquatic invertebrate assemblage changed, with a constant reduction of EPT species, along a gradient from <50 μS cm^{-1} to >30,000 μS cm^{-1} (Kefford et al., 2011). A study on German streams found strong turnover of aquatic insects when the natural background EC was exceeded by >400 μS cm^{-1} (Le et al., 2021). In our study region, the natural background EC is ≤100 μS cm^{-1} (Stöppel, 2005), and we found EC values in a range of 93 μS cm^{-1} to 381 μS cm^{-1} in forested sites. In agricultural sites, however, we found EC in the range of 179 μS cm^{-1} to 1899 μS cm^{-1}, corresponding to levels that have been shown to drive community change.

Pesticides, the second major driver of adult aquatic insect assemblage in our study, are globally present in streams (Beketov et al., 2013; Malaj et al., 2014; Stehle & Schulz, 2015). A country-wide monitoring study of streams across Germany, including some of the same sites as our study, found that pesticides regularly exceeded concentrations considered harmful for aquatic insects and, similarly, were the main driver for assemblage changes of juvenile vulnerable aquatic insects during their aquatic life stage (Liess et al., 2021). In the future, aquatic insect assemblage changes due to pesticides may increase because of increasing global pesticide use (Bernhardt et al., 2017; Kattwinkel et al., 2011) and increasing pesticide toxicity towards aquatic insects (Schulz et al., 2021). These trends could lead to higher variability in emergence (Uno & Pneh, 2020), therefore altering foraging opportunities for predators (Armstrong et al., 2016). Further, pesticide toxicity has been shown to cause earlier insect emergence (Cavallaro et al., 2018), which may affect the growth rate, population biomass, and maturity rate of predators (Sato et al., 2016) and may lead to a mismatch in predator and prey cycles.

3.6 Conclusion

Our study, which focused on different land-use types in temperate streams, revealed differences in aquatic insect abundance, biomass, and phenology between forested and agricultural sites and identified several land-use-related drivers associated with changes in order-level assemblages. These differential effects may vary with different land-use practices within land-use categories, as well as geographically. Therefore, we suggest that future studies should consider land-use intensity over a range of ecosystem types.

The aggregated parameters total abundance and biomass of insect emergence, which have frequently been used in meta-analyses, were to some extent associated with different land use related drivers and showed partly different trends than the orders of aquatic insects. Thus, we question their suitability to evaluate the anthropogenic influence on ecosystems and their use may result in missing complex responses to environmental change.

Overall, we were able to quantify the export of aquatic insects in the primary emergence period in different land-use types as well as seasonal patterns. These results can be included in modelling, for example, of food-web dynamics or meta-ecosystems. Further, the knowledge of important land-use-related drivers associated with aquatic insects enables to implement of specific management strategies to protect aquatic insect assemblages.

3.7 Acknowledgments

We thank Tim Ostertag, Moritz Schäfer, Lukas Reinhard and Laura Kieffer for assistance lyophilizing and weighing aquatic insects, Nadin Graf for assistance during identification of aquatic insects, and Brooke Cassell for language editing. This study was supported by the “Pilotstudie zur Ermittlung der Belastung von Kleingewässern in der Agrarlandschaft mit Pflanzenschutzmittel-Rückständen” funded by the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (FKZ 3717 63 403 0) and the German Helmholtz long-range strategic research funding. Additional funding was supplied by the MOSES (Modular Observation Solutions for Earth Systems) and the German Research Foundation (DFG; Project Number: 216374258). Katharina Ohler was funded by the German Academic Scholarship Foundation.

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4. Land use alters cross-ecosystem transfer of high value fatty acids by aquatic insects

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This chapter is adapted from the article under review in 2023 in Environmental Sciences Europe.

4.1 Abstract

Many aquatic insects emerge as adults from water bodies to complete parts of their life cycle in terrestrial ecosystems and are potential prey for riparian predators. The benefits of riparian predators from aquatic insects include higher contents of long-chain polyunsaturated fatty acids (PUFA) compared to terrestrial insects. Aquatic insects are therefore considered a high-quality food. Food containing high levels of PUFA can enhance growth and immune response of spiders. However, agricultural stressors like nutrient increase, pesticides and habitat degradation can affect the biomass of aquatic insects and in turn the diet of spiders. Studies quantifying the influence of land use on fatty acid (FA) profiles of emergent aquatic insects and riparian predators are lacking. We quantified differences in exports of FA, saturated FA, monounsaturated FA, and PUFA, FA profiles of aquatic insects and spiders between forested and agricultural sites over the primary emergence period within one year. The FA export to the riparian food web is crucial to understand energy fluxes between ecosystems. Furthermore, we monitored environmental variables to identify associations between agricultural stressors and FA profiles of aquatic insects and spiders.

We found differences in FA export and profiles of aquatic insects between land-use types. The quantity of total FA export via aquatic insects was lower in agricultural sites (95% CI: 1147 – 1313 µg m^{-2}) in comparison to forested sites (95% CI: 1555 – 1845 µg m^{-2}), while the biomass export was higher in agricultural sites. Additionally, in spring the PUFA export was significantly lower (up to 0.06 µg d^{-1} m^{-2}) in agricultural than forested sites. Agricultural stressors explained only little variation in the FA profiles of aquatic insects, e.g., 4% for caddisflies and 12% for non-biting midges. Percentage of shading and pool habitats were identified as most important variables explaining the variation in FA profiles.

The quality of aquatic insects as food source for riparian spiders was smaller in agricultural than forested sites, which can decrease the fitness of riparian predators. To improve our capacity to predict potential adverse effects in the riparian food web, future studies should identify the mechanisms underlying a lower PUFA content.

4.2 Introduction

Globally, intensive agriculture is a major land-use type (Václavík et al., 2013). In agricultural areas, stream ecosystems are threatened by enhanced nutrient inputs, pesticides, and habitat degradation, which in turn jeopardizes biodiversity and human water security (Collen et al., 2014; Reid et al., 2019; Vörösmarty et al., 2010).

Stream ecosystems and adjacent terrestrial ecosystems are closely connected via the exchange of matter and organisms (Baxter et al., 2005). Many aquatic insects e.g., Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies) and some Diptera (flies) emerge as adults from water bodies into terrestrial ecosystems. There, they are potential prey for riparian predators like spiders, birds, and bats (Gray, 1993; Kato et al., 2004; Sullivan et al., 1993). Additionally, agriculture is linked to the loss of terrestrial invertebrates (Ewald et al., 2015; Hallmann et al., 2017; Seibold et al., 2019). Therefore, riparian predators can benefit from aquatic insects complementing the food source of terrestrial invertebrates (Graf et al., 2020; Krell et al., 2015; Stenroth et al., 2015).

Furthermore, aquatic insects are considered a high-quality food source because they typically contain elevated levels of fatty acids (FA) compared to terrestrial insects (Hixson et al., 2015; Parmar et al., 2022; Twining et al., 2019). Especially, levels of polyunsaturated FA (PUFA) of aquatic insects can be ten times higher than in terrestrial insects (Hixson et al., 2015; Parmar et al., 2022). These differences originate from the base of the food web propagating to higher trophic levels: Aquatic primary producers like diatoms are capable of synthesizing long-chain PUFA (Ahlgren et al., 1992; Kainz et al., 2004; Strandberg et al., 2015), while terrestrial vascular plants cannot (Sayanova & Napier, 2004). Additionally, many animals are not able to produce PUFA de novo and therefore depend on dietary intake of these compounds (Twining, Bernhardt, et al., 2021). Animals like some bird and spider species that can synthesize PUFA still are constrained by high energetic costs and, thus, may only produce PUFA in the absence of other sources (Mathieu-Resuge et al., 2022; Twining, Bernhardt, et al., 2021; Twining, Parmar, et al., 2021). Consequently, food containing high levels of long chain PUFA has been shown to enhance growth and immune response of spiders and birds (Fritz et al., 2017; Twining et al., 2016). Furthermore, FA in general have been linked to the increased growth of spiders (Mayntz & Toft, 2001).

Emergence of aquatic insects is variable over time and reveals seasonal patterns (Nakano & Murakami, 2001; Ohler, Schreiner, Link, Liess, et al., 2023) and it has been shown that the timing of emergence can control growth rate, population biomass and maturity rate of terrestrial predators (Sato et al., 2016; Uno, 2016). Therefore, accounting for temporal dynamics is important when aiming to predict effects of total FA export via aquatic insects to terrestrial ecosystems (Gounand et al., 2018).

It is known that agricultural stressors like increased nutrient concentration in stream water, pesticides and habitat degradation affect aquatic insects e.g., by changing the composition of aquatic insect assemblages (Ohler, Schreiner, Link, Liess, et al., 2023; Stenroth et al., 2015) and increasing or decreasing their biomass, depending on the aquatic insect order (Ohler, Schreiner, Link, Liess, et al., 2023; Raitif et al., 2018). Furthermore, agricultural stressors can affect riparian spiders by altering the amount of aquatic insects in their diet (Graf et al., 2020), reducing their richness as well as abundance (Graf et al., 2019).

Most studies thus far have focused on PUFA profiles of aquatic insects and riparian predators without considering potential effects of agricultural stressors (e.g., (Martin-Creuzburg et al., 2017; Moyo et al., 2017; Parmar et al., 2022; Twining et al., 2019; Twining, Parmar, et al., 2021)). One mesocosm study on Chironomidae (non-biting midges), including nutrient elevation and predation, found that FA export was highest at intermediate phosphate concentrations and that biomass of non-biting midges was the best predictor for FA export (Scharnweber et al., 2020). However, under laboratory conditions, with toxicant exposure (copper, pesticides, Bacillus thuringiensis var. israelensis) during larval stages of non-biting midges, no effect on FA profiles of adult non-biting midges and a tendency to decreased FA content in spiders was found (Pietz, Kolbenschlag, et al., 2023). Similarly, one field study on emergent aquatic insects in two streams included stream-bed characteristics and physicochemical variables like nutrients but did not find an association of these variables with the FA profiles of aquatic insects (Kowarik et al., 2022). In a field study, conducted in agricultural and forested streams focusing on vegetation (herbaceous and woody), the taxonomy of spiders at family level was the best predictor for FA content of spiders (Ramberg et al., 2020).

To our knowledge, field studies that quantified the influence of land use and associated stressors on FA profiles of emergent aquatic insects and riparian predators as well as FA export via aquatic insects are lacking. However, this would be important to estimate the effect of changing quality in terms of FA export to the riparian food web, which is crucial to understand energy fluxes between ecosystems and to predict effects on the subsidized food web (Marcarelli et al., 2011; Osakpolor et al., 2023; Pichon et al., 2023). Therefore, we aimed to quantify differences in total FA export and FA profiles of aquatic insects as well as riparian spiders between forested and agricultural sites of ten streams over the primary emergence period within one year (March – September). As spiders can prey on emergent aquatic insects, their FA profiles may be affected by changes in FA profiles of emergent aquatic insects. We collected emergent aquatic insects and riparian spiders and measured their FA profiles. In addition, we monitored a range of environmental variables to identify potential associations between agricultural stressors and the FA profiles of aquatic insects and spiders. We compared (1) total FA export via aquatic insects between both land-use types, (2) FA profiles of aquatic insects and spiders between agricultural and forested sites and (3) examined associations between agricultural stressors and the FA profiles of aquatic insects and spiders.

4.3 Methods

4.3.1 Study sites

To cover the primary emergence period (Corbet, 1964), our study was conducted from 22nd March to 13th September 2018 in southwestern Germany. In 10 parallel, fine substrate-dominated, mostly small, first and second order highland streams, an upstream forested site, and a downstream site where agricultural land use dominated were selected (Figure C.1). The mean distance between the upstream and downstream sites within a stream was 5.5 (range: 1.4 – 14.0) km and the maximum distance between parallel streams was 50 km. All streams originated in the Palatinate Forest, a forested low mountain range. The sites were mostly free from large wastewater treatment plants and industrial facilities. Viticulture was the main agricultural land use. It has been shown that environmental variables were similar across different types of agricultural land use including viticulture, cereals, and corn in this region (Voß et al., 2015). The stream size and order of all study sites was comparable, for details see Ohler et al. (Ohler, Schreiner, Link, Liess, et al., 2023).

4.3.2 Agricultural stressors associated with fatty acid profiles

We recorded physicochemical variables every three weeks and hydromorphological structure in March, July, and August to determine land-use-related variables associated with FA profiles of aquatic insects and riparian spiders. For instance: Electrical conductivity (EC), nitrate concentration, air and water temperature, oxygen saturation, the percentage of pool habitats and the percentage of shading (Table C.1). Furthermore, in-stream pesticide concentrations were determined from 49 event-driven samples taken during heavy rainfall events and 85 grab samples taken every three weeks. Glass bottle samplers (Liess & von der Ohe, 2005) and automated samplers (MAXX TP5, Rangendingen, Germany) took event-driven samples whenever the water level increased more than 5 cm. The samples were filtered (either automatically on site or manually in the lab) to retain particles, which were then analyzed for pesticides bound on particles. More information on pesticide sampling, analysis and exposure are described in Halbach et al. (Halbach et al., 2021) and Liess et al. (Liess et al., 2021, 2022).

The pesticide class of pyrethroids typically enters streams bound on particles (Gan et al., 2005) and has a high relative toxicity for aquatic insects (Rico & Brink, 2015; Rubach et al., 2010). Therefore, the concentration of pesticides bound on particles in event samples was used to estimate the bioavailable concentration in water cd following Schäfer et al. (2011) and Toro et al. (1991) with the equation:

$$c_d=\frac{c_{tot}}{f_{oc}\cdot k_{oc}+1} \quad (4.1)$$

where f_{oc} is the fraction of organic carbon in the sample, c_{tot} is the total concentration on the suspended particles, and k_{oc} is the soil organic carbon-water partitioning coefficient, which was extracted from the Pesticide Property Data Base (PPDB, Lewis et al., 2016) and PubChem (National Center for Biotechnology Information, 2021) database (Table C.2).

The logarithmic sum of toxic units (sumTU) was calculated to estimate the toxicity of the pesticide mixture (Schäfer et al., 2013):

$$\text{sumTU}=\log\sum_{i=1}^{n}\frac{c_i}{EC_{50i}} \quad (4.2)$$

where c_i is the concentration of the single pesticide, EC_{50i} the acute effect concentration of the pesticide towards the most sensitive freshwater invertebrate species, and n is the number of pesticides. The R package Standartox (version 0.0.1, Scharmüller et al. 2020) was used to compile the EC_{50} values from the ECOTOX database (US EPA, 2021). If the EC_{50} values were missing in Standartox, the values were complemented from the PPDB (Lewis et al., 2016) or Malaj et al. (2014, Table C.3). The maximum pesticide toxicity (maximum sumTU of all samplings per site and season; hereafter pesticide toxicity) was used in the analysis because it may be responsible for the strongest ecological response.

4.3.3 Spider and aquatic insect sampling

We chose *Tetragnatha* sp. to determine effects of FA in aquatic-terrestrial food webs, as these spiders frequently colonize riparian areas and prey on aquatic insects (Kato et al., 2004) with orb webs spanning over streams (Reitze & Nentwig, 1991). Whenever feasible only female and adult spiders of the species *T. montana* were collected to minimize variation in feeding, because feeding differs between and within spider species (Foelix, 2011). In the subsequent FA analysis 73% of the spiders were adult female T. montana, for more details see Ohler et al. (Ohler, Schreiner, Link, Brack, et al., 2023). Up to ten spiders were gathered with a maximum distance of 1 m from the stream by hand in spring (14th – 16th May 2018), summer (16th – 19th, 23rd, 26th July 2018) and autumn (10th – 13th September 2018).

Emergence traps with a basal area of 0.25 m^2 and a bottle trap without any solution (Cadmus et al., 2016) were used to sample aquatic insects continuously. Two traps were installed at every site covering pool and riffle habitats. This sampling method likely underestimated the fraction of stoneflies that emerge by walking on the banks. As previous studies estimated only < 1% to 3% (Gray, 1989; Jackson & Fisher, 1986) of aquatic emergent insects returning to water bodies, we assume that most sampled aquatic insects would have reached the riparian area. Twice a week the traps were emptied by replacing the bottle trap.

The spiders as well as aquatic insects were transported on ice until they were euthanized in liquid nitrogen and identified in the laboratory on ice. Under a stereo microscope, spiders were identified to species level using the key by Roberts (1995) and aquatic insects to family level with the following keys Bährmann and Müller (2015), Nilsson (1996b, 1996a), Schäfer and Brohmer (2010). Subsequently, spiders and aquatic insects were lyophilized to complete dryness and weighed to the nearest 0.1 µg.

4.3.4 Fatty acid analysis

For FA analysis the major orders of aquatic insects, i.e., mayflies, stoneflies, caddisflies, and flies, were chosen. In total 21 FA with 18 or more carbon atoms were included in the analysis. Since non-biting midges (Chironomidae) dominated the emergence of flies (Diptera) (Ohler, Schreiner, Link, Liess, et al., 2023), only their FA profiles were analyzed. The samples of aquatic insects collected over approximately two weeks (Table C.4) were pooled on order level prior to analysis, which is commonly done in FA analysis (e.g., Kowarik et al., 2022; Martin-Creuzburg et al., 2017). An analysis on family level would have exceeded financial and labor capacities, though FA profiles may differ across families (Scharnweber et al., 2020). Hence, the FA analysis at order level will reflect the FA profiles of the families present in one site.

After the addition of an internal standard (C17:0 200 µg mL^{-1}; C23:0 250 µg mL^{-1}, Sigma-Aldrich) the FA of all samples were extracted following Folch et al. (Folch et al., 1957) with chloroform/methanol (GC-grade, 5 mL, v:v; 2:1) at – 20 °C over night. Then the samples were filtered with a syringe filter (PTFE, 13 mm, 0.45 µm, BGB), evaporated until dryness at 40 °C under nitrogen and redissolved in methanol. The volume of methanol depended on the weight of the sample (maximum ratio of weight to volume: 3:10), for details see Ohler et al. (Ohler, Schreiner, Martin-Creuzburg, & Schäfer, 2023). All samples were stored under nitrogen at – 20 °C until derivatization. Methanolic trimethylsulfonium hydroxide (TMSH, 0.2 M, 10 µL, Macherey-Nagel) was used to derivatize FA to fatty acid methyl esters (FAME) in the sample (20 µL) at room temperature for 60 minutes. A gas chromatograph with a flame ionization detector (Varian CP-3800, Varian Inc) equipped with a DB-225 capillary column (30 m x 0.25 mm x 0.25 µm, Agilent J&W) was used to analyze FAME. The FAME were identified and quantified with external standards (Supelco 37 component FAME mix, 18:1n-7 FAME, ALA FAME, Sigma-Aldrich). OpenChrom (Wenig & Odermatt, 2010) was used for identification and R (version 4.2.0, R Core Team, 2022) for quantification. Further details are given in Ohler et al. (Ohler, Schreiner, Martin-Creuzburg, & Schäfer, 2023).

4.3.5 Data analysis

The export of FA, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA via aquatic insects was assessed with hierarchical generalized additive models (HGAM) following Pedersen et al. 2019). This gives information about the amount of these compounds available for riparian predators. HGAM allow to identify seasonal patterns of FA, SFA, MUFA and PUFA export as well as differences between land-use types in the amount exported. The sum of all FA, SFA, MUFA and PUFA of aquatic insects in total as well as on order level were used in the HGAM. The export of FA, SFA, MUFA and PUFA was normalized for the sampling area and duration. Group-level smoothers without a global smoother for land use and land use crossed with order were applied. That means each group could differ in its shape without restriction. HGAM including one smoothing parameter for all group levels (model S, same wiggliness) yielded lower Bayesian Information Criterion (BIC, Table C.5) than HGAM fitted with one smoothing parameter for every group level (model I, different wiggliness). Additionally, stream was incorporated as random effect smoother. The HGAM were fitted with the R-package mgvc (version 1.8-36, Wood, 2011). The effect of land use was quantified with the 95% confidence interval (CI) of the difference between the mean fit (mean per time point) for forest and agriculture. At $\alpha = 0.05$ non-overlapping CI were considered statistically significant. Furthermore, the mean export of FA, SFA, MUFA and PUFA per area over the whole sampling period was estimated by using the mean fits of the HGAM in forest as well as agriculture. The temporal resolution of agricultural stressors was too low to include them in model selection in HGAM.

To identify differences in the FA profiles between land-use types, in every season, FA profiles (FA ≥ 18 carbon atoms) of aquatic insects, i.e. mayflies, stoneflies, caddisflies, non-biting midges, and spiders between forested and agricultural sites were compared with analysis of similarity (ANOSIM; 999 permutations, Euclidean distance, R-package vegan version 2.5-7, Oksanen et al., 2020). For this purpose, the content of a single FA was calculated as the proportion of the total FA content (proportion of FA) to assess potential effects of land use on the FA profiles. In autumn, a comparison of FA profiles of stoneflies between land-use types was not possible, because no stoneflies were caught in agricultural sites during autumn. The p-values were adjusted with the Benjamini-Hochberg method (Benjamini & Hochberg, 1995) to decrease the false discovery rate in multiple testing. Similarity percentage (SIMPER) analyses with the R-package vegan version 2.5-7 (Oksanen et al., 2020) were conducted whenever ANOSIM resulted in significant differences between land-use types to identify the specific FA contributing to the differences.

Redundancy analysis (RDA) was conducted to identify agricultural stressors associated with changes in FA profiles of aquatic insects and spiders. For this purpose, the mean of each FA (expressed as proportion of total FA) and environmental variables per season was calculated for spiders and aquatic insects in total. The proportion of single FA was used to determine, if agricultural stressors were in general associated with FA profiles. Furthermore, the mean of each FA per season was calculated for single orders mayflies, stoneflies, caddisflies and non-biting midges. The latter was done, because data aggregation may hamper the identification and evaluation of associations with stressors (Jähnig et al., 2021; Ohler, Schreiner, Link, Liess, et al., 2023). We chose pesticide toxicity, percentage of shading, EC, oxygen saturation, percentage of pool habitats, phosphate and nitrate concentration as well as air and water temperature as variables potentially expressing agricultural influence based on the results of previous studies in the region (Englert et al., 2015; Fernández et al., 2015; Voß et al., 2015). Additionally, we included the variables stream and season. Water temperature and EC were only included in RDA for aquatic insects and air temperature only in RDA for spiders. Furthermore, the biomass of aquatic insects was included in RDA for spiders only. The variables were chosen a priori. Temperature is known to affect FA profiles of organisms, because, for example with rising temperature, organisms can modify their PUFA content to decrease fluidity of cell membranes (Arts & Kohler, 2009; Fuschino et al., 2011). Shading, phosphate, and nitrate concentration can affect primary producers and in turn the trophic transfer of FA by altering the food availability for aquatic insects (Carlson et al., 2016; Griffiths et al., 2013; Stenroth et al., 2015; Terui et al., 2018). Furthermore, the variables considered in this study can affect the biomass, abundance, and assemblage composition of aquatic insects (Graf et al., 2020; Ohler, Schreiner, Link, Liess, et al., 2023; Raitif et al., 2018; Stenroth et al., 2015) and the diet, abundance, as well as assemblage composition of spiders (Graf et al., 2019, 2020). The biomass of aquatic insects determines the potential amount of prey with aquatic origin for spiders (Graf et al., 2020). Before the analysis the environmental variables were checked for collinearity. No collinearity was present (highest $r = 0.5$) and all environmental variables were independent from each other. Additionally, the variables were standardized, which includes mean centering and standardization to unit variance. Variable selection for the agricultural stressors was conducted with automatic forward stepwise model selection using the maximization adjusted R2 (ordiR2step, R-package vegan version 2.5-7 (Oksanen et al., 2020). Stream and season were included in the starting model. After model selection a partial RDA with stream and season as covariates was conducted to identify the variation in FA profiles originating only from the agricultural stressors. All data analysis was conducted with R (R Core Team, 2022) and figures were generated with the R-package ggplot2 version 3.4.1 (Wickham, 2016). The R code and data are available (Ohler, Schreiner, Link, Brack, et al., 2023).

4.4 Results

4.4.1 Comparing fatty acid export between agricultural and forested sites

Overall, 1555 – 1845 µg m^{-2} (95% CI) FA, 425 – 516 µg m^{-2} SFA, 178 – 204 µg m^{-2} MUFA, and 942 – 1114 µg m^{-2} PUFA were exported in forested and 1147 – 1313 µg m^{-2} FA, 329 – 403 µg m^{-2} SFA, 135 – 151 µg m^{-2} MUFA, and 670 – 744 µg m^{-2} PUFA in agricultural sites over the study period. Differences between land-use types in FA, SFA, and MUFA export were not significant (i.e., non-overlapping 95% CI at $\alpha = 0.05$) over the whole study period for individual time points (Figure 4.1a, b, c). However, during spring the PUFA export was significantly higher (up to 0.06 µg d^{-1} m^{-2}) in forested than agricultural sites (Figure 4.1d). The FA, SFA, MUFA and PUFA export was highest in spring and decreased until autumn in both land-use types and the seasonal patterns of these compounds were similar within land-use types (Figure 4.1a, b, c, d).

In autumn, the FA, SFA, MUFA, and PUFA export at single time points of mayflies was significantly higher in forested than agricultural sites (Figure 4.2a, e, i, m), the latter approximately 0.01 µg d^{-1} m^{-2}. The FA, SFA, MUFA as well as PUFA export of mayflies peaked during spring in both land-use types (Figure 4.2a, e, i, m), but individual time points were not significantly different.

In contrast, the FA, SFA, MUFA, and PUFA export via non-biting midges was significantly higher in forested than agricultural sites during spring and the beginning of summer (Figure 4.2b, f, j, n). The FA export ranged from 0.04 (± 0.02) to 0.08 (± 0.04) µg d^{-1} m^{-2} (mean fit HGAM ± 2 standard errors) and from 0.02 (± 0.01) to 0.03 (± 0.02) µg d^{-1} m^{-2}, respectively. In both land-use types FA, SFA, MUFA, and PUFA export via non-biting midges reached its minimum in summer.

The export of FA, MUFA, and PUFA via caddisflies was significantly higher in agricultural than forested sites during autumn, but no significant differences at individual time points were observed for SFA export via caddisflies (Figure 4.2c, g, k, o). In agricultural sites the FA export of caddisflies ranged from 0.05 (± 0.02) to 0.007 (± 0.03) µg d^{-1} m^{-2}, and the PUFA export from 0.04 (± 0.01) to 0.05 (± 0.02) µg d^{-1} m^{-2} whereas the FA export of caddisflies ranged from 0.02 (±0.02) to 0.03 (±0.01) µg d-1 m-2, and the PUFA export was approximately 0.02 µg d^{-1} m^{-2} in forested sites. Additionally, in summer the FA export of caddisflies peaked at 0.07 (± 0.03) µg d^{-1} m^{-2} in agricultural sites, while in forested sites a plateau around 0.05 µg d^{-1} m^{-2} was observed in summer (Figure 4.2c).

Starting at the second half of spring at single time points, the FA, SFA, MUFA, and PUFA export via stoneflies was significantly higher in agricultural than forested sites, though in forested sites the maximum FA export 0.02 (± 0.01) µg d^{-1} m^{-2} (beginning of spring) was the same as the maximum FA export 0.02 (± 0.01) µg d^{-1} m^{-2} (end of spring) in agricultural sites (Figure 4.2d, h, I, p).

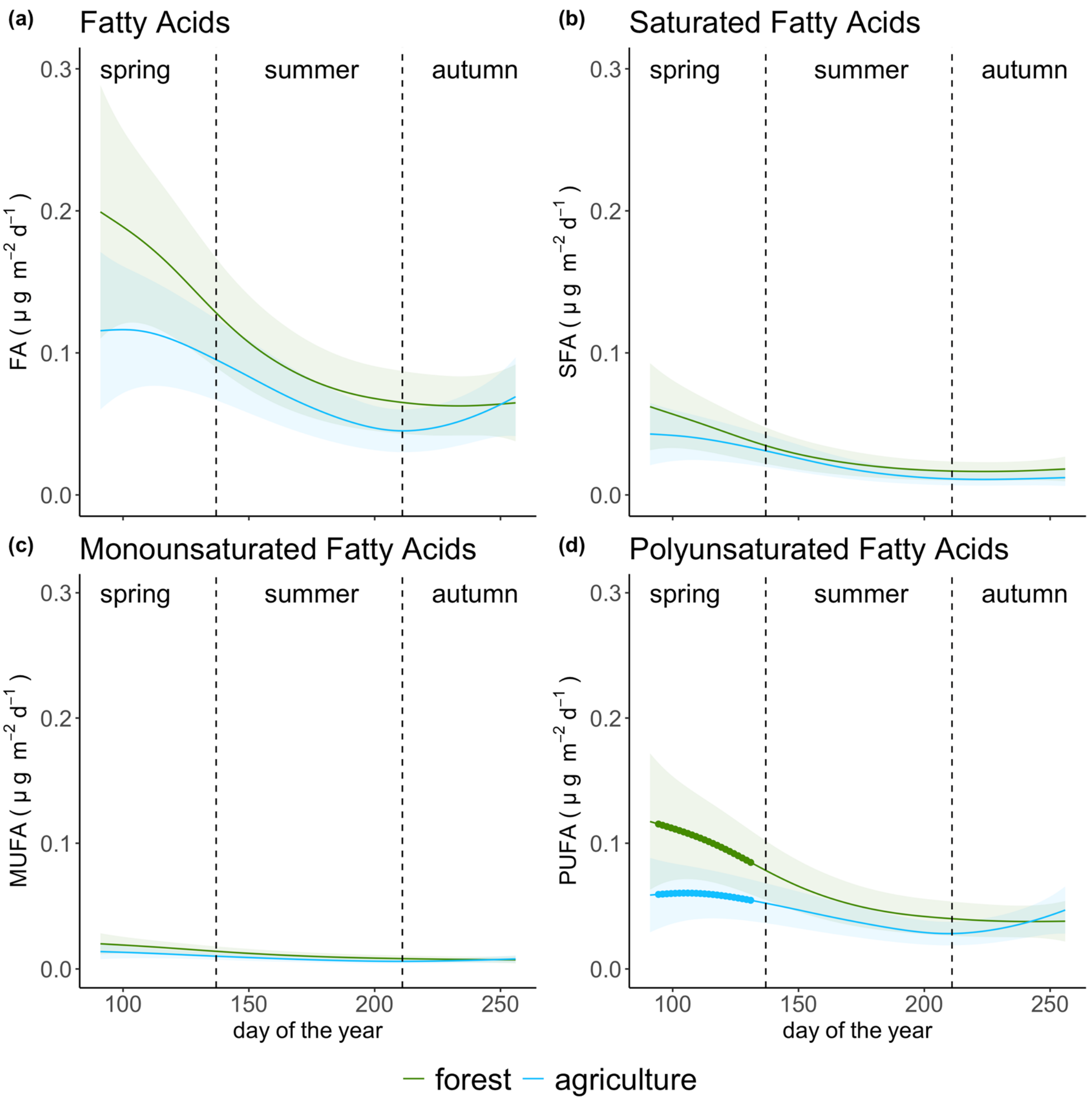

Figure 4.1: Modelled seasonal patterns of fatty acid (FA), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) export from streams of the total emergence including non-biting midges, mayflies, stoneflies, and caddisflies. The values were derived with hierarchical generalized additive models (HGAM) fitted with 354 observations. Solid lines represent the predicted mean fit values of the HGAM, and the ribbon shows ± 2 standard errors around the mean fit. Dots indicate significant differences (non-overlapping 95% confidence intervals at alpha = 0.05) between agricultural and forested sites. Blue shows the seasonal patterns in agriculture and green in forest for (a) FA export, (b) SFA export, (c) MUFA export, and (d) PUFA export. Seasons: spring: 18th March – 16th May, summer: 17th May – 26th July, autumn: 27th July – 13th September.

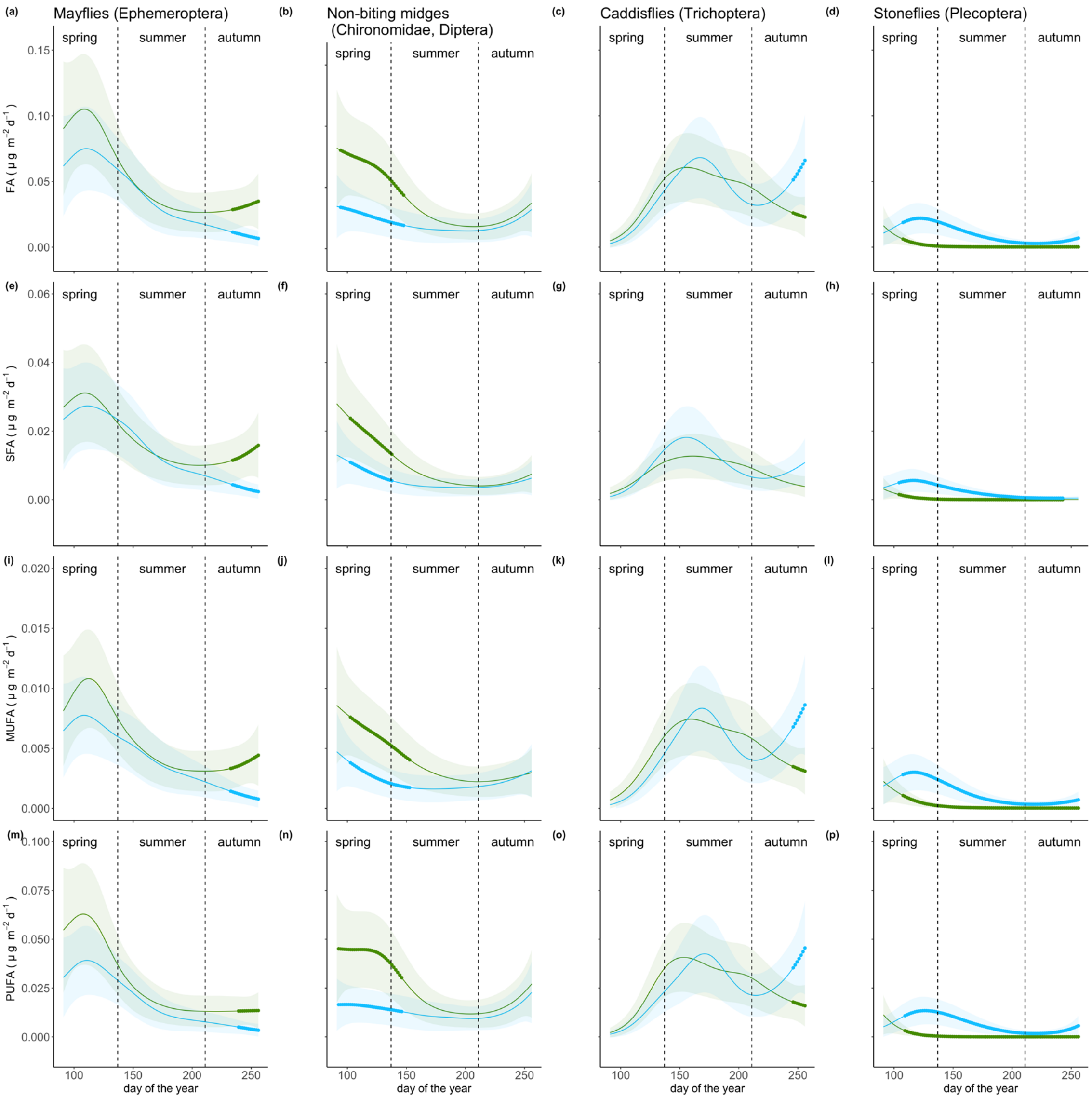

Figure 4.2: Modelled seasonal patterns of fatty acid (FA), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) export from streams of aquatic insect orders. The values were derived with hierarchical generalized additive models (HGAM) fitted with 998 observations. Solid lines represent the predicted mean fit values of the HGAM, and the ribbon shows ±2 standard errors around the mean fit. Dots indicate significant differences (non-overlapping 95% confidence intervals at alpha = 0.05) between agricultural and forested sites. Blue shows the seasonal patterns in agriculture and green in forest for (a-d) mayflies, (e-h) non-biting midges, (i-l) caddisflies, and (m-p) stoneflies. Seasons: spring: 18th March – 16th May, summer: 17th May – 26th July, autumn: 27th July – 13th September. Beware that the y-axis scale varies.

4.4.2 Comparing fatty acid profiles between agricultural and forested sites

We did not find differences in FA profiles of spiders and stoneflies between agricultural and forested sites (Table 4.1, Figure C.2). FA profiles of mayflies (ANOSIM: R = 0.13, p-value = 0.014) exhibited significant differences (i.e., p-value < 0.05) between land-use types in spring (Table 4.1). The FA eicosapentaenoic acid (20:5n-3, EPA), alpha-linolenic acid (18:3n-3, ALA), linoleic acid (18:2n-6c, LIN), elaidic acid (18:1n-9t, ELA), octadecanoic acid (18:0, ODA) and eicosanoic acid (20:0, EA) contributed most to these differences (Table 2). All of these FA, except EPA, tended to have higher proportions in mayflies in forested than agricultural sites (Table 4.2, Figure C.2). Additionally, these FA explained between 22% (EPA) and 6% (ELA, LIN) of the differences.

The FA profiles of non-biting midges differed significantly between land-use types in spring (ANOSIM: R = 0.12, p-value = 0.036, Table 4.1). The FA contributing most to these differences were: gamma-linolenic acid (18:3n-6, GLA), ALA, EPA, ODA and EA, of which GLA explained most of the differences (21%) and EA the least (7%, Table 4.2). GLA, ODA and, EA tended to reach higher proportions in non-biting midges in forested than agricultural sites, while ALA and EPA tended to have higher proportions in aquatic insects in agricultural than forested sites (Table 4.2, Figure C.2).

Furthermore, in summer caddisflies revealed significant differences in FA profiles between forested and agricultural sites (ANOSIM: R = 0.1, p-value = 0.026, Table 4.1). ALA, EPA, LIN, EA, ODA and GLA contributed most to these differences. The former three FA tended to have higher proportions in caddisflies in agricultural sites than forested sites and the latter three FA in forested sites (Table 4.2, Figure C.2). Overall, these FA explained between 20% (ALA) and 7% (LIN) of the differences in FA profiles of caddisflies between land-use types (Table 4.2).

Table 4.1: Results of the analysis of similarities (ANOSIM) for the fatty acid (FA) profiles. Spiders and aquatic insects per order were compared within the same season between forested and agricultural sites. The Benjamini–Hochberg method was used to adjust the p-values. Since stoneflies were not caught in agriculture in autumn, no ANOSIM could be conducted. Differences between forested and agricultural sites were indicated by R values: R < 0.25 barely separated, R < 0.5 clearly separated with some overlap, R > 0.75 well separated (Jaschinski et al., 2011). R < 0 greater dissimilarity within than between groups (Chapman & Underwood, 1999). Bold values indicate significant p-values (i.e., p-values < 0.05).

Organism group	Season	R	p-value	Sample size
Spiders	spring	-0.02	0.858	91
	summer	-0.02	0.858	52
	autumn	0.02	0.858	87
Mayflies	spring	0.13	**0.014**	58
	summer	0.08	0.06	69
	autumn	0.07	0.858	32
Stoneflies	spring	-0.16	0.858	20
	summer	0.14	0.858	14
Caddisflies	spring	0.15	0.504	16
	summer	0.1	**0.026**	69
	autumn	0.05	0.858	39
Non-biting midges	spring	0.12	**0.036**	65
	summer	0.1	0.06	76
	autumn	-0.01	0.858	55

Table 4.2: Results of similarity percentage (SIMPER) analyses conducted when significant differences between forested and agricultural sites in fatty acid (FA) profiles were found with analysis of similarity (ANOSIM). Average is the contribution of FA to the average between-group dissimilarity, ratio is the average to standard deviation ratio, average agriculture is the average abundance in agricultural sites and average forest the average abundance in forested sites. FA with the closest higher cumulative contribution to 0.7 are presented. EPA: Eicosapentaenoic acid (20:5n-3), ALA: alpha-linolenic acid (18:3n-3), GLA: gamma-linolenic acid (18:3n-6), LIN: Linoleic acid (18:2n-6c), ELA: Elaidic acid (18:1n-9t), ODA: Octadecanoic acid (18:0), EA: Eicosanoic acid (20:0).

Order	**Season**	**FA**	**Average**	**Standard deviation**	**Ratio**	**Average agriculture**	**Average forest**	**Cumulative contribution**	**Single contribution**
Mayflies	spring	EPA	0.09	0.06	1.51	0.31	0.19	0.22	0.22
		ALA	0.05	0.05	0.99	0.09	0.12	0.35	0.13
		ODA	0.05	0.05	1.13	0.23	0.27	0.48	0.13
		EA	0.04	0.04	1.08	0.05	0.10	0.58	0.10
		ELA	0.03	0.03	0.82	0.03	0.05	0.64	0.06
		LIN	0.03	0.03	0.89	0.07	0.08	0.70	0.06
Caddisflies	summer	ALA	0.09	0.06	1.36	0.22	0.16	0.20	0.20
		EPA	0.06	0.05	1.33	0.21	0.17	0.34	0.14
		EA	0.05	0.04	1.33	0.10	0.15	0.47	0.13
		ODA	0.05	0.05	1.13	0.11	0.16	0.59	0.12
		GLA	0.04	0.03	1.16	0.06	0.08	0.68	0.09
		LIN	0.03	0.02	1.22	0.05	0.04	0.75	0.07
Non-biting midges	spring	ALA	0.1	0.07	1.39	0.22	0.19	0.20	0.20
		GLA	0.1	0.08	1.19	0.11	0.21	0.41	0.21
		EPA	0.08	0.05	1.40	0.18	0.06	0.57	0.16
		ODA	0.05	0.04	1.27	0.24	0.25	0.68	0.11
		EA	0.03	0.03	1.19	0.06	0.07	0.75	0.07

4.5 Agricultural stressors associated with fatty acid profiles

The partial RDA (first axis: F = 3.7062, p-value = 0.025, second axis: F = 1.9292, p-value = 0.359) of the FA profiles of all analyzed aquatic insects (non-biting midges, mayflies, stoneflies, caddisflies) included water temperature, EC, percentage of pool habitats, oxygen saturation as well as percentage of shading (Figure 4.3a) and explained 5% of the variation in FA profiles (Table C.7). For instance, FA profiles of aquatic insects of forested sites were associated with increasing percentage of shading and FA profiles of aquatic insects of agricultural sites with increasing percentage of pool habitats.

In the final partial RDA (first axis: F = 7.5766, p-value = 0.007, second axis: F = 3.2750, p-value = 0.084), the variables oxygen saturation, phosphate as well as nitrate concentration, EC, water temperature, percentage of pool habitats and shading, explained 12% of the variation of FA profiles of non-biting midges (Figure 4.3b, Table C.6). Increasing percentage of shading and oxygen saturation were associated with FA profiles of non-biting midges in forested sites.

No RDA axes (first axis: F = 2.8377, p-value = 0.152, second axis: F = 1.7128, p-value = 0.431) were significant for mayflies and stoneflies (first axis: F = 1.7548, p-value = 0.249, second axis: F = 1.0624, p-value = 0.419). The agricultural stressors EC, pesticide toxicity, phosphate as well as nitrate concentration were selected for the final partial RDA for mayflies and percentage of shading and phosphate concentration for stoneflies (Figure 4.3c, d). The agricultural stressors explained for mayflies and stoneflies 6% of the variation in FA profiles (Table C.6).

For caddisflies, the final partial RDA (first axis: F = 2.9864, p-value = 0.023, second axis: F = 0.9749, p-value = 0.784) contained percentage of pool habitats, pesticide toxicity and shading (Figure 4.3e), though only 4% of variation were explained by the agricultural stressors (Table C.6). Pesticide toxicity was associated with the FA profiles of aquatic insects in agricultural sites.

For spiders no RDA axes were significant (first axis: F = 2.4217, p-value = 0.253, second axis: F = 2.1278, p-value = 0.230) and air temperature, percentage of shading, phosphate as well as nitrate concentration explained 3% in the variation of the FA profiles in the partial RDA (Figure 4.3f).

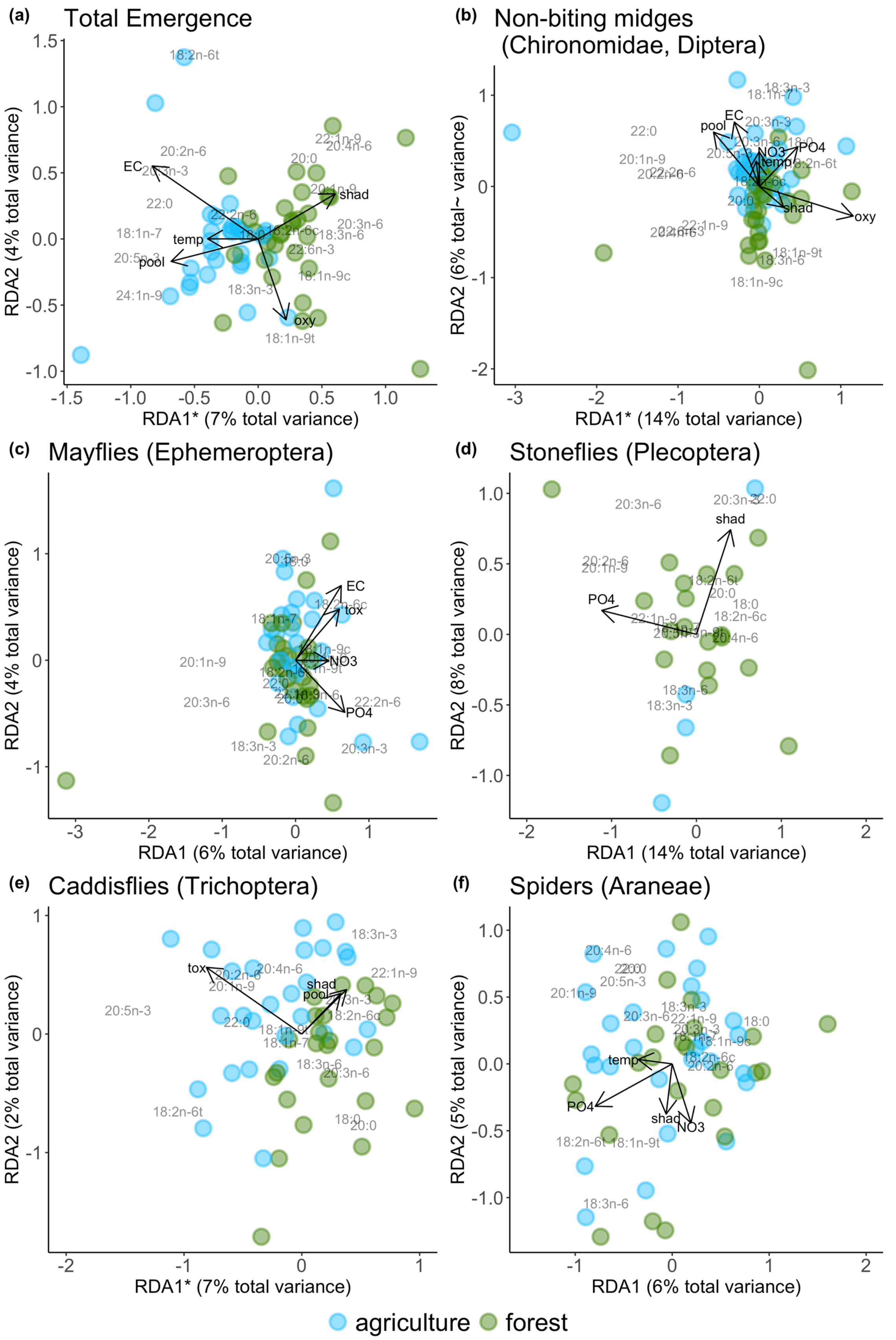

Figure 4.3: Plot of the partial redundancy analysis (RDA) with stream and season as covariates. Colors indicate land-use type: blue = agriculture, green = forest. Asterisks at axes mark significance. NO3: Nitrate concentration, PO4: Phosphate concentration, oxy: Oxygen saturation, pool: Percentage pool habitats, temp: Temperature (for spiders: air temperature, for emergent aquatic insects: water temperature), tox: Pesticide toxicity, EC: Electrical conductivity, shad: Percentage of shading.

4.6 Discussion

4.6.1 Comparing fatty acid export between agricultural and forested sites

The export of total FA of aquatic insects was approximately 26 – 29% higher in forested than agricultural sites, although the biomass of aquatic insects was 61 – 68% higher in agricultural than forested sites (Ohler, Schreiner, Link, Liess, et al., 2023). At individual time points only significant differences of the total PUFA export were observed: In spring more PUFA were exported in forested than agricultural sites. In contrast, at individual time points the biomass of aquatic insects was higher in agricultural than forested sites in spring (Ohler, Schreiner, Link, Liess, et al., 2023). Additionally, the biomass of non-biting midges and mayflies was higher in agricultural than forested sites (Ohler, Schreiner, Link, Liess, et al., 2023), while the FA, SFA, MUFA and PUFA export via non-biting midges and mayflies was higher in forested than agricultural sites. This indicates that the FA, SFA, MUFA, as well as PUFA content in aquatic insects is lower in agricultural than forested sites, and in turn the quality of aquatic insects in terms of FA, SFA, MUFA and PUFA export is decreased in agricultural sites in comparison to forested sites.

The agricultural site was always downstream of the forested site. Hence, the results may partially be influenced by a location effect, where downstream sites are typically larger (Vannote et al., 1980). Though, the distance between sites within one stream was low and a similar study found that invertebrate populations from the upstream and downstream site were connected (Schneeweiss et al., 2023). Thus, the spatial location effect is likely negligible compared to the influence of land use. One reason for the lower FA, SFA, MUFA, and PUFA export in agricultural sites may have been energy costs due to agricultural stressors (for details of agricultural stressors see "4.3 Agricultural stressors associated with FA profiles"). Typically, in moderate stress conditions the cost for maintenance increases to meet the enhanced energy demand for protection against stressors and the repair of damages (Calow & Forbes, 1998; I. M. Sokolova et al., 2012). This can lead to a consumption of energy reserves like lipids like neutral lipid FA (Azeez et al., 2014; Lannig et al., 2006; Sokolova et al., 2012). Additionally, ingested FA, including PUFA, may be directly oxidized (β oxidation) to carbon dioxide and water to generate adenosine triphosphate (ATP, Gilbert, 1967; Tocher, 2003). The β oxidation of FA is a very efficient ATP source, which can facilitate ATP dependent mechanisms like the elimination of toxicants, detoxification and the repair or replacement of damaged molecules (Sokolova, 2021). Furthermore, agricultural stressors like pesticides can alter the sex ratio of emergent aquatic insects (Hahn et al., 2001) and FA profiles as well as export can differ between male and female aquatic insects (Gerber et al., 2022; Pietz, Kainz, et al., 2023; Scharnweber et al., 2020). For example, female non-biting midges were associated with higher SFA levels and a higher total FA content, while male non-biting midges were associated with higher PUFA levels (Pietz, Kainz, et al., 2023). Future studies, including amongst others, the sex ratio of aquatic insects and the energy costs to cope with agricultural stressors can help to better understand the decrease in FA, SFA, MUFA, as well as PUFA export in agricultural sites, despite the higher biomass export in comparison to forested sites.

The smaller PUFA export in agricultural sites may have consequences for riparian predators like decreased growth and impaired immune response (Fritz et al., 2017; Mayntz & Toft, 2001; Twining et al., 2016). The extent of the effects on riparian predators depends on their foraging strategy (Twining, Parmar, et al., 2021) and riparian predators may need to invest more time and energy in foraging to meet their PUFA demand, if the PUFA content in their food sources is decreased (Schoener, 1971; Senécal et al., 2021; Twining et al., 2018). This may in turn impair their fitness (Naef-Daenzer & Keller, 1999; Schoener, 1971; Twining et al., 2018).

The difference in FA export via stoneflies should be interpreted with caution, because only four observations (three in spring, one in summer) were available for agricultural sites, while in forested sites 33 observations (17 in spring, 13 in summer, three in autumn) were used in the HGAM. Furthermore, our sampling method missed stoneflies emerging by crawling on land, which may have led to an underestimation of the FA, SFA, MUFA and PUFA export via certain stonefly families. Notwithstanding, previous studies in our study region found only few stoneflies in agricultural streams (Englert et al., 2015; Schneeweiss et al., 2022; Voß & Schäfer, 2017). Our sampling intervals may have allowed aquatic insects to utilize FA while being trapped for maximum 2 – 3 days, thereby resulting in a potential underestimation of FA export. Given that the sampling intervals were similar in both land-use types, this very likely does not affect comparisons between land-use types. Furthermore, the consumption of aquatic prey by riparian predators may also occur several days after the day of their emergence. Thus, the sampling interval may provide a realistic estimation of FA available for riparian predators. However, without being trapped the FA profiles of emergent aquatic insects feeding as adults (non-biting midges, stoneflies, some caddisflies, Armitage et al., 1995; Brittain, 1990; Petersson & Hasselrot, 1994) may also change due to the consumption of terrestrial food sources. How the feeding as adults will affect the FA profiles of emergent aquatic insects will depend, for instance, on the assimilation time of terrestrial-derived FA in the tissue of adult emergent aquatic insects and their ability to synthesize FA (Galloway & Budge, 2020). Additionally, we omitted the FA content of other fly families than non-biting midges in the total export of FA, which also lead to an underestimation of the total FA export. Though, the biomass of non-biting midges peaked at least a factor of ten higher than the biomass of other fly families (Ohler, Schreiner, Link, Liess, et al., 2023).

In spring, the PUFA export was higher than in the other seasons in both land-use types. Therefore, during spring riparian predators may have benefited most from the nutritional quality in the sense of PUFA of aquatic insects, because PUFA can enhance growth, reproductive success, and immune response in riparian predators (Fritz et al., 2017; Mayntz & Toft, 2001; Twining et al., 2016, 2018). Especially, for riparian birds breeding in spring this is favorable, because PUFA intake via aquatic insects seems to be crucial for their reproductive success (Shipley et al., 2022; Twining et al., 2018).

4.6.2 Differences of fatty acid profiles between agricultural and forested sites

We found differences in FA profiles of mayflies, caddisflies and non-biting midges between agricultural and forested sites (Table 4.1, 4.2, Figure C.2). In all three orders, ALA, EPA, ODA and EA contributed most to the differences in FA profiles. EPA tended to have higher proportions in agricultural than forested sites, while ODA and EA tended to reach higher proportions in forested sites (Table 4.2, Figure C.2).

The differences across FA profiles may have originated from direct effects on aquatic insects. For instance, agricultural stressors probably required aquatic insects of agricultural sites to invest more energy into maintenance and repair processes (Calow & Forbes, 1998; lSokolova et al., 2012) compared to insects of forested sites. Thereby, FA may have been used to meet the increased energy demand (Tocher, 2003). Specific agricultural stressors are discussed in the section “4.3 Agricultural stressors associated with FA profiles”.

Furthermore, the differences in FA profiles of aquatic insects may have originated from indirect effects in the aquatic food web because FA are transferred from primary producers to higher trophic levels (Kainz et al., 2004; Strandberg et al., 2015). In headwater streams, conditioned leaves may be an important food source (Graça & Canhoto, 2006; Vannote et al., 1980). Conditioned leaves are colonized by microorganisms like aquatic fungi, which have been shown to alter the FA content of leaves (Zubrod et al., 2017). The FA octadecanoic acid (18:0, ODA) is commonly found in aquatic fungi (Arce Funck et al., 2015; Zubrod et al., 2017) and tended to be higher in forested than agricultural sites. The percentage of shading tended to be smaller in agricultural than in forested sites (Figure C.3), which can lead to increased primary production in comparison to forested sites (Griffiths et al., 2013). Therefore, the tendency of higher eicosapentaenoic acid (20:5n-3, EPA) levels of aquatic insects in agricultural sites may have originated from the relatively high EPA levels in aquatic primary producers (Hixson et al., 2015; Kainz et al., 2004; Taipale et al., 2013). EPA is an important membrane compound and serves as precursor for many bioactive molecules, e.g. eicosanoids (Arts et al., 2001; Stanley-Samuelson et al., 1988), this may affect the quality of emergent aquatic insects as food source for riparian predators. While the lower FA, SFA, MUFA, and PUFA content compromises the quality of emergent aquatic insects, the potential increase in single FA like EPA enhances the quality. Therefore, a higher EPA content may buffer potential negative effects of an overall lower FA content.

In addition, a turn-over of aquatic insect families between forested and agricultural sites was shown (Ohler, Schreiner, Link, Liess, et al., 2023) and may have contributed to the differences between FA profiles, driven by differences in the functional feeding groups and the trophic transfer of FA (Scharnweber et al., 2020). For instance, in summer four caddisfly families (Goeridae, Glossosomatidae, Phryganeidae, Philopotamidae) emerged only in forested sites and two caddisfly families (Lepidostomatidae, Limnephilidae) only in agricultural sites (Ohler, Schreiner, Link, Liess, et al., 2023). The latter two families are shredders, while the families emerging only in the forested sites belonged to the functional feeding groups grazers, shredders, collectors, and predators.

Although, we found differences in FA profiles of mayflies, caddisflies, and non-biting midges between forested and agricultural sites, we did not find any differences in FA profiles of spiders between forested and agricultural sites. Spiders are capable of extracting nutrients selectively from their prey to avoid nutritional imbalances (Mayntz et al., 2005). Moreover, spiders usually consume aquatic and terrestrial insects (Graf et al., 2020; Krell et al., 2015), thus also terrestrial insects contribute to the spiders' FA profile. Additionally, spiders are able to synthesize EPA de novo (Mathieu-Resuge et al., 2022), while it is unknown if they can also synthesize other FA. Therefore, the synthesis of EPA by spiders may have masked potential land-use related differences. In previous studies the EPA content in ground dwelling spiders correlated with the biomass of stoneflies (Kowarik et al., 2022), PUFA profiles of riparian spiders were more similar to the PUFA profiles of emerging aquatic insects than terrestrial insects (Kowarik et al., 2021), and riparian spiders relied more on the PUFA content of aquatic emergent insects than spiders further away from a forested lake (Twining et al., 2019).

However, it remains unclear how other riparian predators may have been affected by land use in our study, because the amount of aquatic insects in the diet of riparian predators can vary with the foraging strategy. For instance, ground-hunting and web-building spiders differ in their proportion of aquatic insects in their diet and in environmental factors affecting the amount of consumed aquatic insects (Graf et al., 2020; Krell et al., 2015). Additionally, birds that are aerial insectivores may consume more aquatic insects than gleaners, bark-probers, as well as ground-foragers (Schilke et al., 2020) and therefore may rely more on aquatic insect consumption to meet their PUFA demand (Twining et al., 2016, 2019) than gleaners (Twining, Parmar, et al., 2021). Future studies including riparian predators with different foraging strategies, are needed to understand the effect of land use on FA profiles in the riparian food web better.

4.6.3 Agricultural stressors associated with fatty acid profiles

Generally, environmental variables associated with impaired habitat quality for aquatic insects and spiders (Graf et al., 2019, 2020; Ohler, Schreiner, Link, Liess, et al., 2023; Raitif et al., 2018; Stenroth et al., 2015) were less favorable in agricultural than forested sites e.g., higher pesticide toxicity as well as lower percentage of pool habitats and less shading (Figure C.3, Table C.1). Nonetheless, little variation in FA profiles of aquatic insects in total and on order level was explained by these variables.

Primary production and nutrient availability in streams depend on light availability and can decrease with the increase of shading (Griffiths et al., 2013). Therefore, shading and nutrients may affect aquatic insects‘ FA profiles by the trophic transfer of FA from primary producers to higher trophic levels (Hixson et al., 2015; Kainz et al., 2004; Ohler, Schreiner, Martin-Creuzburg, & Schäfer, 2023; Taipale et al., 2013). The effect of pool habitats on FA profiles may be explained by differences at the base of the food web (algal primary production, conditioned leaves, (Keithan & Lowe, 1985; Whitledge & Rabeni, 2000) and the occurrence of different functional feeding groups in pool and riffle habitats (Angradi, 1996), which in turn can result in different FA profiles. EC (commonly used to estimate the salinity of water) can cause osmoregulatory stress and can be associated with ions that are toxic for aquatic insects (Cañedo-Argüelles et al., 2013). Together with pesticide toxicity, EC can increase the energy demand of aquatic insects (Calow & Forbes, 1998; Sokolova et al., 2012), followed by FA utilization to fulfill the enhanced energy demand (Tocher, 2003), and thereby altering the FA profiles of aquatic insects. To our knowledge, it is currently not known if specific FA are utilized or FA in general. Increasing temperature, as observed in agricultural sites, can cause FA profile alterations, as organisms adapt their PUFA content to adjust membrane fluidity to higher temperatures (Arts & Kohler, 2009; Fuschino et al., 2011). However, the land-use intensity in the studied agricultural sites was similar. Furthermore, the intensity of agriculture and potentially of agricultural stressors may increase in the future, as for instance globally more pesticides with a higher toxicity towards aquatic insects are used (Bernhardt et al., 2017; Kattwinkel et al., 2011; Schulz et al., 2021).

Though, most variation in all FA profiles was explained by stream and season (Table C.6). The families of aquatic insects differed across streams and seasons. For instance, the mayfly families Arthropleidae and Siphlonuridae emerged only in summer (Ohler, Schreiner, Link, Liess, et al., 2023). Therefore, the composition of aquatic insect assemblages may be more important for the FA profiles than agricultural stressors. This is partly in line with Kowarik et al. (2022), who only found an effect of season on the FA profiles of aquatic insects, but not of environmental variables. Furthermore, the FA profiles of species of non-biting midges were shown to differ (Scharnweber et al., 2020). Future studies identifying underlying mechanisms of the differences between families are needed to estimate the effect of a turn-over of aquatic insect assemblages between land-use types on FA profiles.

4.7 Conclusion

The quantity of PUFA export via aquatic insects was decreased in agricultural sites in comparison to forested sites. Additionally, we found differences in FA profiles of aquatic insects between land-use types. We suggest a decreased quality as food source for riparian predators relying on the dietary intake of PUFA. Future studies are needed to identify the mechanisms behind the lower PUFA content in agricultural sites to implement strategies maintaining the PUFA content in aquatic insects. These strategies may focus on the mitigation of stressors that may affect aquatic insects, for example decreasing of pesticide exposure and reforestation to decrease temperature in agricultural streams. Furthermore, our results can be incorporated in modeling food-webs or meta-ecosystems to increase our understanding of effects of timing, food quantity as well as quality in these systems.

4.8 Acknowledgements

We thank Moritz Schäfer, Tim Ostertag and Laura Kieffer for their assistance in lyophilizing and weighing aquatic insects as well as spiders, Nadin Graf for support during identification of aquatic insects and Kilian Kenngott for technical support during the FA analysis. We thank the four anonymous reviewers for their helpful comments that improved the quality of the manuscript. The field study was facilitated by the “Pilotstudie zur Ermittlung der Belastung von Kleingewässern in der Agrarlandschaft mit Pflanzenschutzmittel-Rückständen” funded by the German Federal Ministry for the

Environment, Nature Conservation and Nuclear Safety (FKZ 3717 63 403 0) and the German Helmholtz long-range strategic research funding. The Modular Observation Solutions for Earth Systems (MOSES) and the German Research Foundation (DFG; project number: 216374258) supplied additional funding. The FA analyses were funded by the DFG (Project Number 326210499/GRK 2360). Katharina Ohler was funded by the German Academic Scholarship Foundation as well as the Interdisziplinäres Promotions- und Postdoczentrum (IPZ) completion scholarship.

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5. General discussion and conclusion

5.1 Higher quantity and altered quality of emergent aquatic insects in agricultural sites

The quantity in terms of total biomass as well as abundance was higher (61 – 68 and 79 – 86%, respectively) in agricultural (95% confidence interval (CI): 0.106 – 0.124 kg m^{-2}, 431,383 – 510,017 ind m^{-2}) than forested sites (95% CI: 0.066 – 0.074 kg m^{-2}, 241,499 – 274,701 ind m^{-2}, chapter 3). By contrast, the FA, SFA, MUFA, and PUFA export via aquatic insects were lower in agricultural sites (26 – 29%, 21 – 23%, 24 – 26%, 29 – 33%, respectively) in comparison to forested sites (chapter 4). The opposite trends in quantity and FA, SFA, MUFA, and PUFA export of emergent aquatic insects may be caused by environmental variables associated with agricultural land use. The increased quantity may be traced back to enhanced nutrient supply and reduced shading, which may increase the biomass of emergent aquatic insects (Carlson et al., 2016; Griffiths et al., 2013; Stenroth et al., 2015; Terui et al., 2018). Other environmental variables associated with agricultural land use like pesticide toxicity, oxygen depletion, and increased electrical conductivity (EC) can stress organisms. Stressed organisms need to invest more energy for maintenance (Calow & Forbes, 1998; Sokolova et al., 2012). As a result, FA, SFA, MUFA, and PUFA may have been utilized to satisfy the enhanced energy demand (Tocher, 2003).

The decreased export of FA and PUFA may reduce the quality of emergent aquatic insects as food source for riparian predators. For instance, FA were shown to increase the growth rate of spiders (Mayntz & Toft, 2001), and PUFA the growth and immune response of birds and spiders (Fritz et al., 2017; Twining et al., 2016), as well as the body condition and fresh weight of spiders (chapter 3). In addition, due to decreased FA and PUFA contents in their food sources, riparian predators may investment more time and energy in foraging to meet their FA and PUFA demand (Schoener, 1971; Senécal et al., 2021; Twining et al., 2018). As a result, this can reduce their fitness (Naef-Daenzer & Keller, 1999; Schoener, 1971; Twining et al., 2018). Further details of the effects of environmental variables associated with agricultural land use on the quantity and quality of emergent aquatic insects are discussed in section 5.2 (Environmental variables associated with agricultural land use linked with quantity and quality of emergent aquatic insects).

Furthermore, differences in the FA profiles between land-use types were observed for mayflies as well as flies in spring and caddisflies in summer (chapter 4). These differences may be explained by varying FA profiles across families resulting from different functional feeding groups and the trophic transfer of FA (Scharnweber et al., 2020). For example, in summer four caddisfly families (Goeridae, Glossosomatidae, Phryganeidae, Philopotamidae) emerged only in forested and two caddisfly families (Lepidostomatidae, Limnephilidae) only in agricultural sites (chapter 6, Figure B14, Table B.9). The first four families comprise the functional feeding groups grazers, shredders, collectors, and predators, while the latter two families are shredders. Additionally, a turnover between agricultural and forested sites was observed (chapter 4, 6, Figure B.5). Further details on changes in the composition of emergent aquatic insect assemblages are displayed in section 5.2 (Turnover and shifted phenology of emergent aquatic insects in agricultural sites).

If differences in the FA profiles were found, the FA contributing most to these differences were identified. Eicosapentaenoic acid (20:5n-3, EPA) was one of these FA and tended to reach higher proportions in agricultural than forested sites (chapter 4, Table 4.2). Since EPA is an important membrane compound and serves as precursor for many bioactive molecules, e.g., eicosanoids (Arts et al., 2001; Stanley-Samuelson et al., 1988), this may affect the quality of emergent aquatic insects as food source for riparian predators. While the reduction in FA, SFA, MUFA, and PUFA content reduces the quality of emergent aquatic insects, the potential increase in single FA like EPA enhances the quality. Therefore, a higher EPA content may buffer potential negative effects of overall FA reduction. Particularly, because many organisms are incapable to synthesize EPA *de novo* (Twining et al., 2021) and EPA synthesis is energetically costly (Mathieu-Resuge et al., 2022; Twining et al., 2021). The FA profiles of spiders between land-use types and effects of dietary PUFA intake on spiders are discussed in section 5.4 (Effect of food sources and agricultural land use on riparian predators).

5.2 Turnover and shifted phenology of emergent aquatic insects in agricultural sites

The number of emergent aquatic insect families was similar between both land-use types, but a turnover and different temporal patterns in the composition of emergent aquatic insect assemblages were identified (chapter 3, 6, Table B.9, Figure B.5). In agricultural sites, emergent aquatic insects with a short generation time tended to occur more often than in forested sites (chapter 3, 6, Table B.7, Figure B.6), which indicates a turnover towards emergent aquatic insects assemblages with a lower vulnerability to environmental variables associated with agricultural land use due to a higher recovery potential (Sherratt et al., 1999; Stark et al., 2004).

No difference in the size classes of emergent aquatic insects was found between both land-use types, but temporal patterns were observed. The temporal patterns in size of emergent aquatic insects were more pronounced in agricultural than forested sites. For instance, small-bodied flies were the main part of biomass during spring in agricultural sites, while in forest the biomass of small-bodied flies, larger-bodied mayflies, and stoneflies was similar (chapter 3, Figure 3.2). Smaller predators like spiders may have benefited more from emergent aquatic insects as food source than larger predators like birds in agricultural sites. In forested sites both may have benefited equally, as taxon-specific traits, such as size, determine which riparian predators may benefit most from emergent aquatic insects as food source (Davis et al., 2011; Stenroth et al., 2015). The smaller size together with the reduced FA, SFA, MUFA, and PUFA content of emergent aquatic insects during spring in agriculture, may especially affect the fitness of birds relying on larger-bodied high quality emergent aquatic insects during reproduction (Shipley et al., 2022; Twining et al., 2018).

The phenology of emergent aquatic insects was shifted in agricultural sites. Total biomass and abundance peaked 3 to 19 days earlier in agricultural than forested sites (chapter 3, Figure 3.1). Additionally, out of 24 families emerging in both land-use types, 17 (71%) families exhibited earlier biomass peaks in agricultural than forested sites (chapter 6, Table B.9, Figure B8 – B15). For locally bound predators a shift in the phenology may have adverse effects, especially if the shift in phenology causes a mismatch of life-cycles. In contrast, mobile predators, exploiting emergent aquatic insects as food source in the landscape, can benefit from a prolonged availability of aquatic insects due to shifts in phenology (Schindler & Smits, 2017). The changes in quantity, quality, and phenology of emergent aquatic insects may affect the riparian food web extensively by altering predator population dynamics (Sato et al., 2016) and as consequence other trophic levels like terrestrial insects and plants. For instance, the availability of emergent aquatic insects may increase predation on terrestrial herbivorous insects and, thereby, reduce herbivory (Graf et al., 2017; Henschel et al., 2001).

5.3 Environmental variables associated with agricultural land use linked with quantity and quality of emergent aquatic insects

The quantity in terms of biomass as well as abundance and quality in terms of FA of emergent aquatic insects were linked with different environmental variables associated with agricultural land use (chapter 4, 5). Pesticide toxicity as well as electrical conductivity were the most important environmental variables associated with agricultural land use for quantity and percentage of shading as well as pool habitats for quality of emergent aquatic insects. Though, environmental variables associated with agricultural land use explained only little variation (3 – 12%, chapter 6) in the FA profiles of emergent aquatic insects, while the changes in the quantity were explained up to 26% by environmental variables associated with agricultural land use (chapter 3). How environmental variables associated with agricultural land use have affected riparian predators is discussed in section 5.4 (Effect of food sources and agricultural land use on riparian predators).

The quality was probably mainly affected by the trophic transfer of FA from the base of the food web (Hixson et al., 2015; Kainz et al., 2004; Taipale et al., 2013) and functional feeding groups of emergent aquatic insect families (Guo et al., 2018; Scharnweber et al., 2020). Increased shading can reduce primary production in streams (Griffiths et al., 2013) and pool habitats reveal differences at the base of the food web in comparison to riffle habitats, namely reduced algal primary production and a higher proportion of conditioned leaves (Keithan & Lowe, 1985; Whitledge & Rabeni, 2000). Additionally, the PUFA profiles, so also likely the FA profiles, within the same species (i.e. same functional feeding group) of emergent aquatic insects can differ, due to the consumed food sources (chapter 2, 6). Different functional feeding groups are typically found in pool and riffle habitats (Angradi, 1996), which may result in different FA profiles, as grazers directly feed on primary producers and other functional feeding groups like shredders or detritivores consume conditioned leaves. The effect of functional feeding groups of emergent aquatic insect families is further supported by the higher proportion (up to 35%) of explained variance in FA profiles, when the effect of stream and season were included (chapter 6, Table C.6), as families with different functional feeding groups emerged across streams and seasons (chapter 6, Figure B8 – B15). For example, in summer the emergence of the grazing caddisfly family Psychomyiidae (tube-making caddisfly) and the predatory caddisfly family Rhyacophilidae (primitive caddisfly), consuming amongst others grazing aquatic insects (Nilsson, 1996), peaked.

The environmental variables associated with agricultural land use linked with changes in the quantity of emergent aquatic insects differed between orders of emergent aquatic insects and total biomass as well as total abundance. For instance, pesticide toxicity decreased the biomass of stoneflies, known to be more vulnerable to pesticides than other aquatic insects, but increased the biomass of less vulnerable emergent aquatic insects and the total biomass (Rico & Brink, 2015; Rubach et al., 2010). Though, total abundance did not react on the pesticide toxicity, due to the observed community turnover. Orders of emergent aquatic insects are known to react differently to environmental variables associated with agricultural land use (Carlson et al., 2016; Raitif et al., 2018; Stenroth et al., 2015). Therefore, aggregated responses like total biomass and total abundance hamper to identify complex effects of land use on emergent aquatic insects (Jähnig et al., 2021). No environmental variables associated with agricultural land use explained the number of EPT families. The number EPT families is also an aggregated response and may hamper to detect effects of land use, when a turnover and no decline of families of emergent aquatic insects is observed (chapter 3). Therefore, aggregated responses should be treated carefully to assess insect trends, drivers of insect assemblages and to implement protection strategies.

5.4 Effect of food sources and agricultural land use on riparian predators

Riparian spiders in one outdoor microcosm experiment exhibited different PUFA profiles across treatments due to their food sources (non-biting midges, Chironomidae) differing in PUFA profiles. Additionally, their fresh weight and body condition were affected by the interaction of treatment and time point (chapter 2, Table 2.4, Figure 2.3). Although, in the other shorter microcosm experiment, conducted at higher temperatures, no differences in the PUFA profiles of spiders and no effects on the fresh weight and body condition of spiders were identified (chapter 2, Table 2.4, Figure 2.3). Therefore, other factors, such as different durations of the experiments and environmental conditions, may have affected the PUFA profiles and responses of spiders.

However, in the field no environmental variables associated with agricultural land use explained the variation in FA profiles of spiders significantly. In addition, no differences in the FA profiles of spiders between forested and agricultural sites were found (chapter 4, Figure, 4.3, Table 4.1), though FA profiles of emergent aquatic insects differed partly between land-use types (chapter 4, section 5.1). In contrast to the microcosm experiments, the spiders probably consumed multiple food sources (Nyffeler, 1999; Uetz et al., 1992), including terrestrial insects (Graf et al., 2020; Krell et al., 2015). Furthermore, spiders may be capable to extract nutrients selectively from their food sources or adapt foraging strategies to avoid nutritional imbalances (Mayntz et al., 2005). Despite the FA profiles of spiders did not differ between land-use types, the total FA content may have differed. A previous study has shown higher FA content in spiders of forested than agricultural sites (Ramberg et al., 2020). As spiders can serve as food source for higher trophic levels like birds (Poulin et al., 2010), an altered FA content may affect higher trophic levels, although the FA profiles remain unchanged. However, it remains open if for other riparian predators the FA profiles were similar between land-use types and not relevantly affected by environmental variables associated with agricultural land use. Especially, birds may rely on the dietary intake of PUFA during reproduction (Shipley et al., 2022; Twining et al., 2018). Future studies including higher trophic levels as well as terrestrial insects to assess the effect of land use on FA profiles of riparian predators are needed.

5.5 Conclusion

The quantity, quality, composition, and phenology of emergent aquatic insects differed between land-use types. Thereby, aquatic-terrestrial linkages may be altered in agricultural sites and, in turn ecosystem functioning, because linkages between ecosystems are crucial for ecosystem functioning (Bauer & Hoye, 2014; Reiss et al., 2009). To increase the knowledge of altered aquatic-terrestrial linkages and its effects on ecosystem functioning the data of the quantity, quality, composition, and phenology of emergent aquatic insects in the primary emergence period and associated seasonal FA profiles of riparian spiders can be included in modeling ecosystem functioning in the riparian ecosystems receiving emergent aquatic insects.

As ecosystem functioning is a requirement for ecosystem services (Harvey et al., 2017; MEA, 2005; Truchy et al., 2015), human-well being may be affected by the changes in quantity, quality, composition, and phenology of emergent aquatic insects in agricultural sites. For instance, decreased fitness of riparian predators, because of reduced food quality in terms of PUFA (Twining et al., 2018), can reduce the ecosystem service pest control and recreational opportunities. Therefore, future studies including for example further trophic levels are needed to estimate the effects of altered quantity, quality, composition, and phenology of emergent aquatic insects on human well-being.

Depending on the environmental context, the weight and body condition of riparian spiders can be affected by the PUFA profiles of their food sources. The environmental context includes for example, duration of PUFA assimilation and environmental variables like temperature. Additionally, environmental variables can affect the PUFA as well as FA profiles of organisms. Future studies including a wide range of environmental variables will improve the understanding of the effects of food quality in terms of FA and PUFA in ecosystems.

Furthermore, pesticide toxicity was one major environmental variable associated with agricultural land use explaining the quantity of emergent aquatic insects and as emergent aquatic insects are a part in the riparian food web, changes in their quantity due to pesticide toxicity can affect other trophic levels. These effects may have far reaching consequences in the entire riparian food web, for instance altered population dynamics (Sato et al., 2016) and population densities (Paetzold et al., 2011). This supports several previous studies (e.g., Liess et al., 2021; Schäfer, 2019; Schulz et al., 2021; Stehle & Schulz, 2015) indicating that the current authorization of pesticides fails its aim preventing not acceptable effects in the environment (Australian Environment Agency, 2009; EFSA, 2013; US Goverment, 2004). As a result, pesticide authorization should be adjusted to the current knowledge of pesticide exposure and effects in the environment.

In contrast to the quantity of emergent aquatic insects, the quality was mainly explained by stream and season differences, not by environmental variables associated with agricultural land use. Our study was conducted in agricultural sites with similar land-use intensity in a temperate region. Therefore, the observed effects of land use on emergent aquatic insects may be increased with increasing land-use intensity. In other climate regions, such as tropic regions with less seasonal temperature changes than temperate regions, aquatic insects may react differently towards environmental variables associated with agricultural land use. As intensive agriculture is a globally dominant land-use type (Václavík et al., 2013), future studies should consider the land-use intensity over a range of ecosystem types to assess the quantity, quality, composition as well as phenology of emergent aquatic insects to enable a global assessment of these variables and gather more information on global insect trends.

The aggregated parameters number of EPT families, total biomass, and abundance were linked with different or no environmental variables associated with agricultural land use or reacted differently than orders of emergent aquatic insects towards the same variables. Therefore, they can miss complex responses to environmental changes and may not be suitable to assess the anthropogenic impact on ecosystems. Furthermore, they are not sufficient to identify global trends of emergent aquatic insect families, especially because a turn-over of families is more likely than an overall decline (Dornelas et al., 2019). To protect aquatic insects and predict global insect trends, less aggregated parameters like the biomass of single emergent aquatic insect orders should be preferably used.

5.6 References

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6. Appendix

6.1 Supplementary material

A Supplementary material: Trophic transfer of polyunsaturated fatty acids across the aquatic-terrestrial interface: an experimental tritrophic food chain approach

Table A.1: Spiders used in the experiment. T is the abbreviation for the genus *Tetragnatha*. NA: Spiders used at the beginning of the experiment to analyze the polyunsaturated fatty acids (PUFA) profiles, immune response, and dry weight.

treatment	**experiment**	**experiment ID**	**original spider ID**	**species**	**sex**	**adult/juvenile**
NA	1	21	21	*T. montana*	m	a
NA	1	22	22	*T. montana*	m	a
NA	1	24	24	*T. montana*	f	a
NA	1	38	38	*T. montana*	m	a
NA	1	39	39	*T. montana*	f	a
NA	1	44	44	*T. montana*	m	a
NA	1	48	48	*T. montana*	f	a
NA	1	49	49	*T. montana*	f	a
NA	1	59	59	*T. montana*	f	a
NA	1	63	63	*T. montana*	f	a
NA	1	65	65	*T. montana*	f	a
NA	1	70	70	*T. montana*	f	a
NA	1	74	74	*T. montana*	f	a
NA	1	78	78	*T. montana*	f	a
NA	1	79	79	*T. montana*	f	a
NA	1	81	81	*T. montana*	f	a
NA	1	86	86	*T. montana*	f	a
NA	1	106	106	*T. montana*	f	a
NA	1	111	111	*T. montana*	f	a
NA	1	115	115	*T. montana*	f	a
algae	1	algae_1	104	*T. montana*	f	a
algae	1	algae_2	75	*T. montana*	f	a
algae	1	algae_3	40	*T. montana*	f	a
algae	1	algae_4	88	*T. montana*	f	a
algae	1	algae_4	122	*T. montana*	f	a
algae	1	algae_5	82	*T. montana*	f	a
algae	1	algae_6	41	*T. montana*	f	a
algae	1	algae_7	118	*T. montana*	f	a
algae	1	algae_8	89	*T. montana*	f	a
algae	1	algae_9	116	*T. montana*	f	a
algae	1	algae_10	34	*T. montana*	f	a
algae	1	algae_11	114	*T. montana*	f	a
algae	1	algae_12	97	*T. montana*	f	a
algae	1	algae_13	103	*T. montana*	f	a
algae	1	algae_14	62	*T. montana*	f	a
algae	1	algae_15	33	*T. montana*	f	a
algae	1	algae_16	37	*T. montana*	f	a
algae	1	algae_17	100	*T. montana*	f	a
algae	1	algae_18	92	*T. montana*	f	a
algae	1	algae_19	105	*T. montana*	f	a

Table A.1: Continued.

treatment	**experiment**	**experiment ID**	**original spider ID**	**species**	**sex**	**adult/juvenile**
algae	1	algae_19	124	*T. sp.*	f	j
algae	1	algae_20	77	*T. montana*	f	a
fish food	1	fishfood_1	93	*T. montana*	f	a
fish food	1	fishfood_2	95	*T. montana*	f	a
fish food	1	fishfood_3	55	*T. montana*	f	a
fish food	1	fishfood_4	53	*T. montana*	f	a
fish food	1	fishfood_5	25	*T. montana*	f	a
fish food	1	fishfood_6	23	*T. montana*	f	a
fish food	1	fishfood_7	84	*T. montana*	f	a
fish food	1	fishfood_7	127	*T. sp.*	f	j
fish food	1	fishfood_8	71	*T. montana*	f	a
fish food	1	fishfood_9	50	*T. montana*	f	a
fish food	1	fishfood_10	107	*T. montana*	f	a
fish food	1	fishfood_11	120	*T. montana*	f	a
fish food	1	fishfood_12	64	*T. montana*	f	a
fish food	1	fishfood_13	121	*T. montana*	f	a
fish food	1	fishfood_14	117	*T. montana*	f	a
fish food	1	fishfood_15	45	*T. montana*	f	a
fish food	1	fishfood_16	108	*T. montana*	f	a
fish food	1	fishfood_17	69	*T. montana*	f	a
fish food	1	fishfood_18	43	*T. montana*	f	a
fish food	1	fishfood_19	52	*T. montana*	f	a
fish food	1	fishfood_20	67	*T. montana*	f	a
oatmeal	1	oatmeal_1	27	*T. sp.*	f	j
oatmeal	1	oatmeal_1	126	*T. montana*	f	a
oatmeal	1	oatmeal_2	85	*T. montana*	f	a
oatmeal	1	oatmeal_3	42	*T. montana*	f	a
oatmeal	1	oatmeal_4	83	*T. montana*	f	a
oatmeal	1	oatmeal_5	54	*T. montana*	f	a
oatmeal	1	oatmeal_5	123	*T. montana*	f	a
oatmeal	1	oatmeal_6	94	*T. montana*	f	a
oatmeal	1	oatmeal_7	80	*T. montana*	f	a
oatmeal	1	oatmeal_8	72	*T. montana*	f	a
oatmeal	1	oatmeal_9	36	*T. montana*	f	a
oatmeal	1	oatmeal_10	60	*T. montana*	f	a
oatmeal	1	oatmeal_11	57	*T. montana*	f	a
oatmeal	1	oatmeal_12	73	*T. montana*	f	a
oatmeal	1	oatmeal_13	113	*T. montana*	f	a
oatmeal	1	oatmeal_14	66	*T. montana*	f	a
oatmeal	1	oatmeal_15	99	*T. montana*	f	a
oatmeal	1	oatmeal_16	68	*T. montana*	f	a
oatmeal	1	oatmeal_17	46	*T. montana*	f	a
oatmeal	1	oatmeal_18	96	*T. montana*	f	a
oatmeal	1	oatmeal_19	56	*T. montana*	f	a
oatmeal	1	oatmeal_20	90	*T. montana*	f	a

Table A.1: Continued.

treatment	**experiment**	**experiment ID**	**original ID**	**species**	**sex**	**adult/juvenile**
NA	2	133	133	*T. montana*	f	a
NA	2	138	138	*T. montana*	f	a
NA	2	163	163	*T. montana*	f	a
NA	2	164	164	*T. montana*	f	a
NA	2	165	165	*T. montana*	f	a
NA	2	168	168	*T. montana*	f	a
NA	2	181	181	*T. montana*	f	a
NA	2	187	187	*T. montana*	f	a
NA	2	202	202	*T. montana*	f	a
NA	2	203	203	*T. montana*	f	a
NA	2	215	215	*T. montana*	f	a
NA	2	216	216	*T. montana*	f	a
NA	2	225	225	*T. montana*	f	a
fish food	2	fishfood_1	198	*T. montana*	f	a
fish food	2	fishfood_1	148	*T. montana*	f	a
fish food	2	fishfood_2	170	*T. montana*	f	a
fish food	2	fishfood_2	147	*T. montana*	f	a
fish food	2	fishfood_3	219	*T. montana*	f	a
fish food	2	fishfood_4	175	*T. montana*	f	a
fish food	2	fishfood_4	136	*T. montana*	f	a
fish food	2	fishfood_4	162	*T. montana*	f	a
fish food	2	fishfood_5	196	*T. montana*	f	a
fish food	2	fishfood_6	180	*T. montana*	f	a
fish food	2	fishfood_7	161	*T. montana*	f	a
fish food	2	fishfood_8	213	*T. montana*	f	a
fish food	2	fishfood_9	158	*T. montana*	f	a
fish food	2	fishfood_10	206	*T. montana*	f	a
fish food	2	fishfood_11	139	*T. montana*	f	a
fish food	2	fishfood_12	214	*T. montana*	f	a
fish food	2	fishfood_12	166	*T. montana*	f	a
fish food	2	fishfood_13	201	*T. montana*	f	a
fish food	2	fishfood_14	131	*T. montana*	f	a
fish food	2	fishfood_15	212	*T. montana*	f	a
fish food	2	fishfood_16	140	*T. montana*	f	a
fish food	2	fishfood_17	185	*T. montana*	f	a
fish food	2	fishfood_18	137	*T. montana*	f	a
fish food	2	fishfood_19	199	*T. montana*	f	a
fish food	2	fishfood_19	177	*T. montana*	f	a
fish food	2	fishfood_20	188	*T. montana*	f	a
leaves	2	leaves_1	207	*T. montana*	f	a
leaves	2	leaves_1	153	*T. montana*	f	a
leaves	2	leaves_2	197	*T. montana*	f	a
leaves	2	leaves_2	184	*T. montana*	f	a
leaves	2	leaves_3	169	*T. montana*	f	a
leaves	2	leaves_4	150	*T. montana*	f	a
leaves	2	leaves_5	152	*T. montana*	f	a
leaves	2	leaves_6	226	*T. montana*	f	a
leaves	2	leaves_7	149	*T. montana*	f	a
leaves	2	leaves_8	142	*T. montana*	f	a
leaves	2	leaves_9	191	*T. montana*	f	a
leaves	2	leaves_10	174	*T. montana*	f	a

Table A.1: Continued.

treatment	**experiment**	**experiment ID**	**original spider ID**	**species**	**sex**	**adult/juvenile**
leaves	2	leaves_11	179	*T. montana*	f	a
leaves	2	leaves_11	209	*T. montana*	f	a
leaves	2	leaves_12	193	*T. montana*	f	a
leaves	2	leaves_13	220	*T. montana*	f	a
leaves	2	leaves_14	227	*T. montana*	f	a
leaves	2	leaves_15	183	*T. montana*	f	a
leaves	2	leaves_15	211	*T. montana*	f	a
leaves	2	leaves_16	194	*T. montana*	f	a
leaves	2	leaves_17	144	*T. montana*	f	a
leaves	2	leaves_17	178	*T. montana*	f	a
leaves	2	leaves_18	176	*T. montana*	f	a
leaves	2	leaves_19	210	*T. montana*	f	a
leaves	2	leaves_19	172	*T. montana*	f	a
leaves	2	leaves_20	192	*T. montana*	f	a
leaves	2	leaves_20	208	*T. montana*	f	a
oatmeal	2	oatmeal_1	221	*T. montana*	f	a
oatmeal	2	oatmeal_2	171	*T. montana*	f	a
oatmeal	2	oatmeal_2	182	*T. montana*	f	a
oatmeal	2	oatmeal_2	218	*T. montana*	f	a
oatmeal	2	oatmeal_3	186	*T. montana*	f	a
oatmeal	2	oatmeal_4	159	*T. montana*	f	a
oatmeal	2	oatmeal_5	204	*T. montana*	f	a
oatmeal	2	oatmeal_6	167	*T. montana*	f	a
oatmeal	2	oatmeal_7	222	*T. montana*	f	a
oatmeal	2	oatmeal_8	132	*T. montana*	f	a
oatmeal	2	oatmeal_9	146	*T. montana*	f	a
oatmeal	2	oatmeal_10	190	*T. montana*	f	a
oatmeal	2	oatmeal_11	130	*T. montana*	f	a
oatmeal	2	oatmeal_12	154	*T. montana*	f	a
oatmeal	2	oatmeal_13	189	*T. montana*	f	a
oatmeal	2	oatmeal_14	195	*T. montana*	f	a
oatmeal	2	oatmeal_15	134	*T. montana*	f	a
oatmeal	2	oatmeal_16	223	*T. montana*	f	a
oatmeal	2	oatmeal_17	157	*T. montana*	f	a
oatmeal	2	oatmeal_18	228	*T. montana*	f	a
oatmeal	2	oatmeal_19	135	*T. montana*	f	a
oatmeal	2	oatmeal_19	217	*T. montana*	f	a
oatmeal	2	oatmeal_20	160	*T. montana*	f	a

Table A.2: Number of samples used in the PUFA analysis, for dry weight and immune response.

experiment	treatment	time point (days)	trophic level	number samples
1	algae	0	spider	7
1	algae	23	chironomid	4
1	algae	23	basic food source	6
1	algae	23	spider	5
1	algae	44	chironomid	6
1	algae	44	basic food source	6
1	algae	44	spider	3
1	fish food	0	spider	6
1	fish food	23	chironomid	6
1	fish food	23	basic food source	6
1	fish food	23	spider	4
1	fish food	44	chironomid	5
1	fish food	44	basic food source	6
1	fish food	44	spider	3
1	oatmeal	0	spider	7
1	oatmeal	23	chironomid	6
1	oatmeal	23	basic food source	6
1	oatmeal	23	spider	5
1	oatmeal	44	chironomid	6
1	oatmeal	44	basic food source	6
1	oatmeal	44	spider	6
2	fish food	0	spider	4
2	fish food	14	chironomid	4
2	fish food	14	basic food source	4
2	fish food	14	spider	10
2	fish food	21	chironomid	2
2	fish food	21	basic food source	2
2	fish food	21	spider	6
2	leaves	0	spider	5
2	leaves	14	chironomid	5
2	leaves	14	basic food source	4
2	leaves	14	spider	10
2	leaves	21	chironomid	4
2	leaves	21	basic food source	6
2	leaves	21	spider	6
2	oatmeal	0	spider	4
2	oatmeal	14	chironomid	4
2	oatmeal	14	basic food source	5
2	oatmeal	14	spider	10
2	oatmeal	21	chironomid	3
2	oatmeal	21	basic food source	2
2	oatmeal	21	spider	6

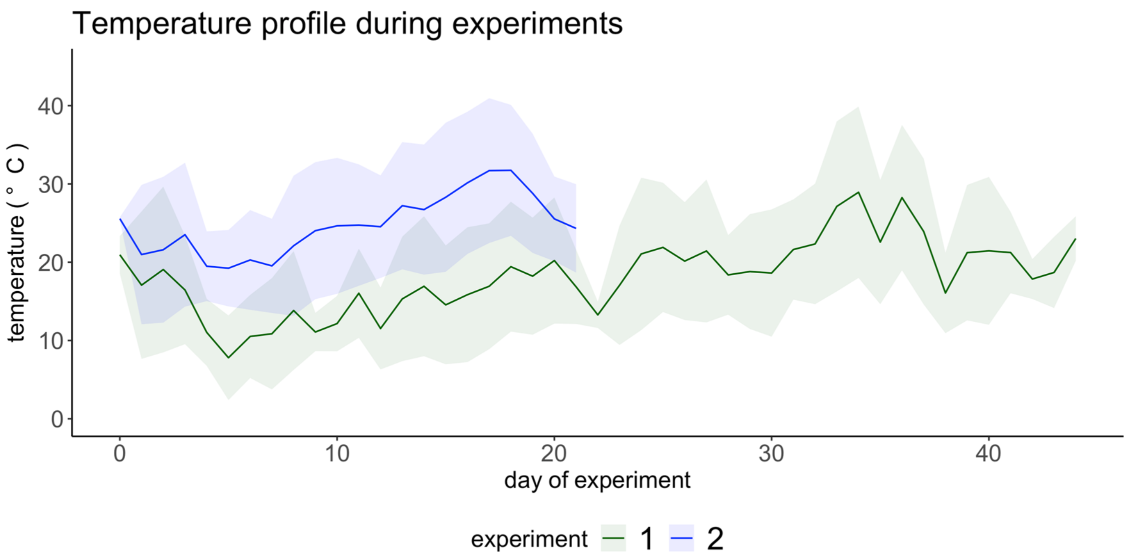

Figure A.1: Temperature profiles during the two microcosm experiments. Green: First experiment. Blue: Second experiment. The lines represent the mean temperature per day and the ribbon the standard deviation of the mean temperature per day. The first experiment took place from 29th of April to the 12th of June 2019 and the second experiment was run from the 8th to 29th of July 2019. The first experiment included algae, fish food and oatmeal as basic food treatment and the second experiment included leaves, fish food and oatmeal.

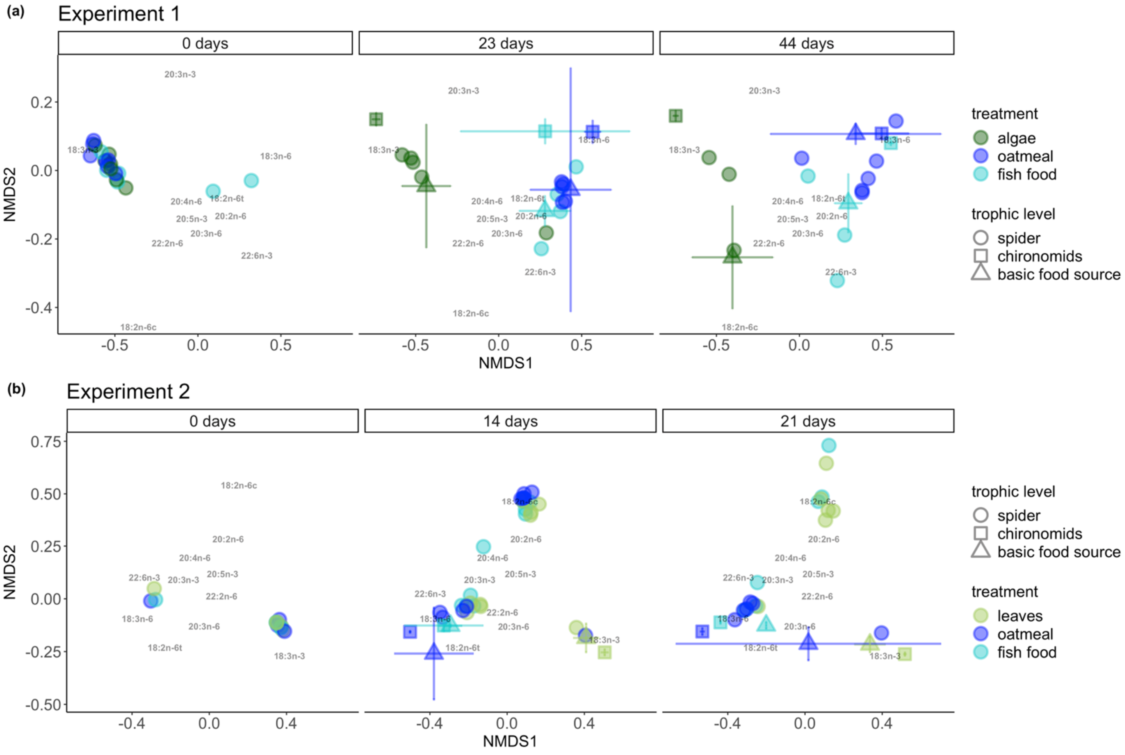

Figure A.2: Non-metric multidimensional scaling (NMDS) of the polyunsaturated fatty acid (PUFA) profiles in the first (A) and second (B) experiment. Colors indicate treatment, shape indicates trophic level. The first experiment included algae, fish food and oatmeal as food treatment, whereas the second experiment included leaves, fish food and oatmeal. For the chironomids and basic food sources mean and standard deviation are presented. On day 0, only PUFA profiles of spiders were analyzed to gain their starting values before they were fed with chironomids of the different treatments and the spiders were assigned randomly to the treatments. Chironomids and basic food sources were sampled and analyzed as soon as the spiders were fed with them. 20:5n-3: eicosapentaenoic acid (EPA), 20:4n-6: arachidonic acid (ARA), 18:3n-3: α-linolenic acid (ALA), 18:2n-6: linoleic acid (LIN), 18:3n-6: ɣ-linolenic acid (GLA), 18:2n-6t: linolelaidic acid (LLA).

Analytical procedure polyunsaturated fatty acids (PUFA)

In this study, we conducted the extraction of PUFAs following a modified procedure by (Folch et al., 1957). PUFAs were extracted in 5 mL chloroform/methanol (v:v; 2:1). After the addition of an internal standard (C17:00 200 µg/mL; C23:0 250 µg/mL, Sigma-Aldrich) the samples were vortexed for 10 s and extracted over night at -20 °C. The amount of internal standard added to the sample depended on the sample weight (Table A.3).

After the extraction, the samples were filtered with a syringe filter (PTFE, 13 mm, 0.45 µm, BGB), to remove all solid particles. Before the samples were filtered, the syringe filters were washed with chloroform/methanol (v:v; 2:1) three times to reduce baseline noise. The filtered samples were evaporated under nitrogen at 40 °C to dryness and dissolved in methanol during vortexing every sample for 30 s. After 60 s the dissolved samples were transferred to GC-vials. Their headspace was filled with nitrogen and the samples were stored at -20 °C until derivatization to fatty acid methyl esters (FAME).

Methanolic trimethylsulfonium hydroxide (TMSH, 0.2 M, Macherey-Nagel) was used as the derivatization agent, because it was shown to be suitable for PUFA derivatization (Butte, 1983; Gómez-Brandón et al., 2008, 2010). Furthermore, derivatization with TMSH is time-saving, because it is a fast and single-step reaction, which can be done at room temperature (Yamauchi et al., 1979). We pipetted 20 µL of the sample and 10 µL of TMSH in a GC-vial, vortexed for 30 s and then the sample was kept at room temperate until it was measured with a GC-FID (Varian CP-3800, Varian Inc). Nitrogen was the carrier gas with a constant flow of 0.7 mL min^{-1} and 1 µL of the sample was injected splitless at 250 °C. The temperature program of the column oven is shown in Table A.4.

Table A.3: Volume of added internal standard and volume of methanol used to dissolve PUFA, depending on the dry weight of the spiders.

Weight sample (mg)	**Volume Internal Standard (µL)**	**Volume methanol (µL)**
<13	50	50
30	50	100
45	100	150
60	100	200
75	150	250
90	150	300

Table A.4: Temperature program of the column oven.

Heating rate (°C min^{-1})	**Temperature (°C)**	**hold time (min)**
0	60	1
30	150	0
10	180	0
2	205	20
10	220	20

Calculation PUFA content

The samples were measured in three campaigns: January – March 2020, May – June 2020 and November 2020. For every campaign, one calibration curve, limit of quantification (LOQ) and recovery were calculated. For all calculations, R version 4.2.0 (R Core Team, 2022) was used.

The external standards SUPELCO 37 Component FAME mix, ALA- and 18:1n-7-FAME (Sigma-Aldrich) were measured in order to obtain the calibration curve. Failed measurements, measurements with bad quality and outliers were omitted from the calibration. We used linear calibration curves with an intercept of 0 because this increased R^2 of the calibration curves and therefore the quality of the calibration.

The LOQ was determined using blank samples. The blank samples went through the same extraction procedure as the samples but did not contain any PUFA. The mean area of the peaks in the blank samples was compared with the mean area of the peaks in the calibration curves. As LOQ the concentration of standard was set, where the mean peak area in the standard was a factor of three higher than the mean peak area in the blank samples. Concentrations in the samples below the LOQ were set to 0.

The recovery of PUFAs was calculated by dividing the concentration of the internal standard 23:0 measured in the samples by the added concentration of 23:0. The recovery was included in the calculation of the PUFA concentration of the samples. The data and R-Scripts are openly available in GitHub at https://doi.org/10.5281/zenodo.7692685.

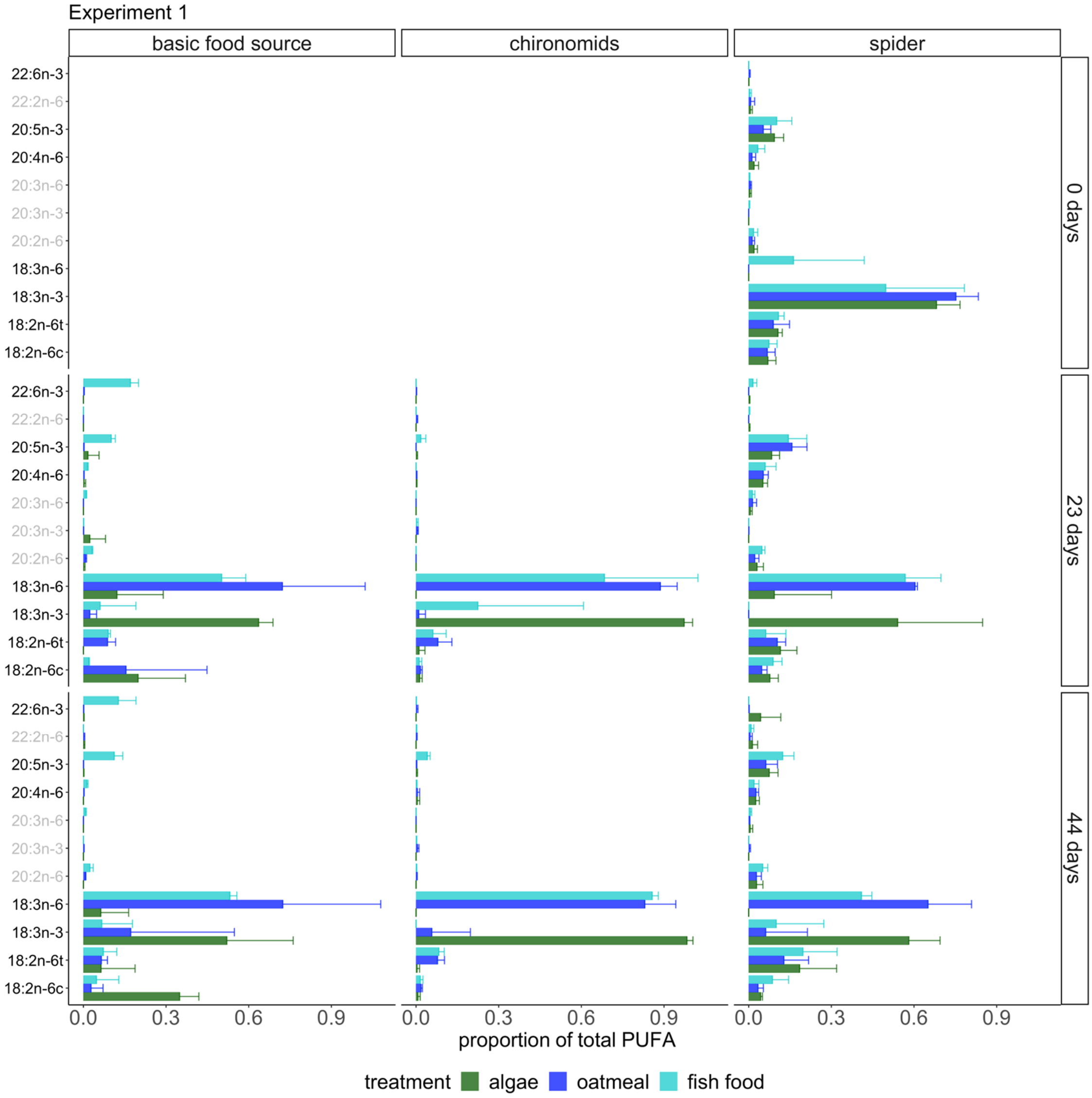

Figure A.3: Mean proportion and standard deviation of polyunsaturated fatty acids (PUFA) in the first microcosm experiment. Colors indicate the treatments algae, oatmeal and fish food. On day 0, only PUFA profiles of spiders were analyzed to gain their starting values before they were fed with chironomids of the different treatments and the spiders were assigned randomly to the treatments. Eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6), α-linolenic acid (ALA, 18:3n-3), ɣ-linolenic acid (GLA, 18:3n-6), linolelaidic acid (LLA, 18:2n-6t) and linoleic acid (LIN, 18:2n-6) are written in black and the other PUFA in grey.

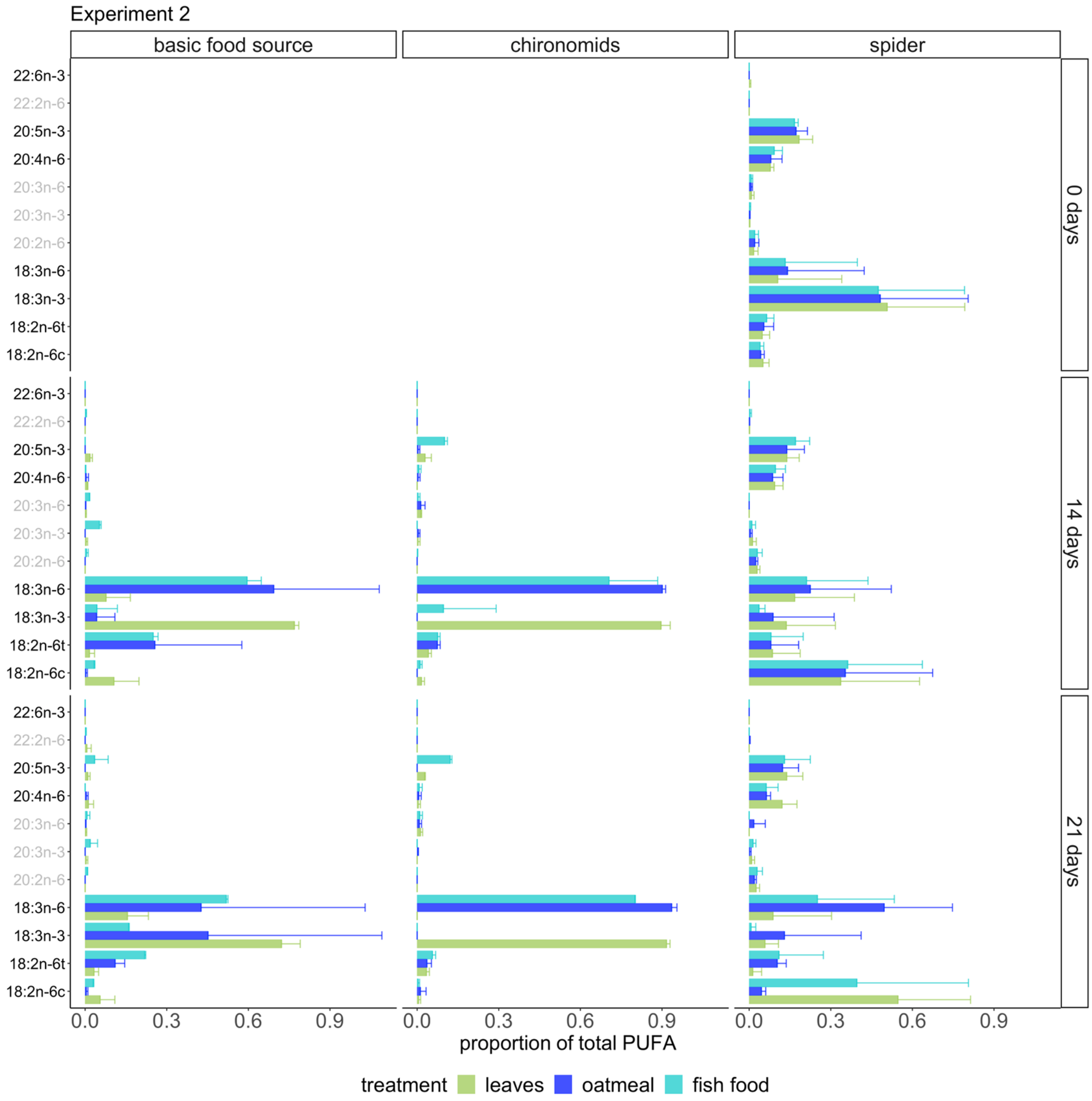

Figure A.4: Mean proportion and standard deviation of polyunsaturated fatty acids (PUFA) in the second microcosm experiment. Colors indicate the treatments leaves, oatmeal and fish food. On day 0, only PUFA profiles of spiders were analyzed to gain their starting values before they were fed with chironomids of the different treatments and the spiders were assigned randomly to the treatments. Eicosapentaenoic acid (EPA, 20:5n-3), arachidonic acid (ARA, 20:4n-6), α-linolenic acid (ALA, 18:3n-3), ɣ-linolenic acid (GLA, 18:3n-6), linolelaidic acid (LLA, 18:2n-6t) and linoleic acid (LIN, 18:2n-6) are written in black and the other PUFA in grey.

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B Supplementary material: Land use changes biomass and temporal patterns of insect cross-ecosystem flows

Results family biomass and abundance

The abundance and biomass of flies were driven by non-biting midges (Chironomidae, Figure B.8 – B.9). Their abundance and biomass peaked in spring at 7 (±3) mg d^{-1} m^{-2} and 56 (±16) ind d^{-1} m^{-2} in agriculture and at 2 (±1) mg d^{-1} m^{-2} and 20 (±6) ind d^{-1} m^{-2} in forest. The other fly families peaked at least a factor of ten lower and at different times. During parts of the sampling campaign, non-biting-midges and drain flies (Psychodidae) revealed significantly higher biomass in agriculture, whereas phantom midges (Chaoboridae), meniscus midges (Dixidae), phantom crane flies (Ptychopteridae), and crane flies (Tipulidae) reached significantly higher levels in forest. By contrast, black flies (Simuliidae), limoniid crane flies (Limoniidae), and ballon/dagger flies (Empididae) showed significantly higher biomass in forest and agriculture at different time points.

The abundance and biomass patterns of mayflies were driven by small mayflies (Baetidae, Figure B.10 – B.11), which peaked in spring in agriculture at 5 (± 2) mg d^{-1} m^{-2} and 3 (± 1) ind d^{-1} m^{-2} as well as in forest at 3 (± 1) mg d^{-1} m^{-2} and 1 (± 1) ind d^{-1} m^{-2}. Other mayfly families peaked at least a factor of ten lower and mainly in summer. Flatheaded mayflies (Arthropleidae) and primitive minnow mayflies (Siphlonuridae) were not found in any agricultural site (i.e., zero biomass and abundance). The biomass of burrowing mayflies was significantly higher in forest than in agriculture, whereas the biomass of small mayflies was significantly higher in agriculture.

Both the abundance and biomass of all stonefly families peaked in spring but in agriculture at least a factor of ten lower than in the forest (Figure B.12 – B.13). Spring stoneflies were the main component of stonefly abundance and biomass, peaking at 1 (± 1) mg d^{-1} m^{-2} and 1 (± 1) ind. d^{-1} m^{-2} in forest. Needle flies (Leuctridae) and green stoneflies (Chloroperlidae) were not sampled in agricultural sites. The abundance of needle flies peaked higher than the abundance of willow flies, and the opposite was shown for biomass. Significant differences were shown for biomass and abundance for all families except green stoneflies.

The main components of caddisfly biomass were net spinning caddisflies (Hydropsychidae) in both land use types (Figure B.14–B.15). In forest, their biomass peaked at 5 (± 2) mg d^{-1} m^{-2} in summer, whereas their biomass in agriculture revealed three overlapping peaks with a maximum of 6 (± 3) mg d^{-1} m^{-2}. In contrast, their abundance peaked only once at 1 (± 1) ind. d^{-1} m^{-2} in agriculture. Furthermore, they accounted for the main part of abundance in agriculture, whereas in forest net-spinning and micro caddisflies (Hydroptilidae) drove abundance patterns, both peaking at 1 (± 1) ind d^{-1} m^{-2}. Biomass of net-spinning, northern (Limnephilidae), tube-making (Psychomyiidae) and long-horned caddisflies (Leptoceridae) was significantly higher in agriculture, whereas biomass of primitive caddisflies (Rhyacophilidae), weighted case makers (Goeridae), micro, and bushtailed caddisflies (Sericostomatidae) was significantly higher in forest. In forested sites no lepidostomatid case makers (Lepidostomatidae) and in agricultural sites no stonecase caddisflies (Uenoidae), weighted case makers, tortoise makers (Glossosomatidae), large caddisflies (Phryganeidae), or finger-net caddisflies (Philopotamidae) were found.

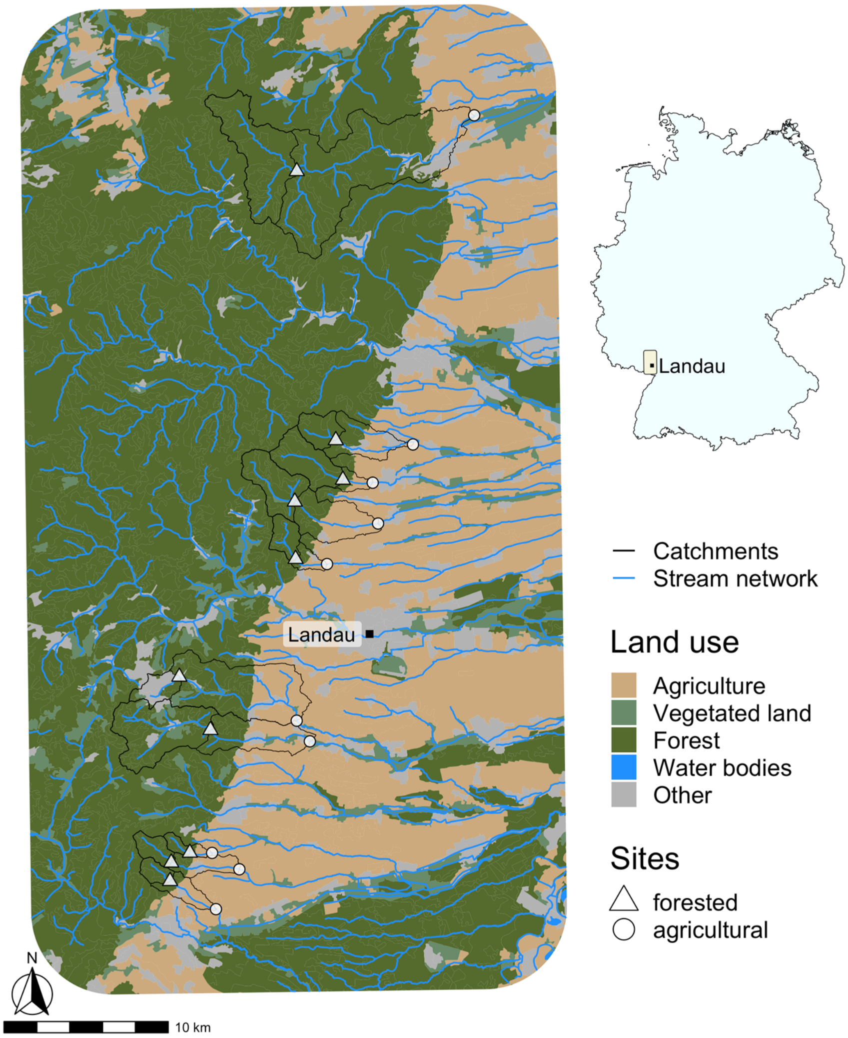

Figure B.1: Stream sampling sites in southwestern Germany (European Environment Agency, 2007). The inset map shows the location of the study site within Germany.

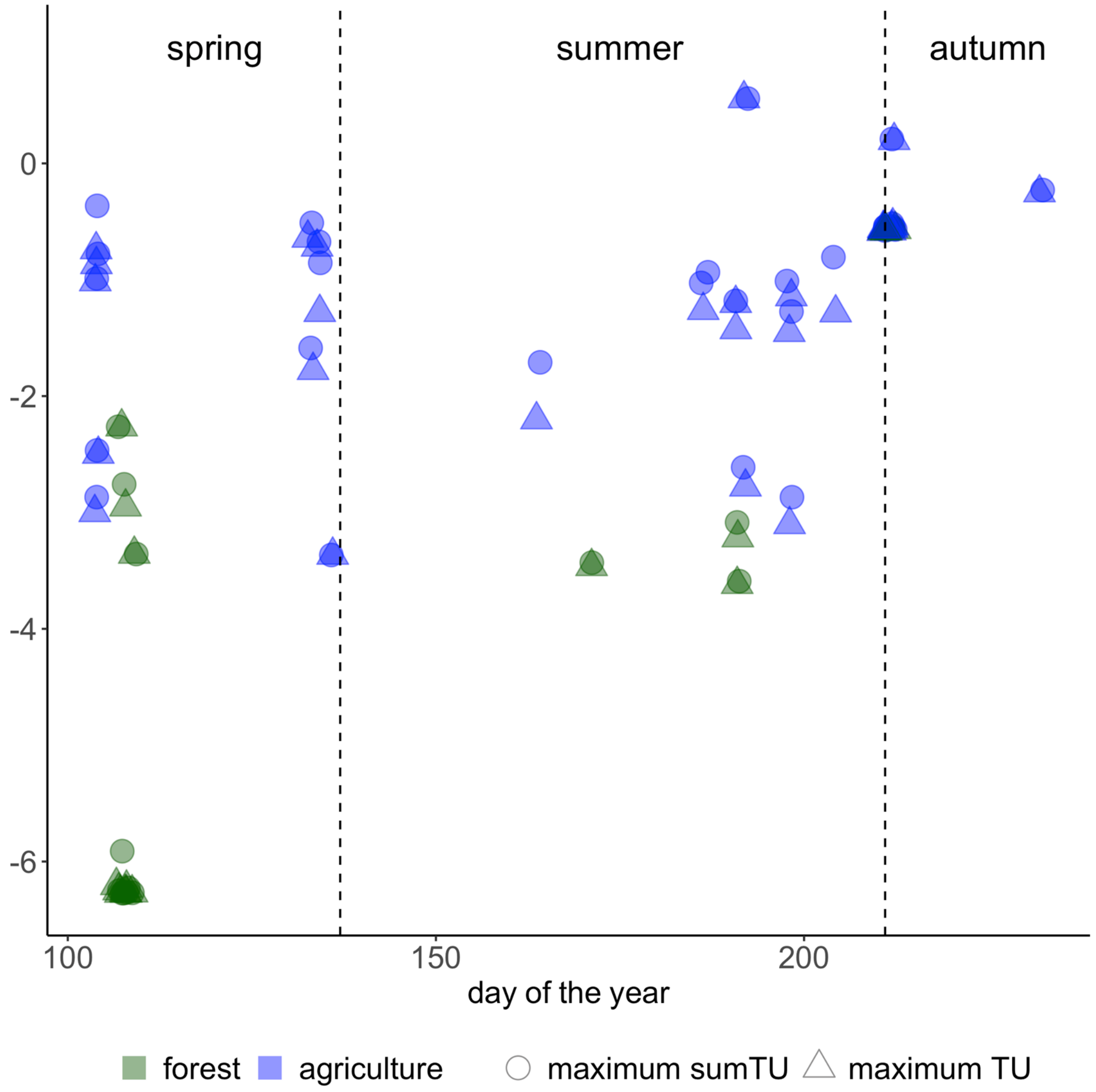

Figure B.2: Maximum logarithmic sum of the toxic units (max sumTU) and maximum toxic unit (max TU) per season and site. Green represents forested sites, and blue represents agricultural sites. Circles show max sumTU, and triangles show max TU.

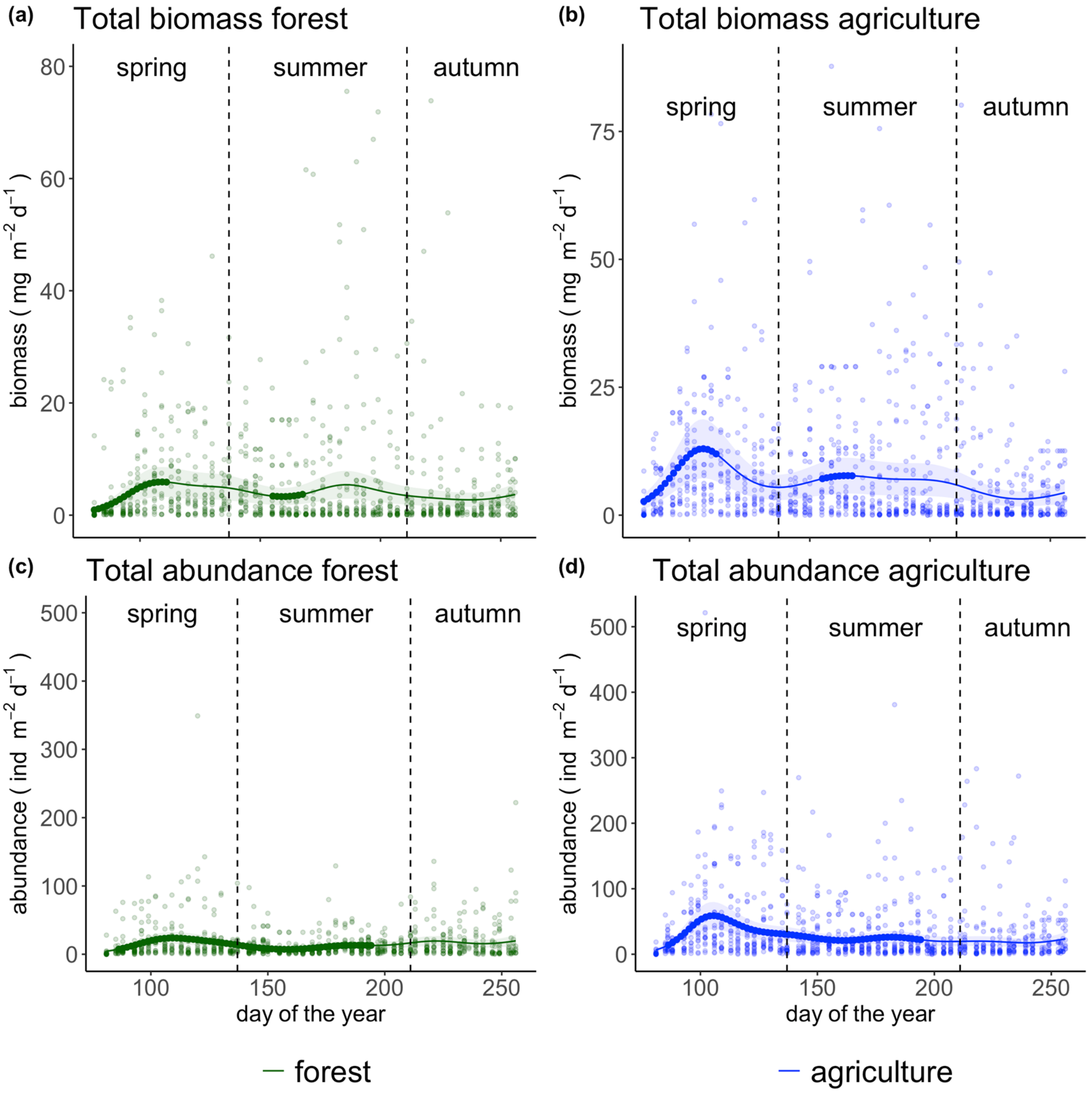

Figure B.3: Seasonal abundance patterns of total abundance and biomass. The solid line shows the predicted mean values of the hierarchical generalized additive models (HGAM) and the ribbon indicates ± 2 standard errors around the mean. The HGAM were fitted with 7644 observations. Green represents the abundance pattern in forest and blue in agriculture. Note that the y-axis scales vary. Solid dots mark significant differences with non- overlapping 95% confidence intervals at alpha = 0.05 between agriculture and forest. Transparent dots show the raw data.

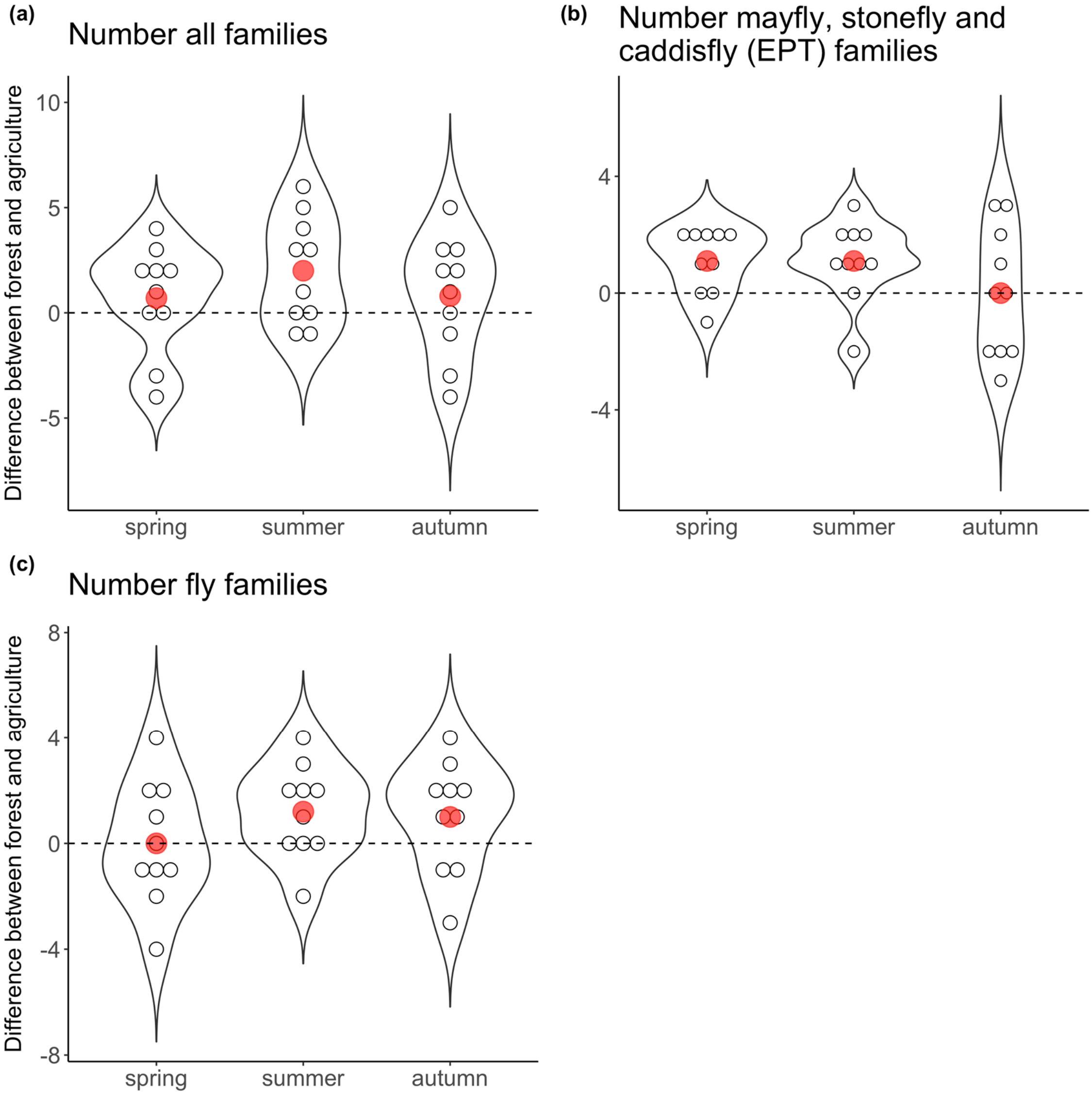

Figure B.4: Violin plots showing differences in the number of all, fly (Diptera), Ephemeroptera, Plecoptera, Trichoptera (EPT; mayfly, stonefly, caddisfly) families between forested and agricultural sites. White dots represent the difference between forested and agricultural sites within the same stream. These differences were calculated by subtracting the sum of families per season in agriculture from the sum of families per season in forest (ten streams, each with one upstream forested site and one downstream agricultural site). Red dots show the mean difference between forested and agricultural sites. Width of the violin plot reflects the frequency distribution of the data. Differences below zero reflect a higher number of families in agricultural sites and above zero reflect a higher number of families in forested sites.

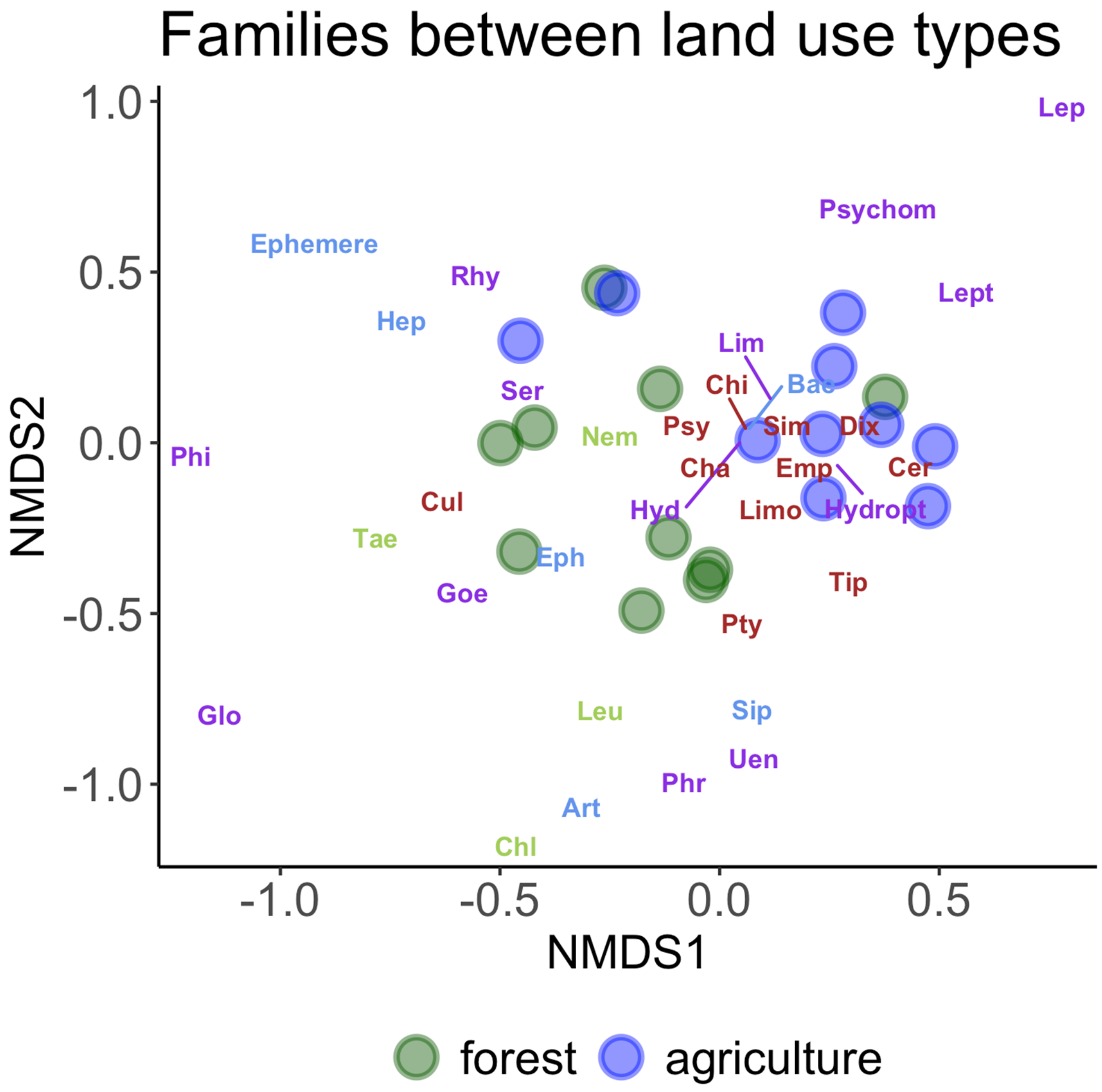

Figure B.5: Non-metric multidimensional scaling of the Jaccard index of the presence-absence data of the aquatic insect families per site. Green dots represent forested sites and blue dots agricultural sites. The colors of the text represent the orders of the aquatic insects: brown: flies; blue: mayflies, green: stoneflies, violet: caddisflies. Family names abbreviations are: Art: Flatheaded mayflies (Arthropleidae), Bae: Small mayflies (Baetidae), Cer: (Ceratopogonidae), Cha: Phantom midges (Chaoboridae), Chi: Non biting midges (Chironomidae), Chl: Green stoneflies (Chloroperlidae), Cul: Mosquitoes (Culicidae), Dix: Meniscus midges (Dixidae), Emp: Ballon/dagger flies (Empididae), Ephemere: Spiny crawler mayflies (Ephemerellidae), Eph: urrowing mayflies (Ephemeridae), Glo: Tortoise makers, (Glossosomatidae), Goe: Weighted case maker (Goeridae), Hep: Stream mayflies (Heptageniidae), Hyd: Net-spinning caddisflies (Hydropsychidae), Hydropt: Micro caddisflies (Hydroptilidae), Lep: Lepidostomatid case makers (Lepidostomatidae), Lept: Long-horned caddisflies (Leptoceridae), Leu: Needle flies (Leuctridae), Lim: Northern caddisflies (Limnephilidae), Limo: Limoniid crane (Limoniidae), Nem: Spring stoneflies (Nemouridae), Phi: (Philopotamidae), Phr: Large caddisflies (Phryganeidae), Psy: Drain flies (Psychodidae), Psychom: Tube-making caddisflies (Psychomyiidae), Pty: Phantom crane flies (Ptychopteridae), Rhy: Primitive caddisflies (Rhyacophilidae), Ser: Bushtailed caddisflies (Sericostomatidae), Sim: Black flies (Simuliidae), Sip: Primitive minnow mayflies (Siphlonuridae), Tae: Willow flies (Taeniopterygidae), Tip: Crane flies (Tipulidae), Uen: Stonecase caddisflies (Uenoidae).

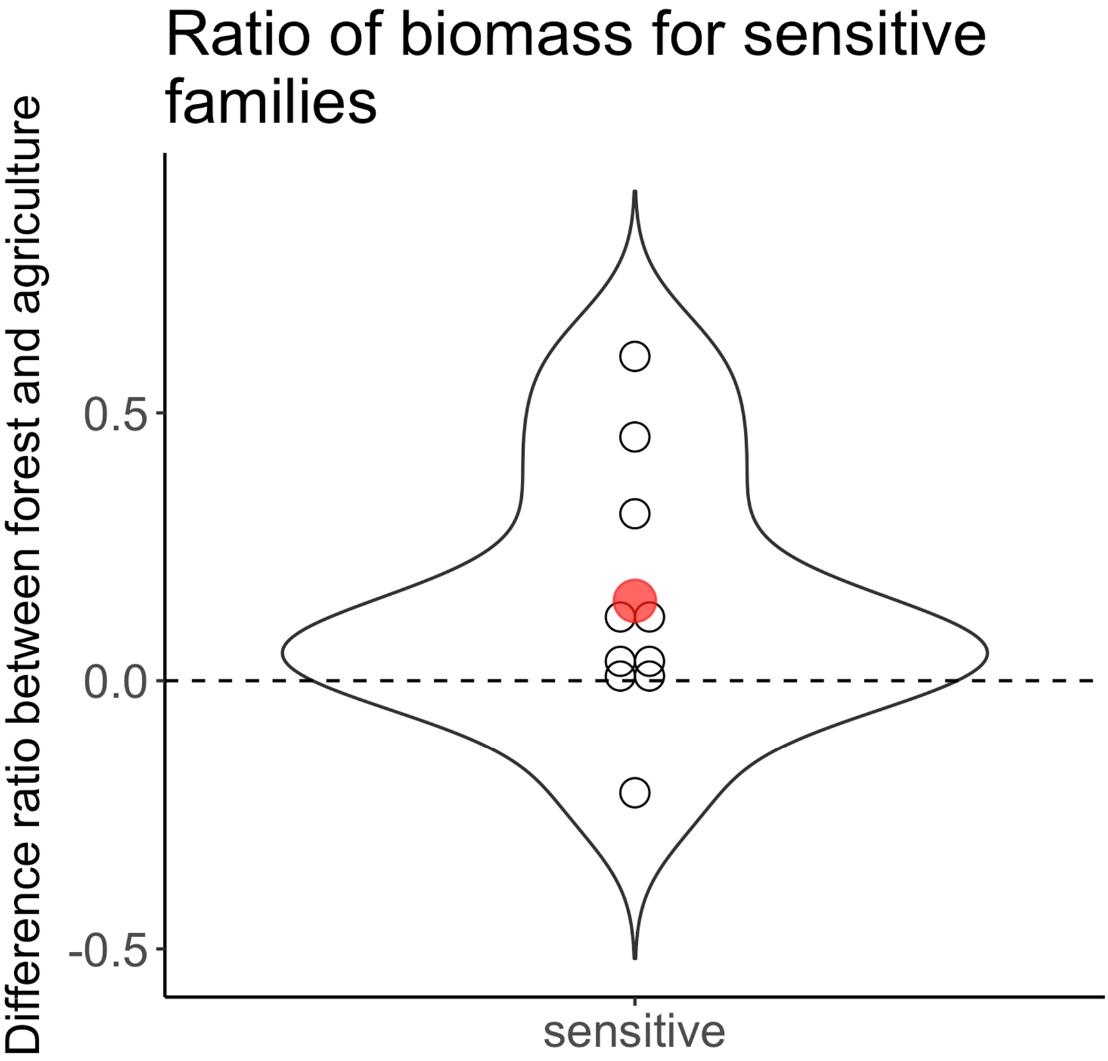

Figure B.6: Violin plot showing the difference of the biomass ratio for families with a generation time ≥ 0.5 year^{-1}, which are classified as sensitive. White dots represent the difference of ratios between forested and agricultural sites within the same stream. These differences were calculated by subtracting the biomass ratio in agriculture from the ratio in forest (ten streams, each with one upstream forested site and one downstream agricultural site). Red dots show the mean of the difference between forested and agricultural sites. Width of the violin plot reflects the frequency distribution of the data. Differences below zero reflect higher values of the biomass ratio in agricultural sites and above zero reflect higher values of the biomass ratio in forested sites.

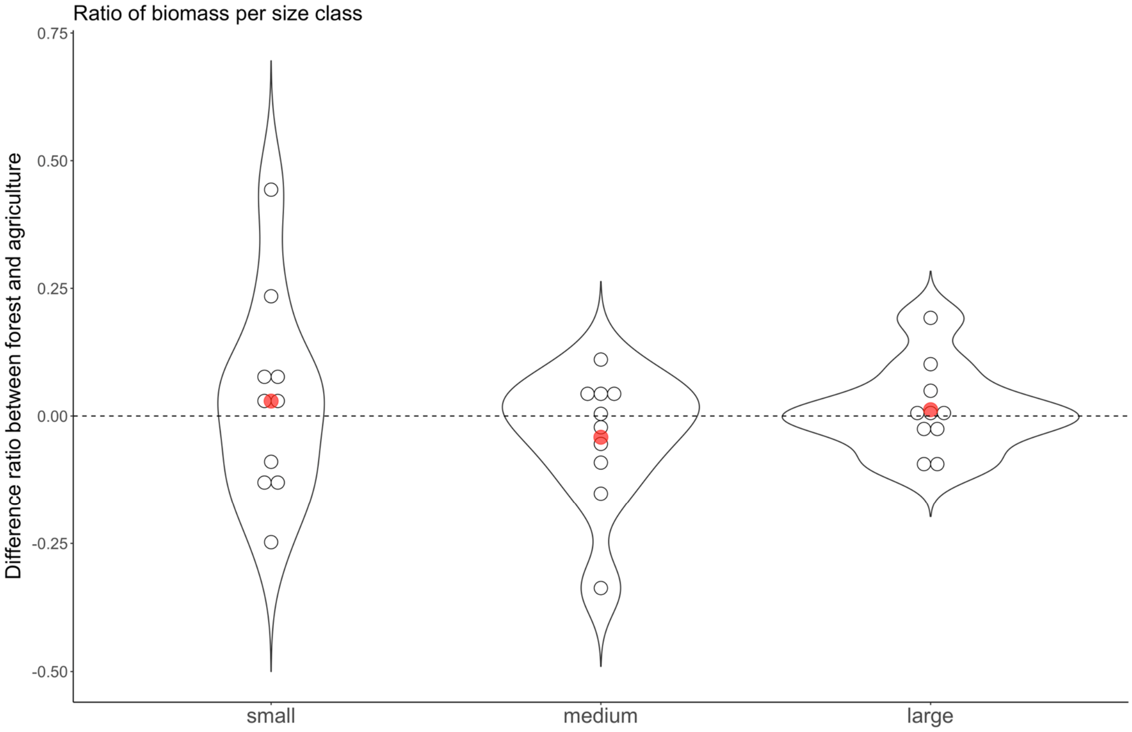

Figure B.7: Violin plot showing the difference of the biomass ratio for all families for three size classes: small (0.25 < size ≤ 1.0 cm), medium (1.0 < size ≤ 2.0 cm) and large (2.0 < size ≤ 8.0 cm). White dots represent the difference of ratios between forested and agricultural sites within the same stream. These differences were calculated by subtracting the biomass ratio in agriculture from the ratio in forest (ten streams, each with one upstream forested site and one downstream agricultural site). Red dots show the mean of the difference between forested and agricultural sites. Width of the violin plot reflects the frequency distribution of the data. Differences below zero reflect higher values of the biomass ratio in agricultural sites and above zero reflect higher values of the biomass ratio in forested sites.

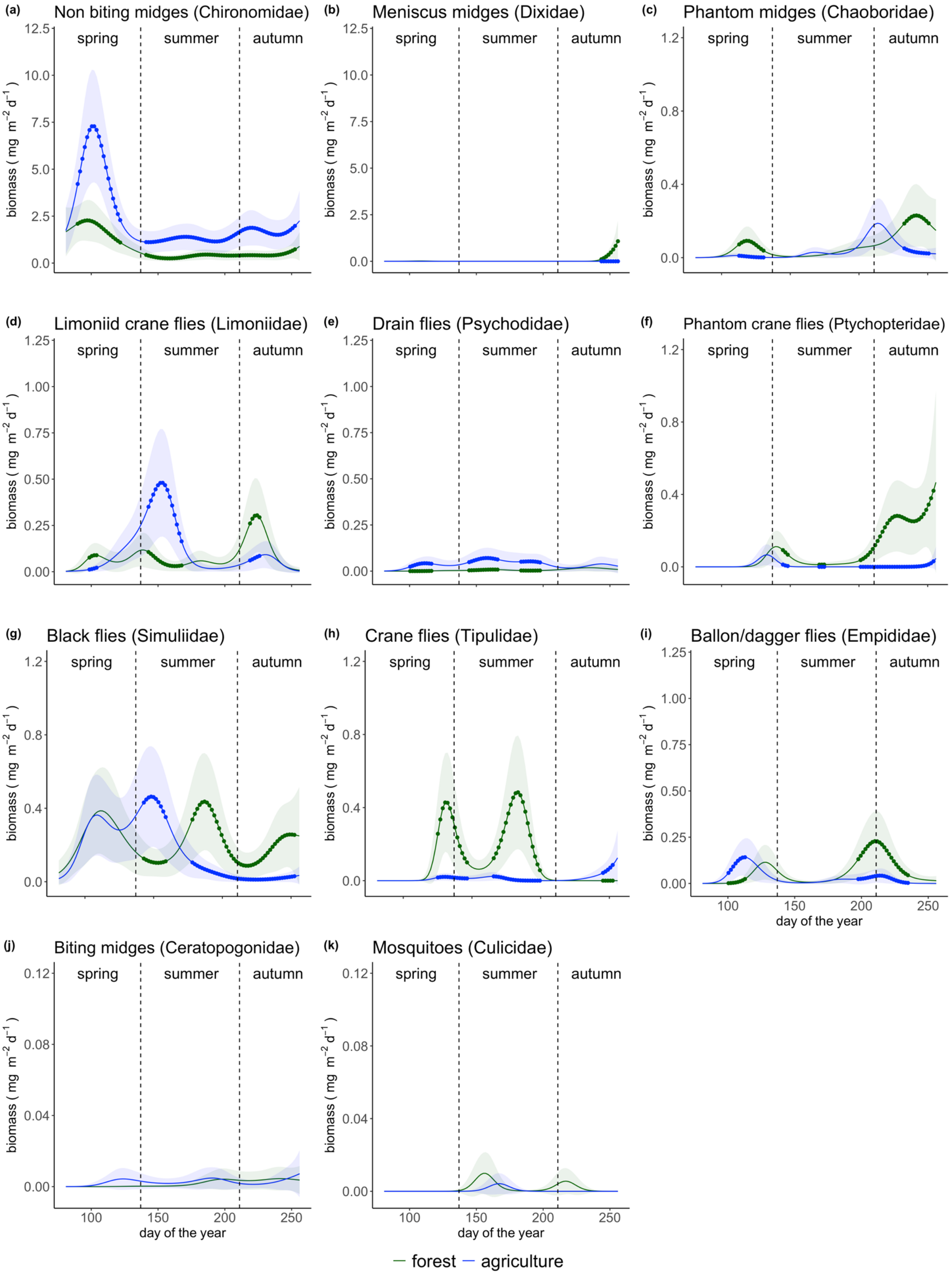

Figure B.8: Predicted seasonal biomass patterns of fly families derived with hierarchical generalized additive models (HGAM) fitted with 64987 observations. The solid line shows the predicted mean values of the HGAM and the ribbon indicates ± 2 standard errors around the mean. Green represents the biomass pattern in forest and blue in agriculture. Note that the y-axis scales vary.

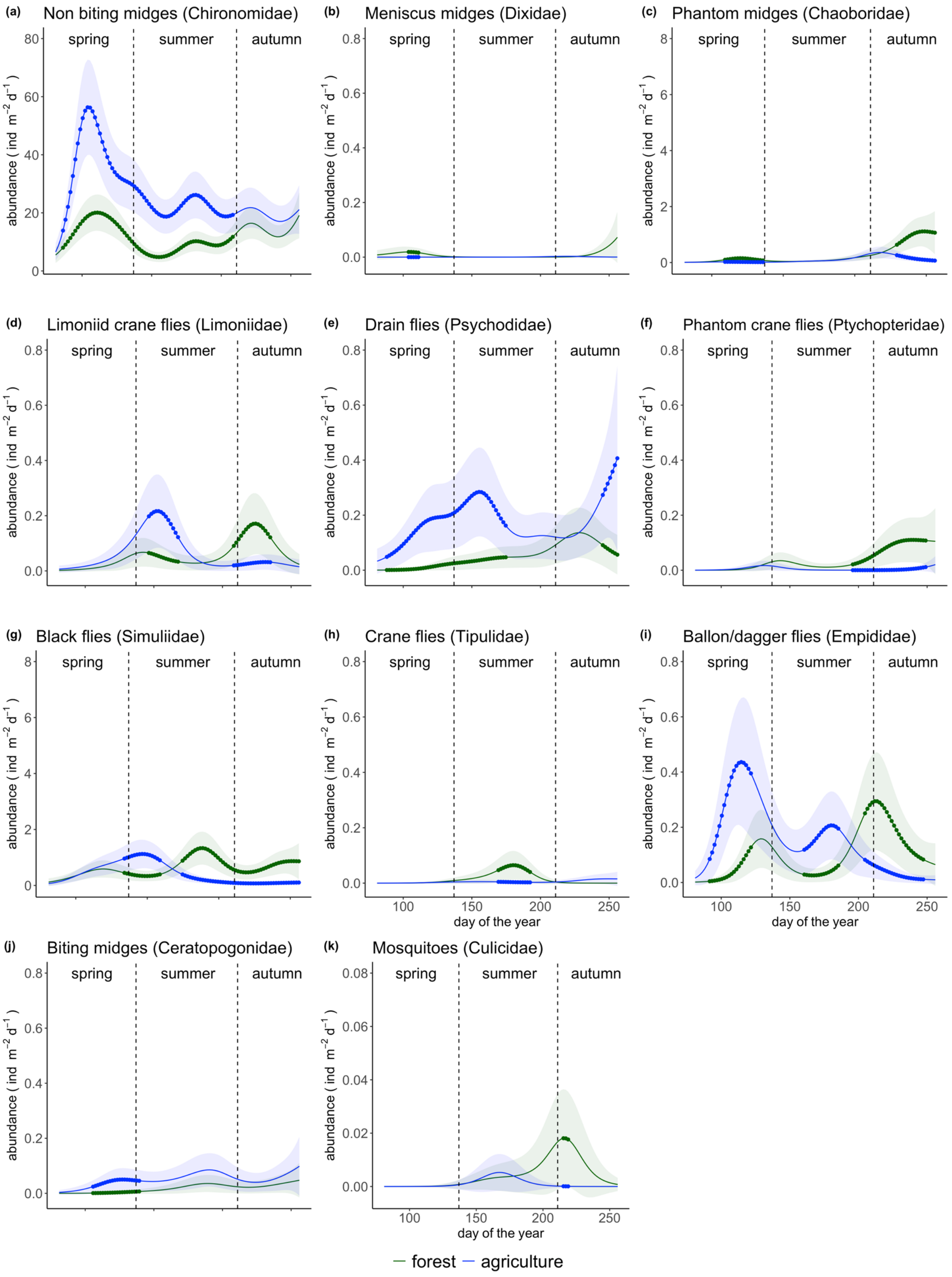

Figure B.9: Predicted seasonal abundance patterns of fly families derived with hierarchical generalized additive models (HGAM) fitted with 64987 observations. The solid line shows the predicted mean values of the HGAM and the ribbon indicates ± 2 standard errors around the mean. Green represents the abundance pattern in forest and blue in agriculture. Note that the y-axis scales vary.

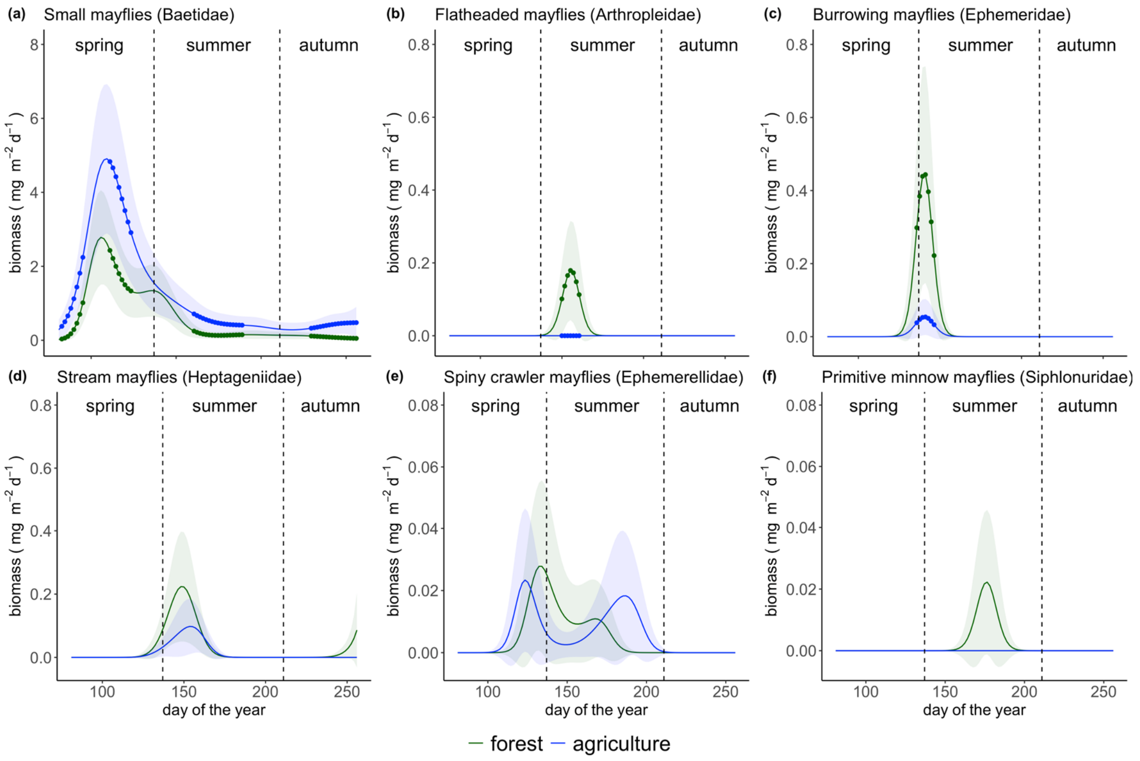

Figure B.10: Predicted seasonal biomass patterns of mayfly families derived with hierarchical generalized additive models (HGAM) fitted with 64987 observations. The solid line shows the predicted mean values of the HGAM and the ribbon indicates ± 2 standard errors around the mean. Green represents the biomass pattern in forest and blue in agriculture. Note that the y-axis scales vary.

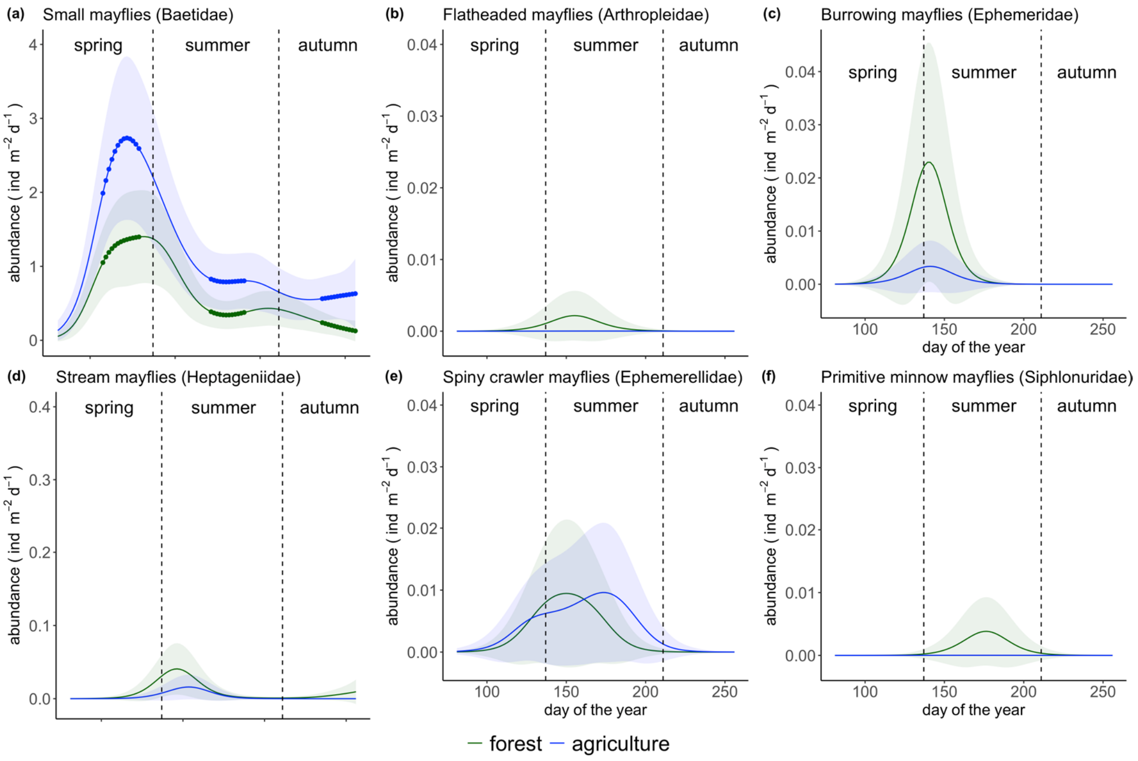

Figure B.11: Predicted seasonal abundance patterns of mayfly families derived with hierarchical generalized additive models (HGAM) fitted with 64987 observations. The solid line shows the predicted mean values of the HGAM and the ribbon indicates ± 2 standard errors around the mean. Green represents the abundance pattern in forest and blue in agriculture. Note that the y-axis scales vary.

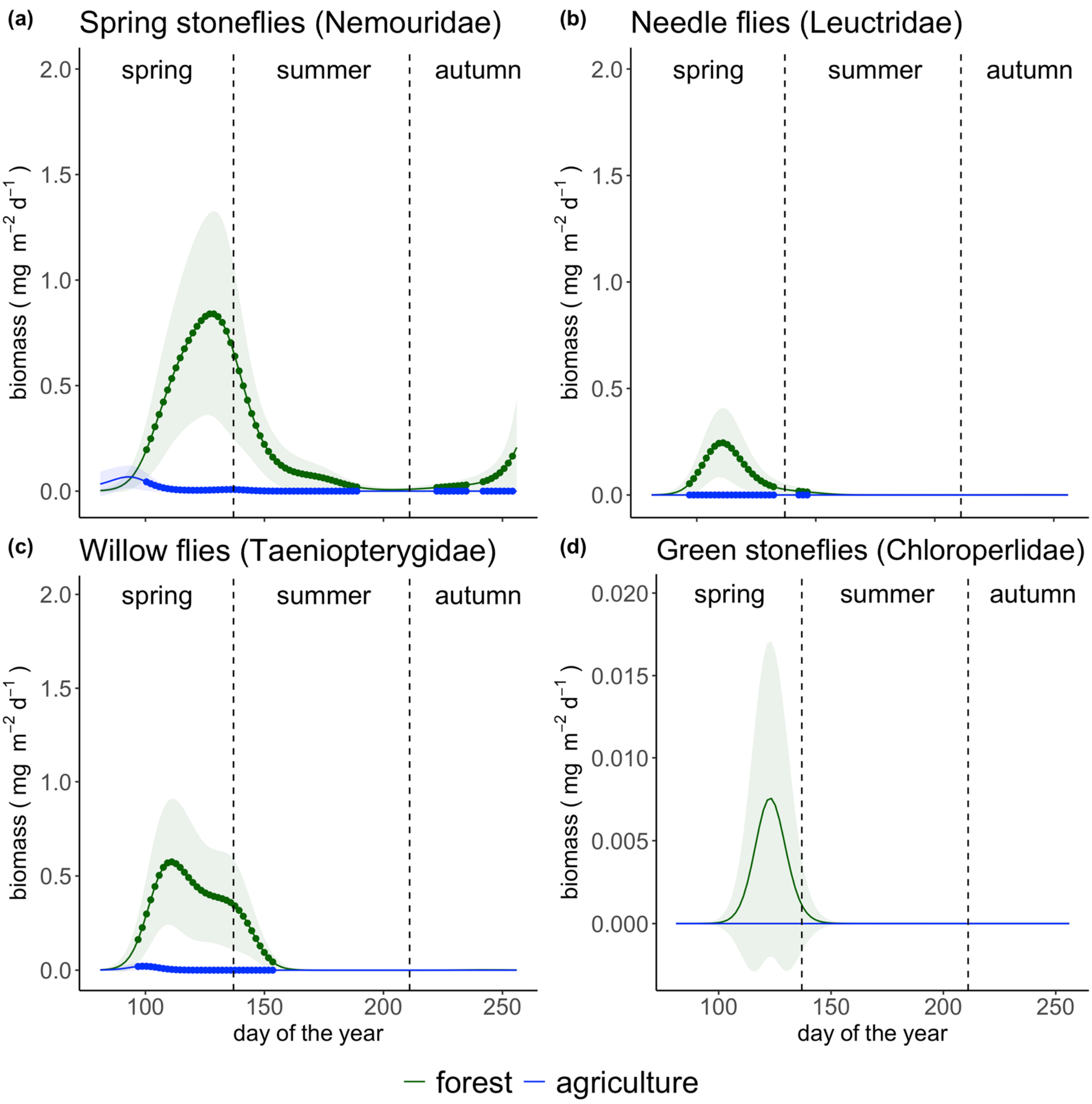

Figure B.12: Predicted seasonal biomass patterns of stonefly families derived with hierarchical generalized additive models (HGAM) fitted with 64987 observations. The solid line shows the predicted mean values of the HGAM and the ribbon indicates ± 2 standard errors around the mean. Green represents the biomass pattern in forest and blue in agriculture. Note that the y-axis scales vary.

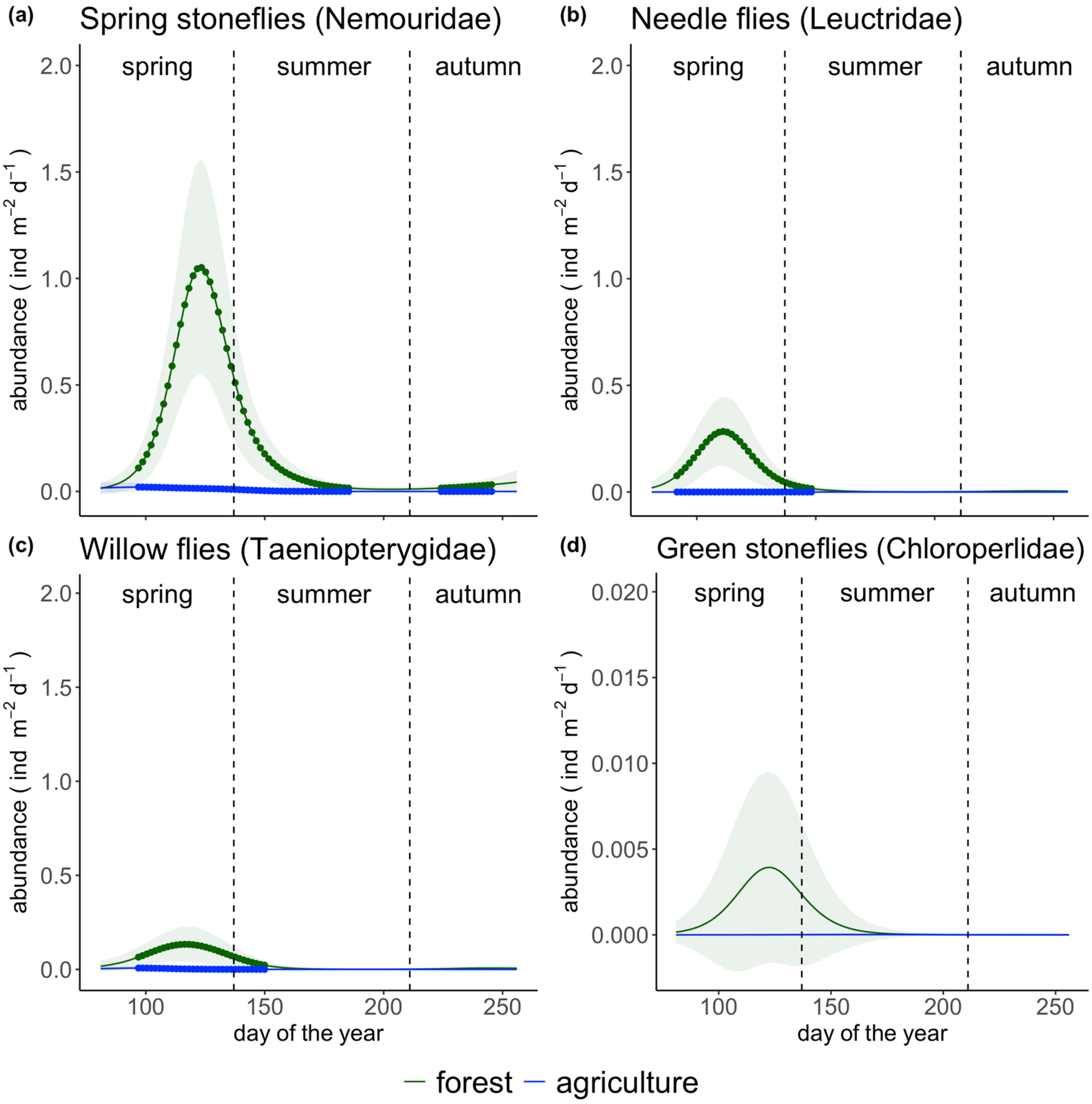

Figure B.13: Predicted seasonal abundance patterns of stonefly families derived with hierarchical generalized additive models (HGAM) fitted with 64987 observations. The solid line shows the predicted mean values of the HGAM and the ribbon indicates ± 2 standard errors around the mean. Green represents the abundance pattern in forest and blue in agriculture. Note that the y-axis scales vary.

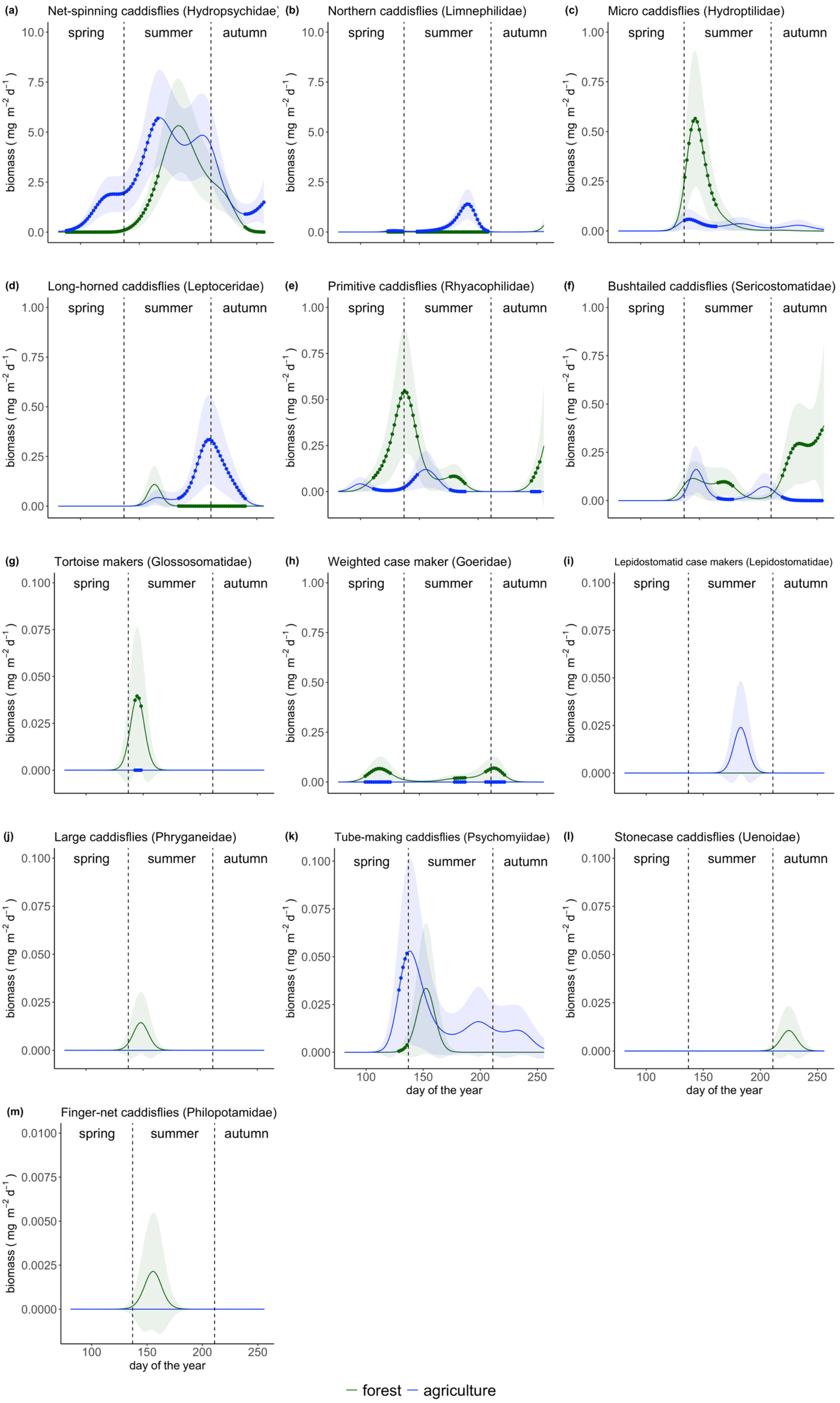

Figure B.14: Predicted seasonal biomass patterns of caddisfly families derived with hierarchical generalized additive models (HGAM) fitted with 64987 observations. The solid line shows the predicted mean values of the HGAM and the ribbon indicates ± 2 standard errors around the mean. Green represents the biomass pattern in forest and blue in agriculture. Note that the y-axis scales vary.

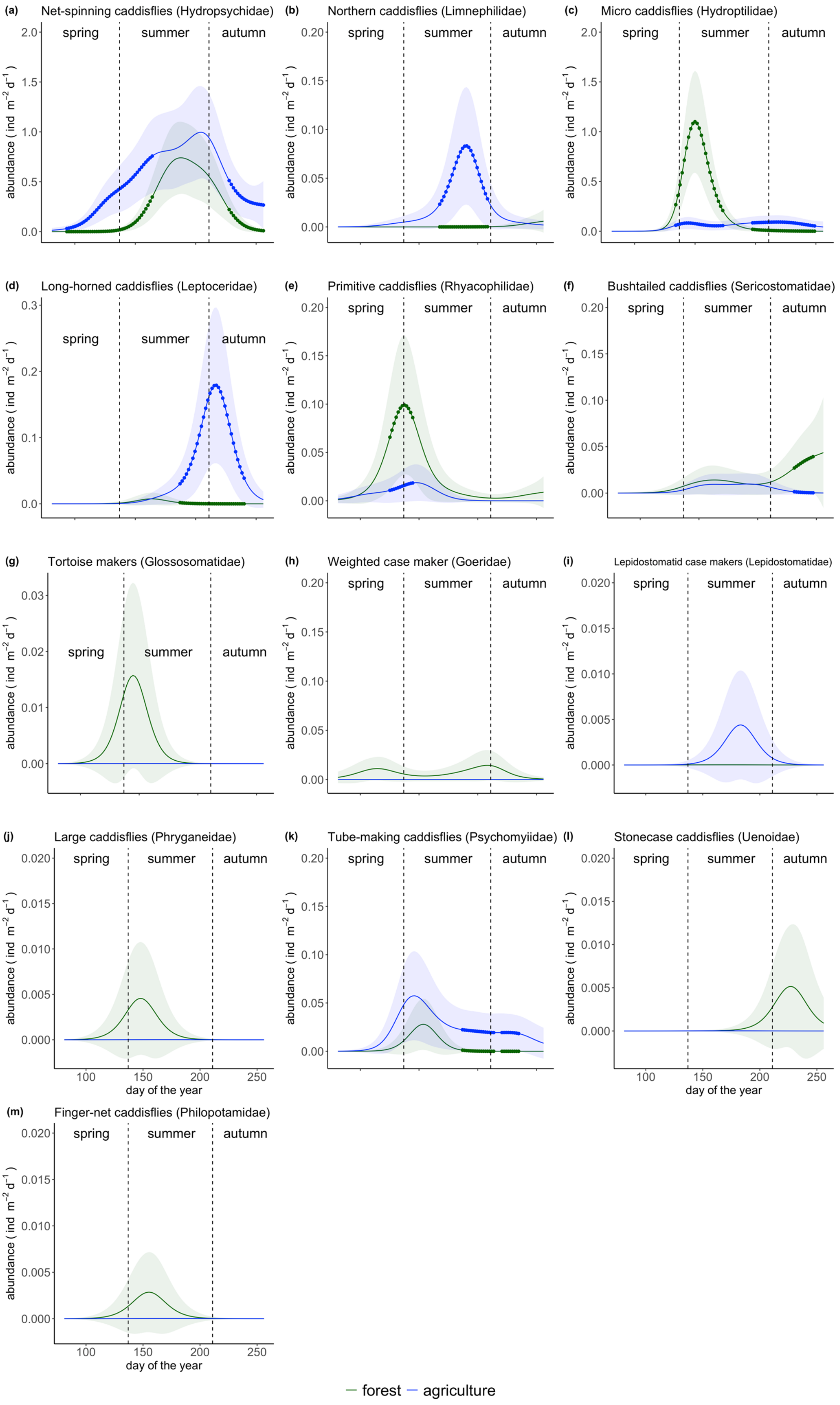

Figure B.15: Predicted seasonal abundance patterns of caddisfly families derived with hierarchical generalized additive models (HGAM) fitted with 64987 observations. The solid line shows the predicted mean values of the HGAM and the ribbon indicates ± 2 standard errors around the mean. Green represents the abundance pattern in forest and blue in agriculture. Note that the y-axis scales vary.

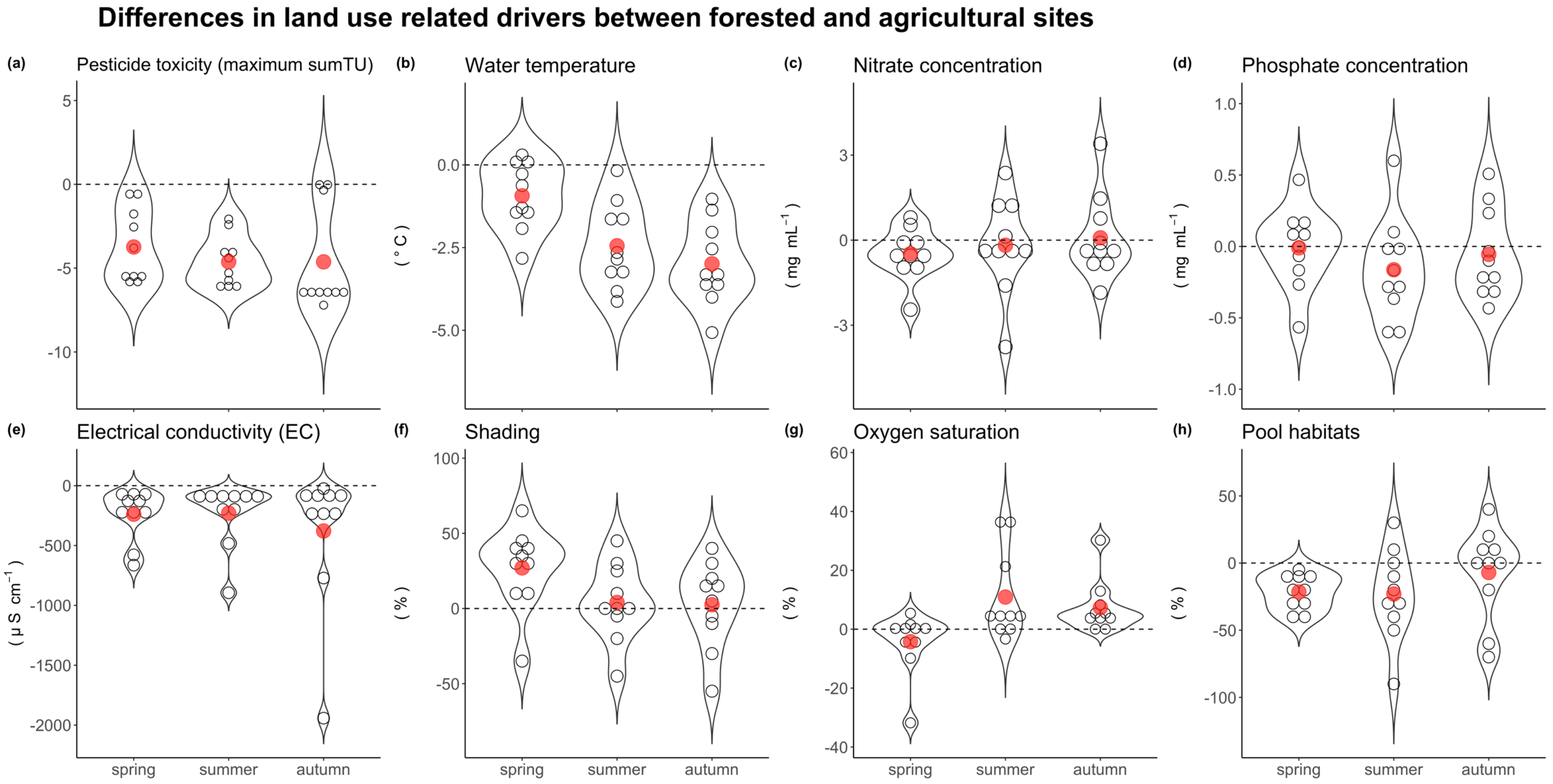

Figure B.5: Violin plots showing the difference of land-use-related drivers between forested and agricultural sites. White dots represent differences between paired forested and agricultural sites. These differences were calculated by subtracting the mean value per season in agriculture from the mean value per season in forest (ten streams, each with one upstream forested site and one downstream agricultural site). Width of the violin plot reflects the frequency distribution of the data. Red dots show the mean of the overall difference between forested and agricultural sites. Differences below zero represent higher values of the land-use-related drivers in agricultural sites and above zero reflect higher values in forested sites.

Table B.1: Name, CAS-number, *k*oc (soil organic carbon–water partitioning coefficient) value of the pyrethroids included in the conversion of the particle-associated concentration into an estimate of the bioavailable concentration in water.

Name	**CAS**	k_{oc}	**Source**
allethrin	584792	1400	Lewis et al., 2016
acrinathrin	101007061	48231	Lewis et al., 2016
etofenprox	80844071	17757	Lewis et al., 2016
permethrin	52645531	100000	Lewis et al., 2016
prallethrin	23031369	1318	(National Center for Biotechnology Information, 2021)

Table B.2: Name, CAS-number, acute EC_{50} value, taxon and source of EC_{50} values of the pesticides included in the calculation of the logarithmic sum of the toxic units (*sumTU*: toxicity).

Name	**CAS**	**Taxon**	EC_{50} **µg L^{-1}**	**Pesticide type**	**Source**
(e)-acetamiprid	135410207	*Chironomus tepperi*	2.22	I	(Scharmüller et al., 2020); (US EPA, 2021)
2-ethoxy-3,3-dimethyl-5-(methylsulfonylmethyl)-2h-1-benzofuran	26225796	*Daphnia magna*	137171.43	H	Scharmüller et al., 2020; US EPA, 2021
2,4-d	94757	*Astacus leptodactylus*	32600	H	Scharmüller et al., 2020; US EPA, 2021
azoxystrobin	131860338	*Gammarus pulex*	270	F	Scharmüller et al., 2020; US EPA, 2021
bentazone	25057890	*Chironomus riparius*	62300	H	Scharmüller et al., 2020; US EPA, 2021
bioallethrin	584792	*Pteronarcys californica*	7.1	I	Scharmüller et al., 2020; US EPA, 2021
boscalid	188425856	*Daphnia magna*	5330	F	Scharmüller et al., 2020; US EPA, 2021
bromoxynil	1689845	*Chironomus riparius*	2113.05	H	Scharmüller et al., 2020; US EPA, 2021
clothianidin	210880925	*Chironomus dilutus*	2.07	I	Scharmüller et al., 2020; US EPA, 2021
cyazofamid	120116883	*Daphnia magna*	413	F	Scharmüller et al., 2020; US EPA, 2021
cyprodinil	121552612	*Daphnia magna*	32	F	Scharmüller et al., 2020; US EPA, 2021
difenoconazole	119446683	*Daphnia magna*	53.03	F	Scharmüller et al., 2020; US EPA, 2021
dimethenamid-p	163515148	*Daphnia magna*	12000	H	Scharmüller et al., 2020; US EPA, 2021
dimethoate	60515	*Chironomus dilutus*	1.29	I	Scharmüller et al., 2020; US EPA, 2021
etofenprox	80844071	*Cheumatopsyche brevilineata*	0.12	I	Scharmüller et al., 2020; US EPA, 2021
fenpropidin	67306007	*Daphnia magna*	1803.14	F	Scharmüller et al., 2020; US EPA, 2021
fipronil	120068373	*Chironomus dilutus*	0.03	I	Scharmüller et al., 2020; US EPA, 2021

Table B.2: Continued.

Name	**CAS**	**Taxon**	***EC*₅₀ µg L⁻¹**	**Pesticide type**	**Source**
fluazinam	79622596	*Brachionus calyciflorus*	1.6	F	Scharmüller et al., 2020; US EPA, 2021
fludioxonil	131341861	*Daphnia magna*	900	F	Scharmüller et al., 2020; US EPA, 2021
flufenacet	142459583	*Hyalella azteca*	2800	H	Scharmüller et al., 2020; US EPA, 2021
imidacloprid	138261413	*Epeorus longimanus*	1.42	I	Scharmüller et al., 2020; US EPA, 2021
kresoxim-methyl	143390890	*Daphnia magna*	285.01	F	Scharmüller et al., 2020; US EPA, 2021
mesotrione	104206828	*Daphnia magna*	840000	H	Scharmüller et al., 2020; US EPA, 2021
methiocarb	2032657	*Chironomus tentans*	1.6	I	Scharmüller et al., 2020; US EPA, 2021
metolachlor	51218452	*Chironomus plumosus*	4089.01	H	Scharmüller et al., 2020; US EPA, 2021
metribuzin	21087649	*Daphnia magna*	4180	H	Scharmüller et al., 2020; US EPA, 2021
napropamide	15299997	*Daphnia magna*	18793.88	H	Scharmüller et al., 2020; US EPA, 2021
permethrin	52645531	*Hyalella azteca*	0.02	I	Scharmüller et al., 2020; US EPA, 2021
pirimicarb	23103982	*Daphnia magna*	17.12	I	Scharmüller et al., 2020; US EPA, 2021
prallethrin	23031369	*Culex quinquefasciatus*	3.15	I	Scharmüller et al., 2020; US EPA, 2021
prochloraz	67747095	*Gammarus pulex*	2180	F	Scharmüller et al., 2020; US EPA, 2021
propiconazole	60207901	*Baetis rhodani*	900	F	Scharmüller et al., 2020; US EPA, 2021
pyraclostrobin	175013180	*Daphnia magna*	54.87	F	Scharmüller et al., 2020; US EPA, 2021
s-metolachlor	87392129	*Gammarus pulex*	9470.87	H	Scharmüller et al., 2020; US EPA, 2021
spiroxamine	118134308	*Daphnia magna*	4164.13	F	Scharmüller et al., 2020; US EPA, 2021
tebuconazole	107534963	*Gammarus fossarum*	1347	F	Scharmüller et al., 2020; US EPA, 2021

Table B.2: Continued.

Name	**CAS**	**Taxon**	***EC*₅₀ µg L⁻¹**	**Pesticide type**	**Source**
terbuthylazine	5915413	*Daphnia magna*	32849.35	H	Scharmüller et al., 2020; US EPA, 2021
thiacloprid	111988499	*Chironomus tepperi*	1.06	I	Scharmüller et al., 2020; US EPA, 2021
thiamethoxam	153719234	*Chironomus*	35	I	Scharmüller et al., 2020; US EPA, 2021
triadimenol	55219653	*Daphnia magna*	2500	F	Scharmüller et al., 2020; US EPA, 2021
trifloxystrobin	141517217	*Daphnia magna*	26.72	F	Scharmüller et al., 2020; US EPA, 2021
dimethomorph	110488705	*Daphnia magna*	10600	F	Scharmüller et al., 2020; US EPA, 2021
fluroxypyr	69377817	*Daphnia magna*	100000	H	Scharmüller et al., 2020; US EPA, 2021
fluxapyroxad	907204313	*Daphnia magna*	6780	F	Lewis et al., 2016
metamitron	41394052	*Daphnia magna*	5700	H	Lewis et al., 2016
metazachlor	67129082	*Daphnia magna*	33000	H	(Malaj et al., 2014)
nicosulfuron	111991094	*Daphnia magna*	90000	H	Lewis et al., 2016
propamocarb	24579735	*Daphnia magna*	106000	F	Lewis et al., 2016
prosulfocarb	52888809	*Daphnia magna*	510	H	Lewis et al., 2016
epoxiconazol	135319732	*Chironomus riparius*	62.5	F	Lewis et al., 2016
dimethachlor	50563365	*Daphnia magna*	24000	H	Lewis et al., 2016
mecoprop_P	16484778	*Daphnia magna*	90940	H	Scharmüller et al., 2020; US EPA, 2021
bixafen	581809463	*Daphnia magna*	1200	F	Lewis et al., 2016
quizalofop_free_acid	76578126	*Daphnia magna*	57700	H	Lewis et al., 2016
isoproturon	34123596	*Daphnia magna*	580	H	Malaj et al., 2014
picoxystrobin	117428225	*Daphnia magna*	24	F	Lewis et al., 2016
dimoxystrobin	149961524	*Daphnia magna*	39.4	F	Lewis et al., 2016
lenacil	2164081	*Daphnia magna*	8400	H	Malaj et al., 2014
pethoxamid	106700292	*Daphnia magna*	23000	H	Lewis et al., 2016
dichlorprop_P	15165670	*Daphnia magna*	100000	H	Malaj et al., 2014
florasulam	145701231	*Daphnia magna*	5500	H	Scharmüller et al., 2020; US EPA, 2021
pyroxsulam	422556089	*Daphnia magna*	100000	H	Scharmüller et al., 2020; US EPA, 2021
foramsulfuron	173159574	*Daphnia magna*	100000	H	Lewis et al., 2016
chloridazon	1698608	*Daphnia magna*	132000	H	Malaj et al., 2014
acrinathrin	101007061	*Daphnia magna*	0.02	I	Lewis et al., 2016

Table B.3: Details for the sampling in 2018 of aquatic insects with emergence traps in forested (F) and agricultural (A) sites. Sampling dates, sampling days per sample, number of samples per trap and site, sum of sampling days, and mean sampling days per sample. Red: marks events where traps were destroyed by vandalism or heavy rainfall and, thus, no insects were collected. Green: time points when emergence was collected.

			March				April									May										June									
Stream	**land use**	**trap**	**22[a]**	**26**	**27**	**29**	**3**	**6**	**9**	**12**	**16**	**19**	**23**	**26**	**30**	**2**	**3[b]**	**7**	**10**	**14**	**15**	**17**	**22**	**24[c]**	**28[c]**	**30[c]**	**4[c]**	**8[c]**	**9[c]**	**11[c]**	**14[c]**	**15**	**18**	**21**	**25**	**28**
Russbach	**F**	**1**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	4	2	5	4		3	3		4	3	4	3
Russbach	**F**	**2**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	4	2	5	4		3	3		4	3	4	3
Otterbach	**F**	**1**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	4	2	5	4		3	3		4	3	4	3
Otterbach	**F**	**2**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	4	2	5	4		3	3		4	3	4	3
Dierbach	**F**	**1**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	4	2	5	4		3	3		4	3	4	3
Dierbach	**F**	**2**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	4	2	5	4		NA	3		4	3	4	3
Klingbach	**F**	**1**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	NA	2	NA	NA		NA	3		4	3	4	3
Klingbach	**F**	**2**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		NA	5	2	NA	2	NA	NA		NA	NA		4	3	4	3
Kaiserbach	**F**	**1**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	NA		3	5	2	NA	2	NA	4		3	3		4	3	4	3
Kaiserbach	**F**	**2**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	NA		3	5	2	NA	2	NA	4		3	3		4	3	4	3
Isenach	**F**	**1**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		5	2	5	2	4	2	5		5	2		4	3	3	4	3
Isenach	**F**	**2**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		5	2	5	2	4	2	5		5	2		4	3	3	4	3
Kropsbach	**F**	**1**	3		5	2	5	3	3	3	NA	3	4	3	4		3	4	3		5	2	5	2	NA	NA	NA	NA		3	3		4	3	4	3
Kropsbach	**F**	**2**	3		5	2	5	3	3	3	NA	3	4	3	4		3	4	3		5	2	5	2	NA	NA	NA	NA		3	NA		NA	3	4	3
Triefenbach	**F**	**1**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		5	2	5	2	NA	2	5	4		3	3		4	3	4	3
Triefenbach	**F**	**2**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		5	2	5	2	NA	2	NA	4		3	3		4	3	4	3
Modenbach	**F**	**1**	3		5	2	5	3	3	3	4	3	4	3	4	2	1	4	3		5	2	5	2	4	2	5	4		3	3		4	3	4	3
Modenbach	**F**	**2**	3		5	2	5	3	3	3	4	3	4	3	4	2	1	4	3		5	2	5	2	NA	NA	NA	4		3	3		4	3	4	3
Hainbach	**F**	**1**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		5	2	5	2	4	2	5	4			6		4	3	4	3
Hainbach	**F**	**2**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		NA	2	5	2	4	2	NA	4			6		4	3	4	3

a: Half of traps installed at 19th and other half at 20th of March

b: One trap destroyed by vandalism

c: Phase with many heavy rainfall events

d: Since 27th of August, only one trap in Hainbach in agricultural site because water level was very low and a lot of material blocked stream

e: Half of traps deinstalled at 12th and other half at 13th of September

Table B.3: Continued.

			March				April									May											June									
Stream	land use	trap	22[a]	26	27	29	3	6	9	12	16	19	23	26	30	2	3[b]	7	10	14	15	17	22	24[c]	28[c]	30[c]	4[c]	8[c]	9[c]	11[c]	14[c]	15	18	21	25	28
Russbach	**A**	**1**	4	4		NA	5	3	3	NA	4	3	4	3	4		3	4	3	NA		3	5	2	3	2	5	4		3	3		4	3	4	3
Russbach	**A**	**2**	4	4		NA	NA	3	3	NA	4	3	4	3	4		3	4	3	NA		3	5	2	3	2	NA	4		3	3		4	3	4	3
Otterbach	**A**	**1**	4	4		NA	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	3	2	NA	4		NA	NA		4	3	4	3
Otterbach	**A**	**2**	4	4		NA	5	3	3	3	4	3	4	3	4		3	4	3	NA		NA	5	2	3	2	NA	4		NA	NA		4	3	4	3
Dierbach	**A**	**1**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	4	2	NA	4		3	3		4	3	4	3
Dierbach	**A**	**2**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	4	2	NA	4		3	3		4	3	4	3
Klingbach	**A**	**1**	4	4		3	5	3	3	3	NA	3	4	3	4		3	4	3	4		3	5	2	4	2	5	4		3	3		4	3	4	3
Klingbach	**A**	**2**	4	4		3	5	3	3	3	NA	3	4	3	4		NA	4	3	4		3	5	2	4	2	NA	4		NA	NA		4	3	NA	3
Kaiserbach	**A**	**1**	4	4		3	5	3	3	3	4	3	4	3	4		3	4	3	4		3	5	2	NA	2	NA	4		3	3		4	3	4	3
Kaiserbach	**A**	**2**	4	4		3	5	3	3	3	NA	3	4	3	4		3	4	3	NA		3	5	2	NA	2	NA	4		3	3		4	3	4	3
Isenach	**A**	**1**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		5	2	5	2	4	2	5		5	2	3		4	3	4	3
Isenach	**A**	**2**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		NA	2	5	2	4	2	5		5	2	3		4	3	4	3
Kropsbach	**A**	**1**	3		5	NA	NA	NA	3	3	NA	3	4	3	4		3	4	3		NA	2	5	2	4	NA	NA	NA		3	NA		NA	3	4	3
Kropsbach	**A**	**2**	3		5	NA	NA	NA	3	3	NA	3	4	3	4		3	4	3		NA	2	5	2	4	NA	NA	NA		3	NA		NA	3	4	3
Triefenbach	**A**	**1**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		5	2	5	2	4	2	5	4		3	3		4	3	4	3
Triefenbach	**A**	**2**	3		5	2	5	3	3	3	4	3	4	3	4		3	4	3		5	2	5	2	4	2	5	4		3	3		4	3	4	3
Modenbach	**A**	**1**	3		5	NA	5	3	3	3	NA	3	4	3	4		3	4	3		5	2	5	2	4	2	NA	4		3	NA		4	3	4	3
Modenbach	**A**	**2**	3		5	NA	NA	3	3	3	NA	3	4	3	4		3	4	3		NA	2	5	2	4	2	NA	4		NA	NA		4	3	4	3
Hainbach	**A**	**1**	3		5	2	5	3	3	3	NA	3	4	NA	4		3	4	3		5	2	5	2	4	2	NA	NA		3	3		4	3	4	3
Hainbach	**A**	**2**	3		5	2	5	3	3	3	NA	3	4	NA	4		3	4	3		5	2	5	2	4	2	NA	NA		3	3		4	3	4	3

a: Half of traps installed at 19th and other half at 20th of March

b: One trap destroyed by vandalism

c: Phase with many heavy rainfall events

d: Since 27th of August, only one trap in Hainbach in agricultural site because water level was very low and a lot of material blocked stream

e: Half of traps deinstalled at 12th and other half at 13th of September

Table B.3: Continued.

			July												August												September									sampling days	
Stream	land use	trap	2	5	9	12	16	17	18	19	23	26	30	31	1	2	6	9	13	16	20	21	22	27[d]	28	30	3	4	6	10	11	12[e]	13[e]	No samples	No lost samples	sum	mean per sample
Russbach	**F**	**1**	4	3	4	3	4			3	4	3	4		2		5	3	4	3	4		2	5		3	4		3	4		2		52	0	179	3
Russbach	**F**	**2**	4	3	4	3	4			3	4	3	4		2		5	3	4	3	4		2	5		3	4		3	4		2		52	0	180	3
Otterbach	**F**	**1**	4	3	4	3	4			3	4	3	4		2		5	3	4	3	4		2	5		3	4		3	4		2		52	0	179	3
Otterbach	**F**	**2**	4	3	4	3	4			3	4	3	4		2		5	3	4	3	4		2	5		3	4		3	4		2		52	0	180	3
Dierbach	**F**	**1**	4	3	4	3	4			3	4	3	4		2		5	3	4	3	4		2	5		3	4		3	4		2		52	0	179	3
Dierbach	**F**	**2**	4	3	4	3	4			3	4	3	4		2		5	3	4	3	4		2	5		3	4		3	4		2		51	1	177	3
Klingbach	**F**	**1**	4	3	4	3	4		2		5	3	4		2		5	3	4	3	4		2	5		3	4		3	4		2		48	4	163	3
Klingbach	**F**	**2**	4	3	4	3	NA		2		5	3	4		2		5	3	4	3	NA		2	5		3	4		3	4		2		44	8	150	3
Kaiserbach	**F**	**1**	4	3	4	3		5		2	4	3	4		2		5	3	4	3	4		2	5		3	4		3	4		2		49	3	166	3
Kaiserbach	**F**	**2**	4	3	4	3		5		2	4	3	4		2		5	3	4	3	4		2	5		3	4		3	4		2		49	3	167	3
Isenach	**F**	**1**	4	3	4	3		5		2	4	3		5		2	4	3	4	3		5			4	2		5	2		5		2	51	0	176	3
Isenach	**F**	**2**	4	3	4	3		5		2	4	3		5		2	4	3	4	3		5			4	2		5	2		5		2	51	0	177	3
Kropsbach	**F**	**1**	4	3	NA	NA		5		2	4	3	4		2		5	3	4	3	4		2	5		3		5	2		5		2	45	7	153	3
Kropsbach	**F**	**2**	4	3	NA	NA		NA		2	4	3	4		2		5	3	4	3	4		2	5		3		5	2		5		2	42	10	142	3
Triefenbach	**F**	**1**	4	3	4	3		5	1		5	3	4		2		5	3	4	3	4		2	5		3		5	2		5		2	51	1	175	3
Triefenbach	**F**	**2**	4	3	4	3		5	1		5	3	4		2		5	3	4	3	4		2	5		3		5	2		5		2	50	2	171	3
Modenbach	**F**	**1**	4	3	4	3	4		2		5	3	4		2		5	3	4	3	4		2	5		3		5	2		5		2	53	0	179	3
Modenbach	**F**	**2**	4	3	4	3	4		2		5	3	4		2		5	3	4	3	4		2	5		3		5	2		5		2	50	3	169	3
Hainbach	**F**	**1**	4	3	4	3	4		2		5	3	4		2		5	3	4	3	4		2	5		3		5	2		5		2	51	0	179	4
Hainbach	**F**	**2**	4	3	4	3	4		2		5	3	4		2		5	3	4	3	4		2	NA		3		5	2		NA		NA	47	3	158	3

a: Half of traps installed at 19th and other half at 20th of March
b: One trap destroyed by vandalism
c: Phase with many heavy rainfall events
d: Since 27th of August, only one trap in Hainbach in agricultural site because water level was very low and a lot of material blocked stream
e: Half of traps deinstalled at 12th and other half at 13th of September

Table B.4: Output of hierarchical generalized additive models (HGAM) to identify temporal emergence patterns. Detailed information about the HGAM can be found in the main text in the data analysis section. Group-levels, type of smoothers, effective degrees of freedom, deviance explained, number of observations, and difference between model I and S of the hierarchical generalized additive models (HGAM). BIC is the Bayesian Information Criterion used to identify the best fit model.

Variable	**Group-levels**	**Type of smoother**		**Effective degrees of freedom**	**Deviance explained**	**Number observations**	**Difference BIC model I and model S**
Biomass	Land use	Factor smoother interaction	s(day, land use)	15.5	23	1911	22
		Random effect smoother	s(stream)	8.8			
	Land use, order	Factor smoother interaction	s(day, land use, order)	61.0	40	7644	37
		Random effect smoother	s(stream)	8.8			
	Land use, family	Factor smoother interaction	s(day, land use, family)	359.2	70	64987	58
		Random effect smoother	s(stream)	8.8			
Abundance	Land use	Factor smoother interaction	s(day, land use)	17.0	31	1911	50
		Random effect smoother	s(stream)	8.8			
	Land use, order	Factor smoother interaction	s(day, land use, order)	56.7	70	7644	112
		Random effect smoother	s(stream)	8.8			
	Land use, family	Factor smoother interaction	s(day, land use, family)	287.0	84	64987	250
		Random effect smoother	s(stream)	8.8			

Table B.5: Number of peaks for total biomass and abundance, time of the peaks in forest and agriculture, and difference in time between peaks in forested and agricultural sites. Day: day of the year. Difference day > 0: peak occurred earlier in agriculture.

Variable	**Peak number**	**Day forest**	**Day agriculture**	**Difference day forest and agriculture**
Biomass	1	109	106	3
	2	185	166	19
Abundance	1	109	106	3
	2	187	182	5
	3	222	219	3

Table B.6: Estimated regression parameters, standard errors (SE), Z *and P* values for the generalized linear mixed-effects models (GLMM) to compare the number of all families and EPT (stonefly, mayfly, and caddisfly) families between forested and agricultural sites.

Model	**Parameter**	**Estimate**	**SE**	***Z value***	***P* value**	σ_{stream}	σ_{season}
Number families	Intercept	2.20	0.09	*24.53*	$< 2 \cdot 10^{-16}$	$2 \cdot 10^{-5}$	0.11
	Land use	-0.14	0.09	-1.55	0.12		
Number EPT families	Intercept	1.35	0.16	8.53	$< 2 \cdot 10^{-16}$	0.16	0.21
	Land use	-0.20	0.14	-1.49	0.14		

Table B.7: Paired *t*-test to compare the generation time and size of aquatic insect families between forested and agricultural sites. A generation time ≥ 0.5/year was classified as sensitive. Size classes: Small (0.25 < size ≤ 1.00 cm), medium (1.00 < size ≤ 2.00 cm) and large (2.00 < size ≤ 8.00 cm). Cohen's d gives the effect size: 0.2 (small effect), 0.5 (medium effect), 0.8 (large effect).

Trait	***t***	**Degrees of freedom**	**Mean of difference**	**95% confidence interval**	***p*-value**	**Cohen's *d***
Sensitive generation time	-1.95	9	-0.15	-0.32 – 0.02	0.08	0.62
Size class small	-0.46	9	-0.03	-0.17 – 0.11	0.66	0.15
Size class medium	1.02	9	0.04	-0.05 – 0.13	0.33	0.32
Size class large	-0.45	9	-0.01	-0.07 – 0.05	0.66	0.14

Table B.8: Number of peaks per flies (Diptera), mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) for biomass and abundance, time of peaks in forest and agriculture, and difference in time between peaks in forested and agricultural sites. Day: day of the year. Difference day > 0: peak occurred earlier in agriculture. Difference day < 0: peak occurred earlier in forest. NA: No peak available in either forest or agriculture and, thus, no difference between peaks can be calculated.

Variable	**Order**	**Peak number**	**Day forest**	**Day agriculture**	**Difference day forest and agriculture**
Biomass	Flies	1	102	102	0
		2	187	157	30
		3	NA	219	NA
	Mayflies	1	108	109	-1
		2	138	NA	NA
	Stoneflies	1	115	93	22
	Caddisflies	1	185	175	10
		2	NA	192	NA
Abundance	Flies	1	109	104	5
		2	184	182	2
		3	222	221	1
	Mayflies	1	134	120	14
		2	205	192	14
	Stoneflies	1	118	100	18
	Caddisflies	1	152	203	-51

Table B.9: Number of peaks per family for biomass and abundance, time of peaks in forest and agriculture, and difference in time between peaks in forested and agricultural sites. Day: day of the year. Difference day > 0: peak occurred earlier in agriculture. Difference day < 0: peak occurred earlier in forest. NA: no peak available in either forest or agriculture and thus no difference between peaks can be calculated.

Variable	**Order**	**Family**	**Peak number**	**Day forest**	**Day agriculture**	**Difference day forest and agriculture**
Biomass	Flies	Phantom midges (Chaoboridae)	1	118	109	9
			2	242	168	74
			3	NA	214	NA
		Non biting midges (Chironomidae)	1	97	102	-5
			2	185	171	14
			3	219	221	-2
		Mosquitoes (Culicidae)	1	155	NA	NA
			2	217	NA	NA
		Meniscus midges (Dixidae)	1	108	NA	NA
		Ballon/dagger flies (Empididae)	1	129	113	16
			2	210	187	23
			3	NA	214	NA
		Limoniid crane flies (Limoniidae)	1	104	153	-49
			2	139	231	-92
			3	182	NA	NA
			4	224	NA	NA
		Drain flies (Psychodidae)	1	162	111	51
			2	235	159	76
			3	NA	191	NA
			4	NA	244	NA
		Phantom crane flies (Ptychopteridae)	1	139	132	7
			2	228	NA	NA
		Black flies (Simuliidae)	1	111	109	2
			2	187	148	39
			3	249	NA	NA
		Crane flies (Tipulidae)	1	130	130	0
			2	184	166	18
	Mayflies	Flatheaded mayflies (Arthropleidae)	1	155	NA	NA
		Small mayflies (Baetidae)	1	106	109	-3
			2	136	NA	NA
			3	192	NA	NA
		Spiny crawler mayflies (Ephemerellidae)	1	132	123	9
			2	168	187	-19

Table B.9: Continued

Variable	Order	Family	Peak number	Day forest	Day agriculture	Difference day forest and agriculture
Biomass	Mayflies	Burrowing mayflies (Ephemeridae)	1	141	141	0
		Stream mayflies (Heptageniidae)	1	148	153	-5
		Primitive minnow mayflies (Siphlonuridae)	1	176	NA	NA
	Stoneflies	Green stoneflies (Chloroperlidae)	1	123	NA	NA
		Needle flies (Leuctridae)	1	111	NA	NA
		Spring stoneflies (Nemouridae)	1	129	93	36
			2	NA	136	NA
		Willow flies (Taeniopterygidae)	1	111	99	12
	Caddisflies	Tortoise makers (Glossosomatidae)	1	145	NA	NA
		Weighted case maker (Goeridae)	1	116	NA	NA
			2	214	NA	NA
		Net-spinning caddisflies (Hydropsychidae)	1	184	129	55
			2	NA	168	NA
			3	NA	203	NA
		Micro caddisflies (Hydroptilidae)	1	146	141	5
			2	NA	184	NA
			3	NA	235	NA
		Lepidostomatid case makers (Lepidostomatidae)	1	NA	184	NA
		Long-horned caddisflies (Leptoceridae)	1	162	166	-4
			2	NA	210	NA
		Northern caddisflies (Limnephilidae)	1	NA	129	NA
			2	NA	191	NA
			3	NA	252	NA
		Large caddisflies (Phryganeidae)	1	148	NA	NA
		Tube-making caddisflies (Psychomyiidae)	1	152	138	14
			2	NA	198	NA
			3	NA	231	NA
		Primitive caddisflies (Rhyacophilidae)	1	138	100	38
			2	180	155	25

Table B.9: Continued.

Variable	**Order**	**Family**	**Peak number**	**Day forest**	**Day agriculture**	**Difference day forest and agriculture**
Biomass	Caddisflies	Bushtailed caddisflies (Sericostomatidae)	1	145	146	-1
			2	171	205	-34
			3	235	NA	NA
		Stonecase caddisflies (Uenoidae)	1	224	NA	NA
Abundance	Flies	Biting midges (Ceratopogonidae)	1	189	127	62
			2	NA	191	NA
		Phantom midges (Chaoboridae)	1	120	111	9
			2	249	217	32
		Non biting midges (Chironomidae)	1	111	104	7
			2	182	182	0
			3	221	221	0
		Mosquitoes (Culicidae)	1	215	168	47
		Meniscus midges (Dixidae)	1	104	NA	NA
		Ballon/dagger flies (Empididae)	1	129	115	14
			2	212	180	32
		Limoniid crane flies (Limoniidae)	1	143	153	-10
			2	224	231	-7
		Drain flies (Psychodidae)	1	228	155	73
			2	NA	201	NA
		Phantom crane flies (Ptychopteridae)	1	143	132	11
		Phantom crane flies (Ptychopteridae)	2	240	NA	NA
		Black flies (Simuliidae)	1	120	146	-26
			2	189	NA	NA
			3	252	NA	NA
		Crane flies (Tipulidae)	1	180	155	25
			2	NA	249	NA
	Mayflies	Small mayflies (Baetidae)	1	132	122	10
			2	205	191	14
		Spiny crawler mayflies (Ephemerellidae)	1	150	173	-23
		Burrowing mayflies (Ephemeridae)	1	139	NA	NA
		Stream mayflies (Heptageniidae)	1	146	153	-7

Table B.9: Continued.

Variable	**Order**	**Family**	**Peak number**	**Day forest**	**Day agriculture**	**Difference day forest and agriculture**
Abundance	Stoneflies	Needle flies (Leuctridae)	1	111	NA	NA
		Spring stoneflies (Nemouridae)	1	123	97	26
		Willow flies (Taeniopterygidae)	1	116	97	19
			2	244	NA	NA
	Caddisflies	Tortoise makers (Glossosomatidae)	1	145	NA	NA
		Weighted-case maker (Goeridae)	1	115	NA	NA
			2	208	NA	NA
		Net-spinning caddisflies (Hydropsychidae)	1	187	205	-18
		Micro caddisflies (Hydroptilidae)	1	150	145	5
			2	NA	219	NA
		Long-horned caddisflies (Leptoceridae)	1	164	217	-53
		Northern caddisflies (Limnephilidae)	1	NA	191	NA
		Tube making caddisflies (Psychomyiidae)	1	153	146	7
			2	NA	224	NA
		Primitive caddisflies (Rhyacophilidae)	1	138	148	-10
		Bushtailed caddisflies (Sericostomatidae)	1	162	164	-2
			2	NA	191	NA
		Stonecase caddisflies (Uenoidae)	1	228	NA	NA

Table B.10: Overview of study results for the research objectives (I – III), information on the statistical analysis and the figures and tables of the results.

Research objective	Response variable	Statistical analysis	Result	
I) Compare total biomass and abundance	Amount	HGAM	Higher in agriculture	Fig. 1 Tab. S4, S5
	Phenology		Difference between agricultural and forested sites	
II) Compare taxonomic and trait composition	Amount order biomass and abundance	HGAM	Fly, mayfly and caddisfly higher in agriculture; Stonefly higher in forest	Fig. 2, 3 Tab. S4, S8
	Phenology order biomass and abundance		Difference between agricultural and forested sites	
	Amount family biomass and abundance		Difference between agricultural and forested sites	Fig. S8 – S15 Tab. S1, S9
	Phenology family biomass and abundance		Difference between agricultural and forested sites	
	Number all families and EPT families	GLMM	No difference between agricultural and forested sites	Fig. S4 Tab. S6
	Turnover of families	ANOSIM	Turnover of families between agricultural and forested sites	Fig. S5
	Size aquatic insects	Paired t-test	No difference between agricultural and forested sites	Fig. S7 Tab. S7
	Generation time aquatic insects		No difference between agricultural and forested sites	Fig. S6 Tab. S7
III) Identify land-use-related drivers	Total biomass	LMM	Increase: Toxicity, EC	Tab. 2
	Fly biomass		Decrease: Shading	
	Mayfly biomass		No explanatory variable	
	Stonefly biomass		Decrease: Toxicity	
	Caddisfly biomass		Increase: Toxicity, EC	
	Total abundance		Increase: Percentage pool habitats, EC Decrease: Shading	
	Fly abundance		Increase: Percentage pool habitats, EC Decrease: Shading, oxygen saturation	
	Mayfly abundance		Increase: Toxicity	
	Stonefly abundance		Decrease: Toxicity	
	Caddisfly abundance		Increase: EC	
	Number EPT families	GLMM	No explanatory variable	

Table B.11: Studies containing biomass and abundance information of aquatic insects in forest (F) and/or agriculture (A). Mean and range of biomass and abundance of aquatic insects.

			Biomass (mg d^{-1} m^{-2})		Abundance (ind d^{-1} m^{-2})		Total number families	
Reference	**Study site and duration**	**Insect order**	**A**	**F**	**A**	**F**	**A**	**F**
Carlson et al. (2016)1	Central Sweden	Total	—	—		A > F		A > F
	4 streams each in F and A	Flies	—	—		A > F		A > F
	Snap shot: 4 days in Jul, Aug, Oct 2009, Apr 2010	Caddisflies	—	—		A > F		A > F
Graf et al. (2020)2	Central Romania	Total	344 (6–1729)	—	57 (0–152)	—	28	—
	19 streams along gradient of A intensity	Flies	131 (6–1318)	—	72 (27–152)	—	10	—
	3 weeks, May 2016	Mayflies	218 (69–541)	—	69 (24–131)	—	7	—
		Stoneflies	—	—	—	—	2	—
		Caddisflies	696 (10–696)	—	31 (0–106)	—	9	—
Krell et al. (2015)3	South-Western Germany	Total	2.97	0.83	119.8	16.61	6	4
	1 stream each in F and A (and meadow)	Flies	0.44	0.14	117	16	3	2
	May–Aug 2012	Mayflies	—	—	1.6	0	1	0
		Stoneflies	0	0	0	0	0	0
		Caddisflies	—	—	1.2	0.61	2	2
Nakano & Murakami (2001)3	Northern Japan 1 stream in F Snap shot: 4 days twice a month May 1997 – Jun 1998	Total	—	2–14	—	—	—	—
Raitif et al. (2018)4	Western France	Total	10 (4–20)	—	—	—	—	—
	12 streams in agriculture	Flies	3 (1–6)	—	—	—	—	—
	Snap shot: 7 days in May, Jun, Jun–Jul, Sep, Nov–Dec 2016, Feb–Mar 2017	Mayflies	2 (0–4)	—	—	—	—	—

Table B.11: Continued.

Reference	Study site and duration	Insect order	Biomass (mg d-1 m-2) A	Biomass (mg d-1 m-2) F	Abundance (ind d-1 m-2) A	Abundance (ind d-1 m-2) F	Total number families A	Total number families F
Poepperl (2000)4	Northern Germany 1 stream in A Apr 1989–Nov 1990	Total	5 (3–5)	—	29 (18–38)	—	15	—
		Flies	3 (2–4)	—	28 (17–37)	—	2	—
		Mayflies	0.3 (0–0.4)	—	0.2 (0–0.3)	—	3	—
		Stoneflies	0.004 (0–0.005)	—	0.005 (0–0.008)	—	1	—
		Caddisflies	1.4 (0.9–1.5)	—	0.7 (0.4–0.9)	—	9	—
Yuen & Dudgeon, (2016)4	Southeastern China 2 streams in F Snap shot: 6 days, once a month May 2013–June 2014	Total	—	3 (2–4)	—	36 (25–49)	—	>18
		Flies	—	1 (1–1)	—	33 (22–43)	—	4
		Mayflies	—	1 (1–1)	—	2 (2–3)	—	5
		Stoneflies	—	0.3 (0.2–0.4)	—	0.7 (0.4–1.1)	—	3
		Caddisflies	—	0.5 (0.1–1.1)	—	0.8 (0.4–1.4)	—	>6
This study5	Southwestern Germany 10 streams,1 site each in F and A Mar–Sep 2018	Total	7 (3–13)	4 (1 - 6)	27 (7–59)	15 (4–24)	25	33
		Flies	3 (1–8)	1 (1 - 2)	27 (7–58)	13 (5–21)	11	11
		Mayflies	1 (0–5)	1 (0–2)	1 (0–3	1 (0–1)	4	6
		Stoneflies	0.01 (0–0.09)	0.03 (0–1)	0.01 (0-0.03)	0.3 (0.01–21)	2	4
		Caddisflies	3 (0–6)	2 (0–4)	1 (0–1)	0.5 (0–1)	8	12

1Other sampling method (sticky traps): only qualitative comparison

2Biomass estimated with length–mass relationship and original values biomass and abundance per trap and day

3Original

4Original values annual biomass and abundance per area

5Calculated of mean fit of HGAMs

Table B.12: Mean and range of all land-use-related drivers of aquatic insects monitored during the field experiment in forested and agricultural sites. Land-use-related drivers used in this study are bold.

Land use related driver	**Unit**	**Forest: mean and range**	**Agriculture: mean and range**
Air temperature[1]	°C	17.5 (12.1-21.5)	18.6 (13.3-23)
Ammonium[2]	mg L-1	0.1 (0-0.3)	0.1 (0-0.2)
Banks	1 – 6 (no – many)	4 (1-6)	2.8 (1-5)
Chloride (2)	mg L-1	15.9 (4.7-32.5)	35.1 (9.3-100)
Copper (2)	mg L-1	0.1 (0-0.1)	0.1 (0-0.2)
Curvature	1 – 7 (meandering – linear)	3.6 (2-6)	5.4 (2-7)
Depth	cm	10.7 (3.3-25.7)	19.9 (3.7-49.3)
Distance field	m	816.9 (5-1000)	19.1 (4-100)
Distance housing	m	750 (200-1500)	740.1 (42.5-1500)
Distance landscape-level	m	4.9 (0.9-12)	1.9 (0.2-10)
Distance street	m	100.5 (8-400)	231.3 (11-700)
Electrical conductivity[1]	µS cm-1	191.8 (92.6-381)	462.2 (178.8-1899)
Flooding of shore	1 – 2 (yes, no)	1.7 (1-2)	1.8 (1-2)
Flow[3]	m/sec	0.2 (0.1-0.4)	0.2 (0.1-0.4)
Height landscape-level	m	2.4 (0.6-6.2)	1.3 (0.1-3)
Islands/Banks	1 – 4 (no – many)	1.7 (1-3)	1.1 (1-3)
Land use: Agriculture	%	0 (0-0)	80.6 (42.5-100)
Land use: Fallow	%	0 (0-0)	18 (5-42.5)
Land use: Forest	%	89.9 (17.5-100)	10 (5-20)
Land use: Housing	%	10 (5-20)	10 (5-15)
Land use: Meadows & Pastures	%	50 (5-100)	23.3 (5-50)
Land use: Other	%	13.8 (5-30)	25 (5-50)
Maximum width	cm	182.6 (68-400)	199.7 (60-450)
Minimum width	cm	97.7 (28-220)	139.5 (16-380)
Nitrate[2]	mg L-1	1.8 (0.5-5.1)	2.1 (1-5.3)
Nitrite[2]	mg L-1	0 (0-0)	0 (0-0)
Oxygen[1]	mg L-1	10.1 (9.1-11.4)	9.3 (6.2-11.6)
Oxygen saturation[1]	%	96.6 (65.6-126.8)	91.9 (63.9-108.5)

Table B.12: Continued.

Land use related driver	**Unit**	**Forest: mean and range**	**Agriculture: mean and range**
Pesticide toxicity	Maximum of logarithmic sum of toxic units for the most sensitive freshwater invertebrate species	-5.4 (-7-(-0.5))	-1.1 (-3.4-0.6)
pH		7.3 (5.8-8.1)	7.7 (6.8-8.3)
Phosphate[2]	mg L-1	0.7 (0.2-1.8)	0.7 (0.2-1.5)
Pools	%	27 (10-80)	44.2 (10-100)
Riffles	%	73 (20-90)	55.8 (0-90)
Shading	%	73 (30-100)	61.8 (5-95)
Shore vegetation cover: Agriculture	%	0 (0-0)	40 (40-40)
Shore vegetation cover: Vegetation Free	%	22.1 (5-40)	21.2 (10-35)
Shore vegetation cover: Forest	%	60.3 (5-100)	26.1 (10-50)
Shore vegetation cover: Meadow	%	76.7 (70-80)	53.7 (7.5-100)
Shore vegetation cover: Reed	%	41.2 (10-72.5)	37.9 (10-80)
Sulfate[2]	mg L-1	19.1 (10-27.7)	37.4 (10-94)
Water temperature[1]	°C	12.6 (9.4-16.6)	14.6 (11.4-18.2)

1 Multi 340i, WTW Germany, was used for measurement

2 Compact-photometer PF-12 with visocolor, Macherey-Nagel, was used for measurement

3 Flowmeter, Höntzsch

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C Supplementary material: Land use alters cross-ecosystem transfer of high value fatty acids by aquatic insects

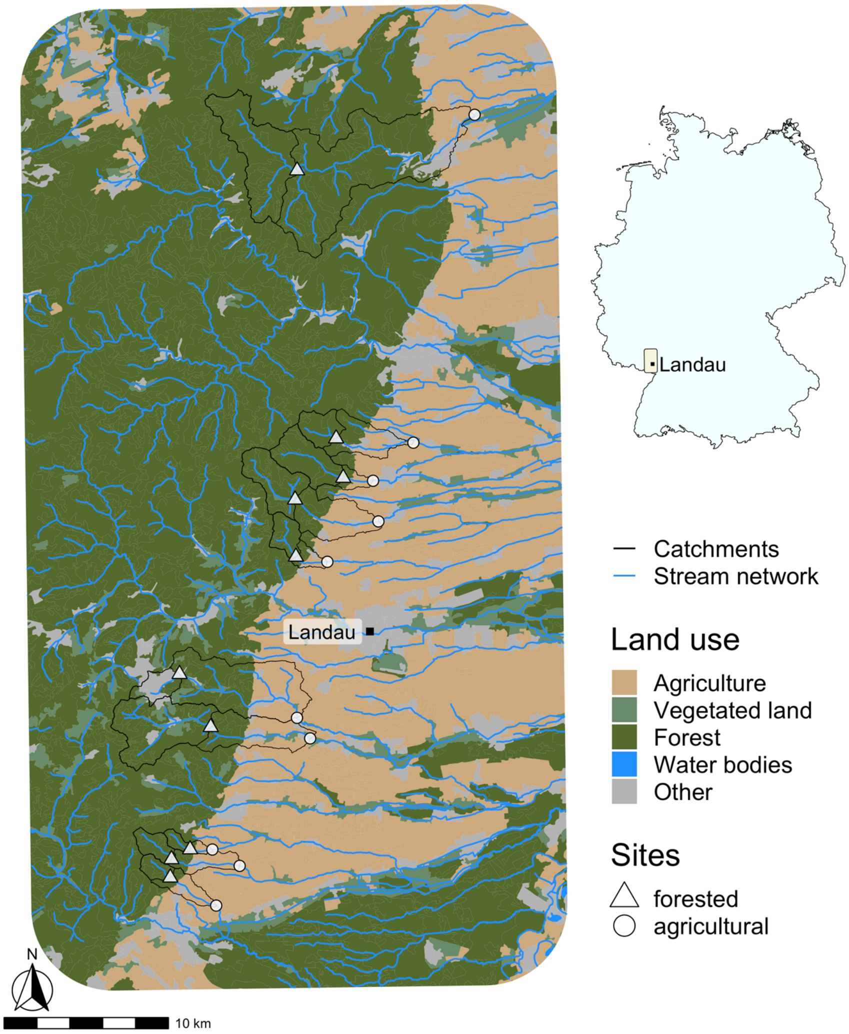

Figure C.1: Sampling sites in south-western Germany (European Environment Agency, 2007). The location of the study sites within Germany are shown in the inserted map.

Table C.1: Mean and standard deviation (sd) of all environmental variables monitored during the field experiment in forested and agricultural sites. Environmental variables used in the Redundancy analysis (RDA) and partial RDA are bold. (1) Multi 340i, WTW Germany, used for measurement, (2) Compact-photometer PF-12 with visocolor, Macherey-Nagel, used for measurement, (3) Flowmeter, Höntzsch, used for measurement.

Environmental variables	**Unit**	**Forest: mean (± sd)**	**Agriculture: mean (± sd)**
Air temperature (1)	**°C**	**18 (±4.6)**	**19.1 (±4.6)**
Ammonium (2)	mg L^{-1}	0.1 (±0)	0.1 (±0)
Banks	1 – 6 (no – many)	4 (±1.4)	2.8 (±1.6)
Biomass emergence	**mg m^{-2} d^{-1}**	**4.7 (±4.8)**	**7.4 (±6)**
Chloride (2)	mg L^{-1}	15.8 (±9.8)	35 (±33.5)
Copper (2)	mg L^{-1}	0.1 (±0)	0.1 (±0)
Curvature	1 – 7 (meandering – linear)	3.6 (±1.4)	5.4 (±1.4)
Depth	cm	10.7 (±4.9)	19.9 (±13.6)
Distance field	m	816.9 (±367)	19.1 (±22.9)
Distance housing	m	750 (±360.4)	740.1 (±368.6)
Distance landscape-level	m	4.9 (±4.3)	1.9 (±2.5)
Distance street	m	100.5 (±118.4)	231.3 (±207.1)
Electrical conductivity (1)	µS cm^{-1}	192.3 (±82.5)	474.2 (±420.6)
Flooding of shore	1 – 2 (yes, no)	1.7 (±0.5)	1.8 (±0.4)
Flow (3)	m s^{-1}	0.2 (±0.1)	0.2 (±0.1)
Height landscape-level	m	2.4 (±1.9)	1.3 (±0.6)
Islands/Banks	1 – 4 (no – many)	1.7 (±0.8)	1.1 (±0.4)
Land use: Agriculture	%	NaN (±NA)	80.6 (±18.5)
Land use: Fallow	%	NaN (±NA)	18 (±16.5)
Land use: Forest	%	89.9 (±20.7)	10 (±7.1)
Land use: Housing	%	10 (±8.7)	10 (±3.5)
Land use: Meadows & Pastures	%	50 (±37.4)	23.3 (±15.3)
Land use: Other	%	13.8 (±11.1)	25 (±22.9)
Maximum Width	cm	182.6 (±80.9)	199.7 (±101.9)
Minimum Width	cm	97.7 (±55.4)	139.5 (±94.7)
Nitrate (2)	**mg L^{-1}**	**1.8 (±1.2)**	**2 (±1)**
Nitrite (2)	mg L^{-1}	0 (±0)	0 (±0)
Oxygen (1)	mg L^{-1}	10.1 (±0.6)	9.3 (±1.1)
Oxygen saturation (1)	**%**	**96.6 (±9.4)**	**91.9 (±10.4)**
Pesticide toxicity	**Maximum of logarithmic sum of toxic unit for the most sensitive freshwater invertebrate species**	**-5.4 (±2.2)**	**-1.1 (±0.9)**
pH		7.3 (±0.5)	7.6 (±0.5)
Phosphate (2)	**mg L^{-1}**	**0.7 (±0.3)**	**0.7 (±0.4)**
Pools	**%**	**27 (±19.3)**	**44.2 (±24.2)**
Riffles	%	73 (±19.3)	55.8 (±24.2)
Shading	**%**	**73 (±17)**	**61.8 (±22.7)**

Table C.1: Continued.

Environmental variables	**Unit**	**Forest: mean (± sd)**	**Agriculture: mean (± sd)**
Shore vegetation cover: Agriculture	%	NaN (±NA)	40 (±NA)
Shore vegetation cover: Vegetation Free	%	22.1 (±15.5)	21.2 (±10.3)
Shore vegetation cover: Forest	%	60.3 (±34)	26.1 (±13.2)
Shore vegetation cover: Meadow	%	76.7 (±5.8)	53.7 (±28.3)
Shore vegetation cover: Reed	%	41.2 (±44.2)	37.9 (±24.7)
Shore vegetation cover: Shrubbery	%	38 (±23.4)	42.2 (±20.1)
Shore vegetation cover: Tall Forbs	%	28.9 (±22.5)	38.5 (±22.9)
Shore vegetation height: Agriculture	m	20 (±NA)	0 (±NA)
Shore vegetation height: Meadow	m	5.2 (±9.9)	0.5 (±0.4)
Shore vegetation height: Forest	m	16.4 (±4)	16.9 (±4.6)
Shore vegetation height: Reed	m	8 (±10.4)	1.9 (±0.4)
Shore vegetation height: Shrubbery	m	4.1 (±5.2)	3 (±2.2)
Shore vegetation height: Tall Forbs	m	3 (±6)	1.3 (±0.7)
Sulfate (2)	mg L^{-1}	18.6 (±5.8)	38.1 (±23.8)
Water temperature (1)	**°C**	**13 (±2.7)**	**15.1 (±3.5)**
Width	cm	136.5 (±59)	173.3 (±93.8)

Table C.2: Name, CAS-number, k_{oc} (soil organic carbon–water partitioning coefficient) value of the pyrethroids used in the calculation of the particle-associated concentration of an estimate of the bioavailable concentration in water.

Name	**CAS**	$\boldsymbol{k_{oc}}$	**Source**
allethrin	584792	1400	Lewis et al., 2016
acrinathrin	101007061	48231	Lewis et al., 2016
etofenprox	80844071	17757	Lewis et al., 2016
permethrin	52645531	100000	Lewis et al., 2016
prallethrin	23031369	1318	National Center for Biotechnology Information, 2021

Table C.3: Name, CAS-number, acute EC50 value, taxon and source of EC50 values of the pesticides used in the calculation of the logarithmic sum of the toxic units (sumTU). Pesticide types are abbreviated as follows: Insecticides (I), Herbicides (H), Fungicides (F).

Name	**CAS**	**Taxon**	**EC50 (µg L^{-1})**	**Pesticide type**	**Source**
(e)-acetamiprid	135410207	*Chironomus tepperi*	2.22	I	Scharmüller et al., 2020; US EPA, 2021
2-ethoxy-3,3-dimethyl-5-(methylsulfonylmethyl)-2h-1-benzofuran	26225796	*Daphnia magna*	137171.43	H	Scharmüller et al., 2020; US EPA, 2021
2,4-d	94757	*Astacus leptodactylus*	32600	H	Scharmüller et al., 2020; US EPA, 2021
acrinathrin	101007061	*Daphnia magna*	0.02	I	Lewis et al., 2016
allethrin	584792	*Pteronarcys californica*	7.1	I	Scharmüller et al., 2020; US EPA, 2021
azoxystrobin	131860338	*Gammarus pulex*	270	F	Scharmüller et al., 2020; US EPA, 2021
bentazone	25057890	*Chironomus riparius*	62300	H	Scharmüller et al., 2020; US EPA, 2021
bixafen	581809463	*Daphnia magna*	1200	F	Lewis et al., 2016
boscalid	188425856	*Daphnia magna*	5330	F	Scharmüller et al., 2020; US EPA, 2021
bromoxynil	1689845	*Chironomus riparius*	2113.05	H	Scharmüller et al., 2020; US EPA, 2021
chloridazon	1698608	*Daphnia magna*	132000	H	Malaj et al., 2014
clothianidin	210880925	*Chironomus dilutus*	2.07	I	Scharmüller et al., 2020; US EPA, 2021
cyazofamid	120116883	*Daphnia magna*	413	F	Scharmüller et al., 2020; US EPA, 2021
cyprodinil	121552612	*Daphnia magna*	32	F	Scharmüller et al., 2020; US EPA, 2021
dichlorprop_P	15165670	*Daphnia magna*	100000	H	Malaj et al., 2014
difenoconazole	119446683	*Daphnia magna*	53.03	F	Scharmüller et al., 2020; US EPA, 2021
dimethachlor	50563365	*Daphnia magna*	24000	H	Lewis et al., 2016
dimethenamid-p	163515148	*Daphnia magna*	12000	H	Scharmüller et al., 2020; US EPA, 2021
dimethoate	60515	*Chironomus dilutus*	1.29	I	Scharmüller et al., 2020; US EPA, 2021
dimethomorph	110488705	*Daphnia magna*	10600	F	Scharmüller et al., 2020; US EPA, 2021
dimoxystrobin	149961524	*Daphnia magna*	39.4	F	Lewis et al., 2016
epoxiconazol	135319732	*Chironomus riparius*	62.5	F	Lewis et al., 2016
etofenprox	80844071	*Cheumatopsyche brevilineata*	0.12	I	Scharmüller et al., 2020; US EPA, 2021
fenpropidin	67306007	*Daphnia magna*	1803.14	F	Scharmüller et al., 2020; US EPA, 2021
fipronil	120068373	*Chironomus dilutus*	0.03	I	Scharmüller et al., 2020; US EPA, 2021
florasulam	145701231	*Daphnia magna*	5500	H	Scharmüller et al., 2020; US EPA, 2021
fluazinam	79622596	*Brachionus calyciflorus*	1.6	F	Scharmüller et al., 2020; US EPA, 2021
fludioxonil	131341861	*Daphnia magna*	900	F	Scharmüller et al., 2020; US EPA, 2021
flufenacet	142459583	*Hyalella azteca*	2800	H	Scharmüller et al., 2020; US EPA, 2021

Table C.3: Continued.

Name	**CAS**	**Taxon**	**EC50 (µg L^{-1})**	**Pesticide type**	**Source**
fluroxypyr	69377817	*Daphnia magna*	100000	H	Scharmüller et al., 2020; US EPA, 2021
fluxapyroxad	907204313	*Daphnia magna*	6780	F	Lewis et al., 2016
foramsulfuron	173159574	*Daphnia magna*	100000	H	Lewis et al., 2016
imidacloprid	138261413	*Epeorus longimanus*	1.42	I	Scharmüller et al., 2020; US EPA, 2021
isoproturon	34123596	*Daphnia magna*	580	H	Malaj et al., 2014
kresoxim-methyl	143390890	*Daphnia magna*	285.01	F	Scharmüller et al., 2020; US EPA, 2021
lenacil	2164081	*Daphnia magna*	8400	H	Malaj et al., 2014
mecoprop_P	16484778	*Daphnia magna*	90940	H	Scharmüller et al., 2020; US EPA, 2021
mesotrione	104206828	*Daphnia magna*	840000	H	Scharmüller et al., 2020; US EPA, 2021
metamitron	41394052	*Daphnia magna*	5700	H	Lewis et al., 2016
metazachlor	67129082	*Daphnia magna*	33000	H	Malaj et al., 2014
methiocarb	2032657	*Chironomus tentans*	1.6	I	Scharmüller et al., 2020; US EPA, 2021
metolachlor	51218452	*Chironomus plumosus*	4089.01	H	Scharmüller et al., 2020; US EPA, 2021
metribuzin	21087649	*Daphnia magna*	4180	H	Scharmüller et al., 2020; US EPA, 2021
napropamide	15299997	*Daphnia magna*	18793.88	H	Scharmüller et al., 2020; US EPA, 2021
nicosulfuron	111991094	*Daphnia magna*	90000	H	Lewis et al., 2016
permethrin	52645531	*Hyalella azteca*	0.02	I	Scharmüller et al., 2020; US EPA, 2021
pethoxamid	106700292	*Daphnia magna*	23000	H	Lewis et al., 2016
picoxystrobin	117428225	*Daphnia magna*	24	F	Lewis et al., 2016
pirimicarb	23103982	*Daphnia magna*	17.12	I	Scharmüller et al., 2020; US EPA, 2021
prallethrin	23031369	*Culex quinquefasciatus*	3.15	I	Scharmüller et al., 2020; US EPA, 2021
prochloraz	67747095	*Gammarus pulex*	2180	F	Scharmüller et al., 2020; US EPA, 2021
propamocarb	24579735	*Daphnia magna*	106000	F	Lewis et al., 2016
propiconazole	60207901	*Baetis rhodani*	900	F	Scharmüller et al., 2020; US EPA, 2021
prosulfocarb	52888809	*Daphnia magna*	510	H	Lewis et al., 2016
pyraclostrobin	175013180	*Daphnia magna*	54.87	F	Scharmüller et al., 2020; US EPA, 2021
pyroxsulam	422556089	*Daphnia magna*	100000	H	Scharmüller et al., 2020; US EPA, 2021
quizalofop_free_acid	76578126	*Daphnia magna*	57700	H	Lewis et al., 2016
s-metolachlor	87392129	*Gammarus pulex*	9470.87	H	Scharmüller et al., 2020; US EPA, 2021
spiroxamine	118134308	*Daphnia magna*	4164.13	F	Scharmüller et al., 2020; US EPA, 2021
tebuconazole	107534963	*Gammarus fossarum*	1347	F	Scharmüller et al., 2020; US EPA, 2021
terbuthylazine	5915413	*Daphnia magna*	32849.35	H	Scharmüller et al., 2020; US EPA, 2021

Table C.3: Continued.

Name	**CAS**	**Taxon**	**EC50 (µg L^{-1})**	**Pesticide type**	**Source**
thiacloprid	111988499	*Chironomus tepperi*	1.06	I	Scharmüller et al., 2020; US EPA, 2021
thiamethoxam	153719234	*Chironomus*	35	I	Scharmüller et al., 2020; US EPA, 2021
triadimenol	55219653	*Daphnia magna*	2500	F	Scharmüller et al., 2020; US EPA, 2021
trifloxystrobin	141517217	*Daphnia magna*	26.72	F	Scharmüller et al., 2020; US EPA, 2021

Table C.4: Time periods, in which samples of emergent aquatic insects were pooled on order level for fatty acid analysis.

Start date	**End date**	**Duration in days**
2018-03-17	2018-04-01	15
2018-04-02	2018-04-15	13
2018-04-16	2018-04-29	13
2018-04-30	2018-05-13	13
2018-05-14	2018-05-16	2
2018-05-17	2018-05-29	12
2018-05-30	2018-06-13	14
2018-06-14	2018-06-27	13
2018-06-28	2018-07-11	13
2018-07-12	2018-07-26	14
2018-07-27	2018-08-12	16
2018-08-13	2018-08-26	13
2018-08-27	2018-09-09	13
2018-09-10	2018-09-13	3

Table C.5: Results of hierarchical generalized additive models (HGAM) to identify seasonal patterns of fatty acid export via aquatic insects. Group-levels, type of smoothers, effective degrees of freedom, deviance explained, number of observations, and difference between model I and S of the HGAM. The Bayesian Information Criterion (BIC) was used to identify the best fit model. FA: fatty acid, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

Variable	**Group-levels**	**Type of smoother**		**Effective degrees of freedom**	**Deviance explained**	**Number observations**	**Difference BIC model I and model S**
FA	land use	factor smoother interaction	s(day, land use)	6.62	0.19	354	7
		random effect smoother	s(stream)	6.65			
	land use, order	factor smoother interaction	s(day, land use, order)	34.02	0.36	998	53
		random effect smoother	s(stream)	6.46			
SFA	land use	factor smoother interaction	s(day, land use)	6.39	0.27	354	20
		random effect smoother	s(stream)	7.76			
	land use, order	factor smoother interaction	s(day, land use, order)	32.54	0.40	998	21
		random effect smoother	s(stream)	7.90			
MUFA	land use	factor smoother interaction	s(day, land use)	5.62	0.17	354	11
		random effect smoother	s(stream)	6.64			
	land use, order	factor smoother interaction	s(day, land use, order)	33.17	0.34	998	74
		random effect smoother	s(stream)	6.16			
PUFA	land use	factor smoother interaction	s(day, land use)	6.53	0.16	354	5
		random effect smoother	s(stream)	6.25			
	land use, order	factor smoother interaction	s(day, land use, order)	35.05	0.35	998	61
		random effect smoother	s(stream)	5.55			

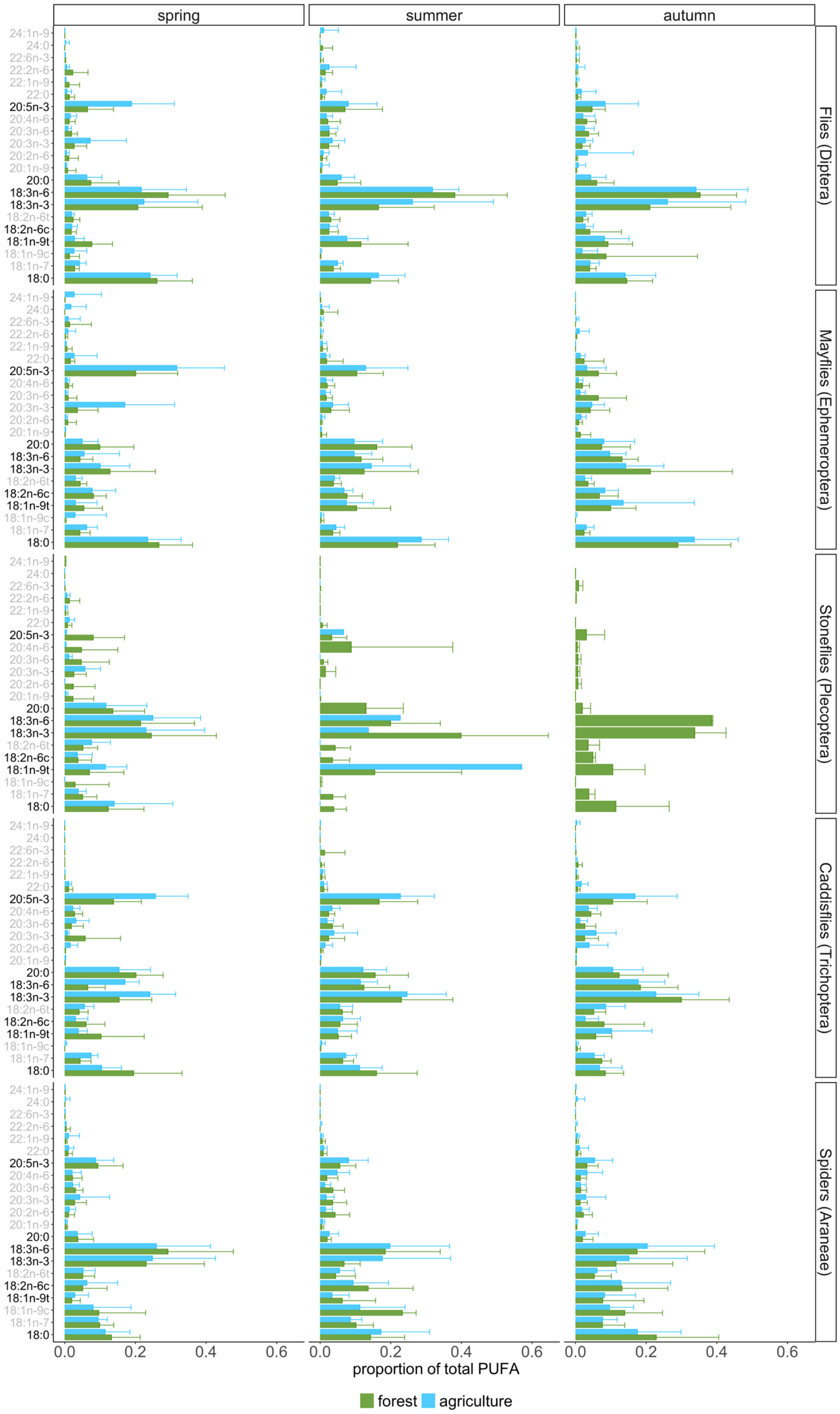

Figure C.2: Mean proportion and standard deviation of fatty acids (FA). Colors indicate the land-use types forest and agriculture. FA identified in Similarity percentage (SIMPER) analysis explaining the differences in FA profiles between land-use types: Eicosapentaenoic acid (20:5n-3, EPA), alpha-linolenic acid (18:3n-3, ALA), gamma-linolenic acid (18:3n-6, GLA), Linoleic acid (18:2n-6c, LIN), Elaidic acid (18:1n-9t, ELA), Octadecanoic acid (18:0, ODA), Eicosanoic acid (20:0, EA) are written in black, other FA in grey. SIMPER analysis was only conducted for flies as well as mayflies in spring and caddisflies in summer, because in other orders and seasons no significant differences between FA profiles was observed.

Table C.6: Results of the partial redundancy analysis (RDA) and RDA. The RDA includes stream and season as regular variable and the partial RDA includes stream as well as season as covariates, to partial out their effect.

			df	**Variance**	**F**	**p-value**	**adjusted R2**
partial RDA	Emergence	model	5	2.14	1.69	0.016	0.05
	Emergence	residual	43	10.90	NA	NA	
	Mayflies	model	4	2.00	1.74	0.041	0.06
	Mayflies	residual	38	10.97	NA	NA	
	Stoneflies	model	2	1.85	1.41	0.152	0.06
	Stoneflies	residual	10	6.57	NA	NA	
	Caddisflies	model	3	1.26	1.60	0.029	0.04
	Caddisflies	residual	36	9.40	NA	NA	
	Flies	model	7	3.61	2.12	0.004	0.12
	Flies	residual	41	9.99	NA	NA	
	Spider	model	4	1.31	1.44	0.063	0.03
	Spider	residual	34	7.70	NA	NA	
RDA	Emergence	model	16	9.10	2.24	0.001	0.25
	Emergence	residual	43	10.90	NA	NA	
	Mayflies	model	15	7.03	1.62	0.002	0.15
	Mayflies	residual	38	10.97	NA	NA	
	Stoneflies	model	13	10.16	1.20	0.153	0.11
	Stoneflies	residual	9	5.84	NA	NA	
	Caddisflies	model	15	7.22	1.92	0.001	0.22
	Caddisflies	residual	35	8.78	NA	NA	
	Flies	model	18	9.01	2.06	0.001	0.24
	Flies	residual	41	9.99	NA	NA	
	Spider	model	15	9.30	2.74	0.001	0.35
	Spider	residual	34	7.70	NA	NA	

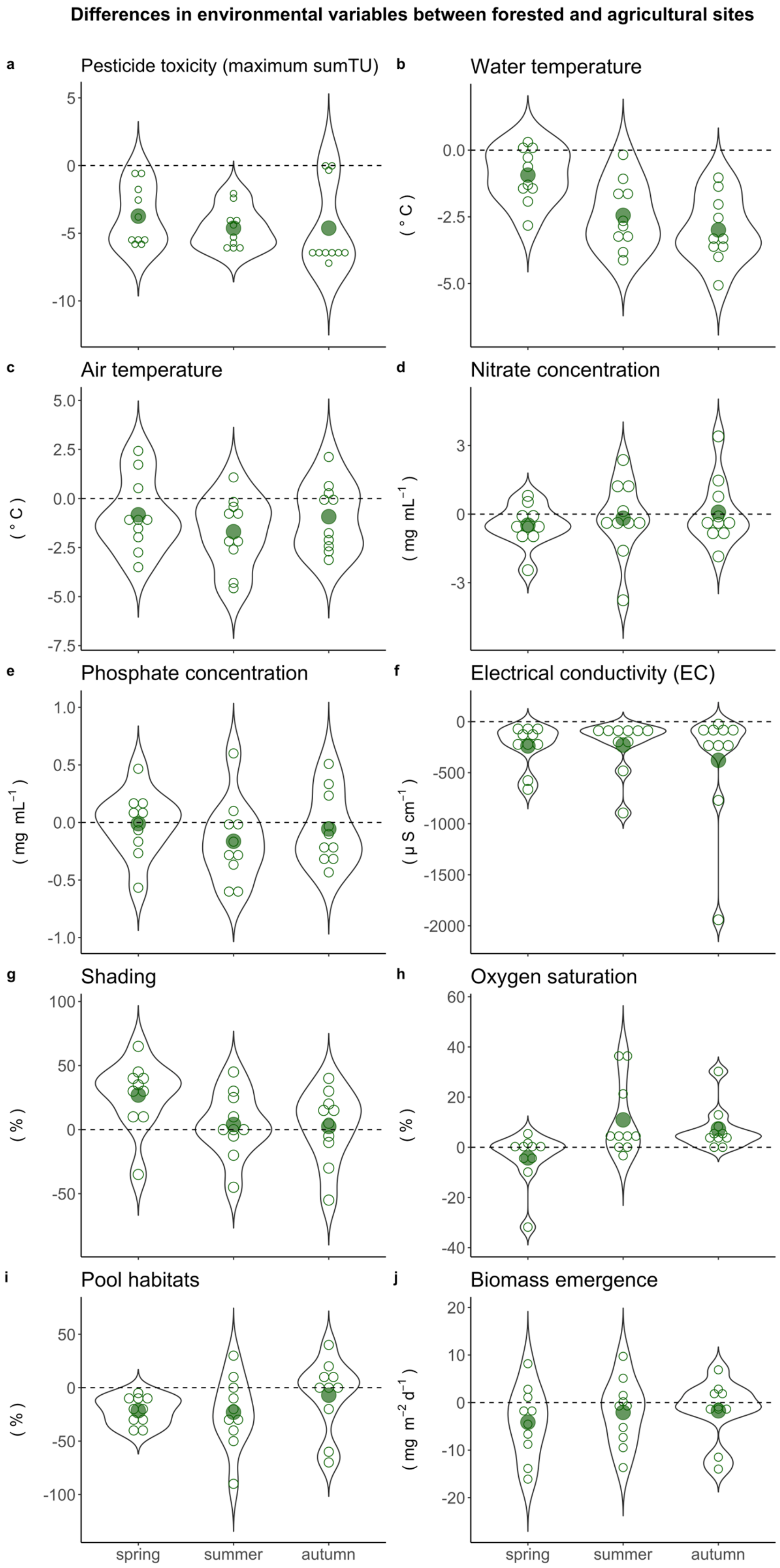

Figure C.3: Differences of environmental variables between forested and agricultural sites visualized with violin plots. White dots show differences between paired forested and agricultural sites. For the differences the mean value per season in agriculture was subtracted from the mean value per season in forest (ten paired upstream forested and downstream agricultural sites). The frequency distribution of the data is reflected by the width of the violin plots. The mean of the overall difference between forested and agricultural sites are represented with green dots. Environmental variables, which had higher values in agricultural than forested sites, show values below zero.

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6.2 Author contributions

Article 1

Title: Trophic transfer of polyunsaturated fatty acids across the aquatic–terrestrial interface: An experimental tritrophic food chain approach

Authors: Katharina Ohler, Verena C. Schreiner, Dominik Martin-Creuzburg, Ralf B. Schäfer

Status: Published in 2023, Ecology and Evolution 13, e9927.

Contributions: Katharina Ohler and Ralf B. Schäfer designed the study; Katharina Ohler conducted the experiment as well as fatty acid analysis, analyzed the data, and drafted the manuscript; all authors discussed the results and revised the manuscript.

Article 2

Title: Land use changes biomass and temporal patterns of insect cross-ecosystem flows

Authors: Katharina Ohler, Verena C. Schreiner, Moritz Link, Matthias Liess, Ralf B. Schäfer

Status: Published in 2023, Global Change Biology, 29, 81 – 96.

Contributions: Katharina Ohler, Verena C. Schreiner, Matthias Liess, Ralf B. Schäfer designed the study and discussed the results; Katharina Ohler, Verena C. Schreiner, Moritz Link selected the sampling sites; Katharina Ohler, Verena C. Schreiner, Moritz Link conducted the field work; Katharina Ohler identified the insects, analyzed the data, and drafted the manuscript; all authors revised the manuscript.

Article 3

Title: Land use alters cross-ecosystem transfer of high value fatty acids by aquatic insects

Authors: Katharina Ohler, Verena C. Schreiner, Lukas Reinhard, Moritz Link, Matthias Liess, Werner Brack, Ralf B. Schäfer

Status: Submitted in 2023, Environmental Sciences Europe.

Contributions: Katharina Ohler, Verena C. Schreiner, Matthias Liess, Ralf B. Schäfer designed the study; Katharina Ohler, Verena C. Schreiner, Moritz Link selected the sampling sites; Katharina Ohler, Verena C. Schreiner, Moritz Link conducted the field work; Katharina Ohler and Lukas Reinhard conducted the fatty acid analysis and identified the spiders; Katharina Ohler identified the insects, analyzed the data, and drafted the manuscript; all authors revised the manuscript.

6.3 Declaration

I, the author of this work, hereby declare that this PhD thesis entitled “Aquatic-terrestrial predator-prey relationships across ecosystem boundaries: How does agricultural land use affect the quantity and quality of stream exports?” contains no material which has been submitted at any university or other tertiary institution for scientific examination.

The work has been prepared independently. All used aids and references as well as involved contributors are clearly declared. I did not use the assistance of a doctoral consultant or similar persons in return for payment. I am aware that a violation of the above-mentioned points can have legal consequences including the withdrawal of the doctoral degree.

Neustadt an der Weinstraße, 19th October 2023

6.4 Curriculum vitae

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10/2015 – 01/2018	**M. Sc. Ecotoxicology**, University Koblenz – Landau Thesis: Influence of agriculture related stressors on aquatic-terrestrial predator-prey relationships in Romania
10/2012 – 09/2015	**B. Sc. Environmental Sciences**, University Koblenz – Landau Thesis: Effect of a fungicide mixture on aquatic leaf litter decomposition
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Publications and conference contributions

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Frisch, K.; Gunold, R.; Link, M.; Reemtsma, T.; Schreiner, V. C.; von Tümpling, W.; Schäfer, R. B.: Quantitative Untersuchung aquatisch-terrestrischer Räuber-Beute-Beziehungen über Systemgrenzen: Wie ändert Landwirtschaft die Quantität des Exports von Fließgewässern? Vortrag. SETAC GLB 2019, Landau, Deutschland.

Frisch, K.; Schreiner, V. C.; Link, M.; Schäfer, R. B.: Study of aquatic-terrestrial predator-prey relationships: How does agriculture alter the quantity and quality of stream exports? Vortrag. SEFS 11, 2019, Zagreb, Kroatien.

Frisch, K.; Graf, N.; Schreiner, V. C.; Szöcs, E.; Entling, M. H.; Schäfer, R. B.: Influence of agricultural related stressors on aquatic-terrestrial predator-prey relationships in Romania. Vortrag. SETAC GLB 2017, Neustadt, Deutschland.