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The effect of riparian buffer properties on spider communities and aquatic-terrestrial food-web linkages – using polyunsaturated fatty acids as trophic biomarkers

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Abstract

Riparian habitats are key habitat interfaces that regulate flows of resource subsidies between aquatic and terrestrial food webs, whilst supporting high biodiversity and providing ecosystem services. However, frequently these habitats are highly degraded, especially in agricultural landscapes. In this study, the effect of riparian buffer properties on the diversity and composition of spider communities along stream channels was investigated. Additionally, trophic connectivity was investigated by analysing the polyunsaturated fatty acid (PUFA) content of the riparian spiders. PUFAs are physiologically essential for animals, and some are exclusively produced by algae in aquatic environments. These aquatic PUFAs can therefore be used as biomarkers to track the uptake of algal-derived aquatic subsidies into terrestrial food webs. As different spider taxa rely on aquatic subsidies to varying degrees, the PUFA content of specific taxa was also examined. This to ascertain to what degree each taxon potentially contributes to the transfer of PUFAs into terrestrial food-webs.

Spiders were collected in Uppland, Sweden, from 10 paired sites (each pair with one unbuffered and one buffered site) in an agricultural landscape, and five reference forest sites. The spiders were identified to family level, and then freeze-dried, pulverized and homogenised, and their fatty acid content then extracted and analysed. Spider diversity, community composition and PUFA data were then statistically analysed using multivariate methods in R to reveal differences and interactions between site types and spider families.

I found that the abundances, community composition and biomass of the riparian spiders differed between site types. This result was largely due to differences in functional types of spiders, with web-building spiders dominating in buffered sites and free-living spiders more common in unbuffered sites. The differences can partly be explained by trait-mediated habitat preferences and local habitat availability. Statistical analyses revealed differences in the PUFA profiles of the spiders, which were largely driven by spider taxonomic identity, but also influenced by site type and an interaction between site type and spider family, as well as stream identity. Overall PUFA content was highest in forest site spiders, however, aquatically-derived PUFAs were similar between site types. Lycosidae spiders had consistently high levels of aquatic PUFAs. Thus, it seems that the assimilation and transfer of aquatic PUFAs from spiders further into terrestrial food-webs may be primarily routed through particular families. Understanding the factors that affect trophic connectivity and flow of resource subsidies is crucial for effective management and restoration of stream-riparian networks. A more varied buffer design may be one mitigation strategy that could benefit both biodiversity and trophic connectivity.

Keywords: riparian buffer, polyunsaturated fatty acid, spiders, trophic connectivity, agricultural landscapes, subsidies

"Soil and water are not two organic systems, but one.
Both are organs of a single landscape;
a derangement in either affects the health of both."

Aldo Leopold

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Abbreviations

FA	Fatty acid
PUFA	Polyunsaturated fatty acid
ALA	Alpha-linolenic acid
LIN	Linoleic acid
ARA	Arachidonic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid

1 Introduction

1.1 Stream-riparian linkages

Streams and their adjacent riparian zones are recognized as key habitats supporting high biodiversity and providing a range of ecosystem services (Naiman & Décamps 1997; Wenger 1999; Keeler *et al.* 2012; Biggs *et al.* 2017). Riparian zones can be defined as the region between the low water mark of the stream channel and the adjacent terrestrial area that is influenced by the stream's hydrology (Naiman & Décamps 1997). Riparian zones thus typically have characteristics of both terrestrial and aquatic ecosystems, and are interfaces between these systems, regulating the flux of resources between them (Naiman & Décamps 1997; Wenger 1999).

That adjacent systems are closely linked through flow and exchange of resources and materials has long been recognised by ecologists (Likens & Bormann 1974). During the 1990's, this view was further developed within food-web ecology by Polis *et al.* (1997) who explicitly defined the concept of "spatial subsidies". Spatial subsidies occur when donor organisms, communities or habitats control the supply of resources (for example nutrients or organisms) from one habitat across boundaries to impact populations and food-web dynamics in recipient habitats (Polis *et al.* 1997). Stream-riparian networks are especially useful models for studying spatial subsidies as the ecosystem boundary between them is relatively well-defined, and the spatial scale is tractable for conducting research (Richardson *et al.* 2010).

Two classic examples of subsidies in stream-riparian networks are the emergence of the adult stages of aquatic insects as prey for terrestrial predators, and the reciprocal input of terrestrial invertebrates to streams as prey for fish. A multitude of studies have looked at these subsidies in stream-riparian networks, focusing on different recipient taxa including birds, lizards and spiders in terrestrial habitats, and fish and detritivorous invertebrates in aquatic habitats (Nakano *et al.* 1999; Nakano & Murakami 2001; Fausch *et al.* 2002; Sabo & Power 2002; Kato *et al.* 2003; Baxter

et al. 2005). Research on the delivery of aquatic subsidies into terrestrial food webs is increasing, with a particular focus on variables that regulate the flow of subsidies, such as season and land use (Kato *et al.* 2003; Baxter *et al.* 2005; Marczak & Richardson 2008; Carlson 2014; Schindler & Smits 2017). The general conclusion of many of these studies is that terrestrial food webs are highly reliant on aquatic subsidies, with abundance, size and biodiversity of terrestrial organisms influenced by fluxes of emerging insects (Sabo & Power 2002; Baxter *et al.* 2005; Marczak & Richardson 2008; Richardson *et al.* 2010; Schindler & Smits 2017). The reliance on aquatic subsidies, however, varies both spatially and temporally. Both season and stream-riparian characteristics affect the timing and identity of emerging insects, and many terrestrial consumers are themselves seasonally constrained by migration and reproduction (Nakano & Murakami 2001; Kato *et al.* 2003; Baxter *et al.* 2005; Schindler & Smits 2017; McKie *et al.* 2018). Given the strong linkages between terrestrial and aquatic habitats, changes in one or both habitats will inevitably affect subsidy magnitude, delivery and impact in the recipient habitat. Increasingly, changes in these habitats and the linkages between them are driven by anthropogenic activities.

1.2 Riparian vegetation: function and threats

Functioning stream-riparian networks are not only vital for sustaining biodiversity but also for human health and well-being. Ecosystem services provided by stream-riparian networks include water purification, nutrient uptake and recreation (Keeler *et al.* 2012; Truchy *et al.* 2015; Biggs *et al.* 2017). Riparian vegetation strongly influences the microclimate in the riparian zone, regulating solar radiation and wind speed, and affecting species composition (Moore *et al.* 2005; Naiman *et al.* 2005). Furthermore, intact riparian vegetation increases habitat complexity due to stratification and successional patterns, which impacts diversity within the riparian zone (Naiman & Décamps 1997; Naiman *et al.* 2005). Characteristics of the riparian vegetation also affects the stream habitat, by controlling light availability and water temperatures, (which affects stream primary production), as well as allochthonous inputs, all in turn affecting in-stream invertebrate community composition (Moore *et al.* 2005; Allan & Castillo 2007; Johnson & Almlöf 2016). This in turn affects the linkages between riparian and stream systems by influencing both the quantity and quality of aquatic insects emerging into the riparian zone. The clearance of riparian vegetation therefore has the potential to alter both riparian and in-stream communities as well as the linkages between them.

Unfortunately, human reliance on, and exploitation of, stream-riparian networks also exposes them to numerous anthropogenic pressures, such as channel

modifications and clearance of riparian vegetation, often affecting network connectivity (Malmqvist & Rundle 2002; Dudgeon *et al.* 2006; Vörösmarty *et al.* 2010; Truchy *et al.* 2015). In agricultural systems riparian zones are often degraded, with little intact native riparian vegetation left. The clearance of riparian wooded vegetation has led to exposed banks, modified vegetation and fragmentation of riparian forest habitats (Corbacho *et al.* 2003; Ollero 2007; Renouf & Harding 2015). Ongoing management activities affecting the riparian zone after deforestation also impacts riparian-stream ecosystems and linkages. Whether the riparian forest vegetation is replaced by managed grass, or regrowth of shrubs and trees is allowed, has significant, contrasting, implications for the changes in the stream-riparian habitats and linkages (Bjelke *et al.* 2016). Furthermore, clearance of riparian wooded vegetation is often accompanied by intensified agricultural land use which has local impacts, and cumulative landscape-scale effects, on stream-riparian networks (Allan 2004). For example, these effects can manifest in the aquatic environment via enhanced sediment inputs from surface runoff and channel erosion (Allan 2004; Burdon *et al.* 2013). Understanding the effects of riparian vegetation clearance and management on communities and the linkages between them is essential for the development of effective restoration and management schemes targeting improved ecological status of stream-riparian networks.

1.3 Riparian buffers as a management measure

As the role and importance of riparian zones for ecosystem services, maintaining biodiversity and sustaining linkages has become increasingly clear, methods to alleviate the impacts of human activities on riparian habitats have been developed. One such mitigation strategy is to leave riparian vegetation in a strip alongside streams and rivers, with the aim that the strip acts as buffer against land-use practices and human activities. Riparian buffers are further expected to act as a refuge for sensitive biodiversity, and to facilitate the maintenance of linkages between stream and terrestrial habitats (Degerman & Bergqvist 2008; Allan & Castillo 2007).

In discussing the characteristics important for the efficiency of riparian buffers the significance of stratified vegetation assemblages, i.e. trees and shrubs, in the riparian zone is often highlighted (Schultz *et al.* 2004; Clark & Reeder 2007; Degerman & Bergqvist 2008; Stutter *et al.* 2012; Renouf & Harding 2015). Furthermore, in the restoration of deforested riparian zones planting of trees and shrubs is generally recommended. Though often an expensive measure, the long-term benefits of reforestation are generally considered to outweigh the short-term costs (Degerman & Bergqvist 2008; Renouf & Harding 2015). Within the forestry sector leaving a wooded riparian buffer zone is a relatively common (but by no means

universal) measure (Richardson *et al.* 2012; Sibley *et al.* 2012; Kuglerová *et al.* 2014). However, within agricultural systems the maintenance or restoration of wooded riparian buffers is not standard practise. Instead, in countries where maintenance of some kind of riparian buffer is legally required, buffers are typically implemented as relatively narrow grass or herbaceous buffer strips (Clark & Reeder 2007; Degerman & Bergqvist 2008; Smiley *et al.* 2011; Stutter *et al.* 2012; Renouf & Harding 2015).

There are several reasons why predominantly grass and herbaceous buffer strips are used. Firstly, a large focus in agricultural landscapes is controlling nutrient and sediment loads in waterways. Grass and herb buffers have the ability to retain nutrients and sediments, though their efficiency has shown to be highly variable (Hickey & Doran 2004; Schultz *et al.* 2004; Degerman & Bergqvist 2008; Stutter *et al.* 2012). Secondly, these grass and herbaceous buffers are relatively inexpensive to implement and maintain both for farmers and governments, requiring that only a minimal area of land is taken out of production, with no extra funding required for planting or management of trees and shrubs (Schultz *et al.* 2004; Clark & Reeder 2007; Stutter *et al.* 2012). The probability of conflict between different stakeholders is thereby also minimized. In Sweden there is an economic incentive for farmers to implement grass buffers on arable land in nitrate-sensitive areas. The regulations specifically state that though a few scattered shrubs and trees are allowed, no forest or 'forest like' areas are permitted to be included within these buffers (Degerman & Bergqvist 2008; Jordbruksverket 2019). The reason for the restriction on trees is a question of definitions of different land use types, arable land by definition are open areas and they are required by law to be kept as such (Jordbruksverket 2019). Finally, there is some evidence to suggest that these grass and herb buffers are important to grassland species, acting as refugia from the surrounding intensively managed matrix (Clark & Reeder 2007; Prieto-Benitez & Mendez 2011). However, these areas are not as species rich as natural grasslands and often benefit generalist species (Clark & Reeder 2007). As efforts, and studies, have largely focused on the role of grass buffers in nutrient and sediment retention in agricultural landscapes, more research is needed to ascertain their general value for biodiversity and trophic linkages between aquatic and terrestrial systems. Additionally, although the general consensus is that trees and shrubs are important for optimal function of riparian buffers, few studies have compared the biodiversity and trophic connectivity of grass/herb buffers and forested buffers within an agricultural landscape. These types of studies are important, not only to understand the effect these buffer types may have on biodiversity and trophic connectivity, but also for the development of best management practices of riparian buffers for landowners and governments.

1.4 Riparian invertebrate consumers: spiders and ground beetles

Riparian invertebrate consumers such as spiders and ground beetles are sensitive to environmental changes and dependent on aquatic subsidies, and are therefore ideal organisms to study aquatic-terrestrial linkages within different riparian buffer types (Kato *et al.* 2004; Baxter *et al.* 2005; Laeser *et al.* 2005; Paetzold *et al.* 2005; Burdon & Harding 2007; Prieto-Benitez & Mendez 2011; Krell *et al.* 2015; Stenroth *et al.* 2015). Habitat complexity in the riparian zone and disturbances such as floods are known to have an impact on riparian invertebrates, with abundances and diversity increasing in systems with dynamic flow-regimes and heterogenous vegetation (Sadler *et al.* 2004; Naiman *et al.* 2005; Greenwood & McIntosh 2008; Lambeets *et al.* 2008). Laeser *et al.* (2005) found that web-building spiders were negatively associated with clearance of riparian vegetation, presumably because of the loss of habitat structures needed for web-building. Variations in riparian invertebrate populations may in turn affect other organism groups. Spiders and ground beetles are prey in both terrestrial and aquatic systems, including for vertebrate predators. As intermediate predators, spiders and beetles thereby function as key links between higher and lower trophic levels (Nakano *et al.* 1999; Nakano & Murakami 2001; Baxter *et al.* 2005). Potential impacts that land use and buffer characteristics may have on broader food web dynamics can therefore be inferred from spider and ground beetle populations. However, not only habitat characteristics impact riparian invertebrate consumers but also prey availability.

All spiders and many ground beetles are predators and tend to aggregate in riparian zones to utilize the emerging insect subsidy (Lindroth 1985; Jocqué & Dippenaar-Schoeman 2007; Schindler & Smits 2017). The reliance of different taxonomic groups on aquatic subsidies varies, reflecting their degree of specialisation on aquatic prey. Web-building Tetragnathidae spiders are often highly reliant on aquatic prey, whilst results for Linyphiidae and Araneidae have been variable (Kato *et al.* 2003, 2004; Krell *et al.* 2015; Stenroth *et al.* 2015). Free-living Lycosidae spiders have also been found to be highly reliant on aquatic subsidies (Paetzold *et al.* 2005; Krell *et al.* 2015; Stenroth *et al.* 2015). Ground beetles are not as commonly studied as spiders, yet many species are hygrophilous, preferring the damp environment of riparian zones (Lindroth 1985). Paetzold *et al.* (2005) found that ground beetles collected on exposed gravel bars in the riparian zone were highly reliant on aquatic prey, with the genus *Bembidion* and *Nebria* entirely reliant on aquatic insects.

Other factors such as timing, distance from stream and land use also affect the ability of riparian consumers to utilize aquatic subsidies. Timing of the aquatic subsidy emergence has a large impact. Timing of the emergence is often taxa specific

and tied to season, which in temperate regions is generally late spring or summer (Kato *et al.* 2004; Paetzold *et al.* 2005; Marczak & Richardson 2008; Carlson 2014). Additionally, land use is also pertinent to timing of emergence as it has an effect of instream characteristics which in turn affects which types of aquatic taxa are present (Johnson & Almlöf 2016; McKie *et al.* 2018). How far the subsidy can disperse from the stream is important for how and where the subsidy can be utilized. The distance aquatic insects can disperse is influenced by species traits and riparian vegetation characteristics, which in turn may be affected by land use (Carlson 2014; Stenroth *et al.* 2015; McKie *et al.* 2018). Often both aquatic invertebrate and terrestrial consumer densities are highest closer to streams (Burdon & Harding 2007; Muehlbauer *et al.* 2014).

It is clear that a range of variables can affect both the timing, dispersal and magnitude (e.g. biomass, abundance) of the subsidy, and the responses of riparian consumers, including their abundances and diversity, which altogether regulate the impact of the subsidy in the recipient habitat. Riparian invertebrates can be both sensitive to habitat degradation and dependent on aquatic subsidies. These aspects mean there is a sound basis to predict that different buffer types within an agricultural landscape will have an impact on these communities' and their link to the adjacent aquatic system. However, few studies have addressed this topic. Quantification of differences in riparian invertebrate consumer populations between buffer types could give an indication of the buffers value for biodiversity. Additionally, dietary tracers can be used to establish the extent to which riparian invertebrate consumers rely on aquatic prey in different buffer types, thereby also giving an indication of the strength of the linkage between aquatic and terrestrial ecosystems.

1.5 Polyunsaturated fatty acids

Aquatic insects are particularly notable for their relatively high concentrations of high quality fatty acids (Gladyshev *et al.* 2009; Schindler & Smits 2017). The transfer of these fatty acids into terrestrial ecosystems is mediated by emerging aquatic insects subsiding terrestrial food webs (Gladyshev *et al.* 2009; Muehlbauer *et al.* 2014; Schindler & Smits 2017). Fatty acids in general play several key roles in living organisms. For example, fatty acids are essential to cell membranes and are important energy stores and sources (Rustan & Drevon 2005; Guschina & Harwood 2009). There are two main groups of fatty acids, saturated fatty acids (SAFA) and unsaturated fatty acids. Unsaturated fatty acids are further grouped in to monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The main structural difference is the presences of a carbon-carbon double bond, SAFAs have none, whilst MUFAs (mono=one) and PUFAs (Poly=many) have double bonds

(Rustan & Drevon 2005). It is these PUFAs that aquatic insects have notably high concentrations of compared to terrestrial prey. Additionally, PUFAs have specific properties that has generated a growing interest and use of them within food-web research.

Several PUFAs are considered essential fatty acids that are vital for the well-being of heterotrophic organisms (Gladyshev *et al.* 2009; Twining *et al.* 2016). Linolenic acid was identified as an essential fatty acid as early as 1930 (Burr & Burr 1930), and today as many as 23 essential fatty acids are known to science (Cunnane 2000). There are two main families of essential fatty acids ω-6 and ω-3, of which Linoleic acid (ω-6) and Alpha-linolenic (ω-3) are parent molecules (Cunnane 2000; Parrish 2009). The position of the first double bond on the methyl end of the carbon chain is on the sixth carbon for ω-6 fatty acids and on the third carbon for ω-3, in Figure 1 the structures of Linoleic acid and Alpha-linolenic acid can be seen, typical of the two families (Parrish 2009).

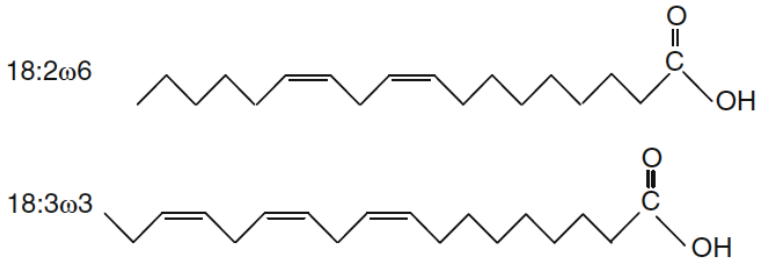

Figure 1. Chemical structures of the polyunsaturated fatty acids Linoleic acid (LIN 18:2ω6) and Alpha-linolenic acid (ALA 18:3ω3) showing the positions of the double bonds typical for ω3 and ω6 groups. (Adapted from Parrish 2009)

The importance of essential PUFAs to heterotrophs is due to two main factors. Firstly, PUFAs are key to several physiological functions in heterotrophs, including membrane and neural functions, reproduction, hormone regulation and cognitive development, and a diet deficient in PUFAs has an negative effect on these functions (Bell *et al.* 1986; Muller-Navarra *et al.* 2000; Gladyshev *et al.* 2009; Twining *et al.* 2016). In general, Docosahexaenoic acid (DHA) and Eicosapentoenoic acid (EPA) which can be synthesized from Alpha-linolenic acid (ALA), and Arachidonic acid (ARA) which is derived from Linoleic acid (LIN), are considered the most important of the essential PUFAs for optimal function (Table 1) (Gladyshev *et al.* 2009, 2013; Parrish 2009). Secondly, though heterotrophs can to a limited degree synthesise LIN and ALA from their derivatives, if those are not present, LIN and ALA cannot be synthesised *de novo* (Cunnane 2000; Parrish 2009). Heterotrophs cannot *de novo* synthesise long-chain PUFAs (C_{20} – C_{22}) unless the precursors LIN or ALA acid are present, and even then, only a limited amount (Cunnane 2000;

Gladyshev *et al.* 2009; Parrish 2009). Higher plants and algae, and to some degree fungi, are thereby the main sources of PUFAs for heterotrophs (Gladyshev *et al.* 2009; Taipale *et al.* 2013; Arce-Funck *et al.* 2015).

Table 1. *Five essential polyunsaturated fatty acids, their structural formulas and some main sources (Torres-Ruiz* et al. *2007; Gladyshev* et al. *2013; Twining* et al. *2016).*

ω-3			**ω-6**		
PUFA	Structure	Source	PUFA	Structure	Source
Alpha-linolenic acid (ALA)	18:3ω3	Plant seeds and nuts, green algae	Linoleic acid (LIN)	18:2ω6	Plant seeds and nuts, green algae
Eicosapentaenoic acid (EPA)	20:5ω3	Micro-algae eg. diatoms, fungi	Arachidonic acid (ARA)	20:4ω6	Macro-algae, Bryophytes
Docosahexaenoic acid (DHA)	22:6ω3	Micro-algae eg. diatoms, fungi			

Higher plants and algae differ in which PUFAs they can produce. Higher plants are generally unable to produce long-chain PUFAs (C_{20} – C_{22}) or convert ALA to EPA (or DHA). Algae, however, can produce high amounts of these long-chain PUFAs, especially of the ω-3 family. Although there are large variations in PUFA profiles between algal groups, aquatic systems are generally considered to be the main source of long-chain PUFAs (Torres-Ruiz *et al.* 2007; Gladyshev *et al.* 2013; Taipale *et al.* 2013; Twining *et al.* 2016). Additionally, green algae are rich in ALA and LIN, and algae and aquatic bryophytes synthesize ARA, thus aquatic systems play a disproportionate role in essential PUFA synthesis (Torres-Ruiz *et al.* 2007).

Abiotic factors can both indirectly and directly influence the production of PUFAs in aquatic systems. Variables such as light, temperature, pH and nutrient levels affect what algae taxa can grow in a specific water body (Hill *et al.* 1995; Stelzer & Lamberti 2001; Allan & Castillo 2007; Larned 2010). Furthermore, abiotic factors also directly affect the synthesis of PUFAs in the algae present (Guo *et al.* 2016). For example, diatoms thrive in cool, moderately shaded, flowing waters (Richardson & Danehy 2007; Allan & Castillo 2007; Law 2011) and are major producers of EPA (Torres-Ruiz *et al.* 2007; Gladyshev *et al.* 2013; Taipale *et al.* 2013). The levels of EPA synthesised are in turn regulated by light, temperature and nutrient levels, with studies showing a relative increase in long-chain PUFA content at low irradiances and temperatures, and high nutrient levels potentially having a negative effect of long-chain PUFA production (Hill *et al.* 2011; Guo *et al.* 2016). Thus, anthropogenic activities that reduce riparian wooded vegetation (shading) and increase eutrophication and sediment loads can alter both algal community composition and the potential PUFA production in aquatic systems. Global warming is also a threat, with increases in temperature potentially affecting PUFA production (Hixson & Arts 2016).

The wide variation in PUFA composition between algal taxonomic groups and the limited abilities of heterotrophs to *de novo* synthesize PUFAs also makes them useful tools in food-web and dietary studies. For example, some PUFAs can be used as trophic biomarkers, either on their own or in combination with other PUFAs. Trophic biomarkers are substances that are usually relatively rare, can be tied to a source and are measurable, and their presence in an organism indicates direct consumption of the source or consumption of the source in lower trophic levels (Iverson 2009). For example, Torres-Ruiz *et al.* (2007) found ARA and Eicosatrieonic acid to be good markers for aquatic bryophytes and could trace these PUFAs in aquatic invertebrates. Additionally, PUFAs are not degraded upon digestion and they are usually stored and accumulate within a consumer (Iverson 2009). These properties enable the use of PUFAs as biomarkers, thus allowing for the quantification of linkages between different habitats.

To date the majority of studies into the trophic transfer of PUFAs have focused on aquatic environments, (Brett & Muller-Navarra 1997; Torres-Ruiz *et al.* 2007; Lau *et al.* 2012; Guo *et al.* 2016; Taipale *et al.* 2016) but there are a growing number of studies linking aquatic and terrestrial food webs with the cross-habitat transfer of PUFAs (Gladyshev *et al.* 2009, 2013; Martin-Creuzburg *et al.* 2017; Moyo *et al.* 2017). However, studies into the PUFA content of riparian invertebrate consumers are extremely limited. Fritz *et al.* (2017) compared the fatty acid profiles of upland and wetland spiders and found that wetland spiders had higher levels of aquatically derived PUFAs and increased immune function compared to upland spiders. As invertebrate consumers can form trophic links between aquatic and terrestrial systems, studying their PUFA content could give an indication of the strength of the linkage between the two ecosystems. Stable isotopes have long been used to ascertain the degree to which different riparian consumers rely on aquatic subsidies (Kato *et al.* 2004; Krell *et al.* 2015). In soil food webs, Pollierer *et al.* (2010) found that fatty acid biomarkers of basal terrestrial resources were clearly detectable in spider consumers and differed depending on diet. Similar studies in riparian zones could be used to link consumer taxa identity to prey reliance and thereby their role in transferring aquatically derived essential PUFAs to terrestrial systems.

1.6 Summary: gaps in current knowledge

Stratified and heterogenous vegetation structure is often recommended to achieve optimal buffer efficiency in regard to biodiversity and trophic connectivity, yet in agricultural landscapes riparian buffers are absent or dominated by grass and herbs. To date, studies comparing forested buffers and unforested buffers in agricultural landscapes are limited and more research is needed to ascertain their role in maintaining biodiversity and trophic connectivity, and to establish efficient management practices of these habitats. Spiders and ground beetles are sensitive to changes in habitat and are reliant on aquatic subsides. Thus, they are ideal model organisms for studying the effect of riparian vegetation properties on biodiversity and linkages between aquatic and terrestrial systems. Few studies have, however, compared how different buffer types influence invertebrate consumer diversity and abundance, and their ability to utilize aquatic subsidies. PUFAs are a useful tool for quantifying the reliance on aquatic subsidies, a measure of the strength of the linkage between aquatic and riparian systems. Research into the PUFA content of different riparian invertebrate taxa is lacking yet could give an indication of the total strength of the linkage at the community level, as well as the role of specific taxa in transferring aquatic PUFAs to terrestrial systems.

2 Aims and hypotheses

In this thesis I report on results of a field investigation assessing how riparian invertebrate diversity and abundance varies with riparian buffer properties (with and without a forested buffer) in the catchment of Ekoln, part of the larger Mälaren basin, in central Sweden. Furthermore, the PUFA content of the invertebrates was analysed to investigate if and how the strength of the linkage between the aquatic and terrestrial systems is influenced by riparian vegetation properties. Differences in PUFA content between taxonomic groups was also investigated, in order to link community composition to the transfer of PUFAs.

I aimed to:

1. Assess the effects of riparian buffer properties on the community composition, diversity, abundances and distribution of riparian invertebrate consumers.
2. Assess the effects of riparian buffer properties on connectivity between aquatic and terrestrial food webs using PUFA biomarkers found in the terrestrial invertebrates as a tool to evaluate the strength of the linkage.
3. Examine the differences in PUFA content between riparian invertebrate taxonomic groups to determine their contribution to the potential transfer of PUFAs from aquatic to terrestrial food webs.

I hypothesised that:

1. Community composition, diversity, abundance and distribution:
    - Riparian invertebrate consumer community composition will differ between unbuffered and buffered sites, with lower abundances of web-building spiders found in unbuffered sites due to lack of vegetation structures appropriate for building webs.
    - Diversity and abundance will be higher at sites with forested buffers as vegetation heterogeneity increases available habitat niches.
    - Additionally, wooded vegetation creates a microclimate that may be beneficial to riparian invertebrate consumers and the dispersal of emerging aquatic insects. Therefore, riparian invertebrate consumers at sites with forested

buffers will be more evenly distributed within the buffer, compared to un-buffered sites where the riparian invertebrate consumers are more likely to be concentrated close to the stream edge.

2. Higher content of long-chain polyunsaturated fatty acids (DHA, EPA & ARA) will be found in terrestrial invertebrates from sites with forested buffers due to two underlying mechanisms.
   - Conditions at buffered sites (i.e. light, temperature) are generally associated with both the algae that produce long-chain PUFAs, and a higher relative production of the these PUFAs.
   - The trophic connectivity between aquatic and terrestrial systems at sites with forested buffers should be stronger than at unbuffered sites.
3. Riparian invertebrate taxonomic groups that have shown to be highly reliant on aquatic subsidies, for example Tetragnathidae and Lycosidae, will have higher long-chain PUFA content, and thereby contribute substantially to linking aquatic and terrestrial systems.

3 Methods

3.1 Study sites

The study sites were situated in the catchment of the Ekoln basin of Lake Mälaren in Uppland, Sweden. As can be seen in the map (Figure 2) the region is a patchwork of agricultural and forest land, with several towns and villages dispersed through the area, including the city of Uppsala (Population approximately 168 000, Uppsala Kommun 2018).

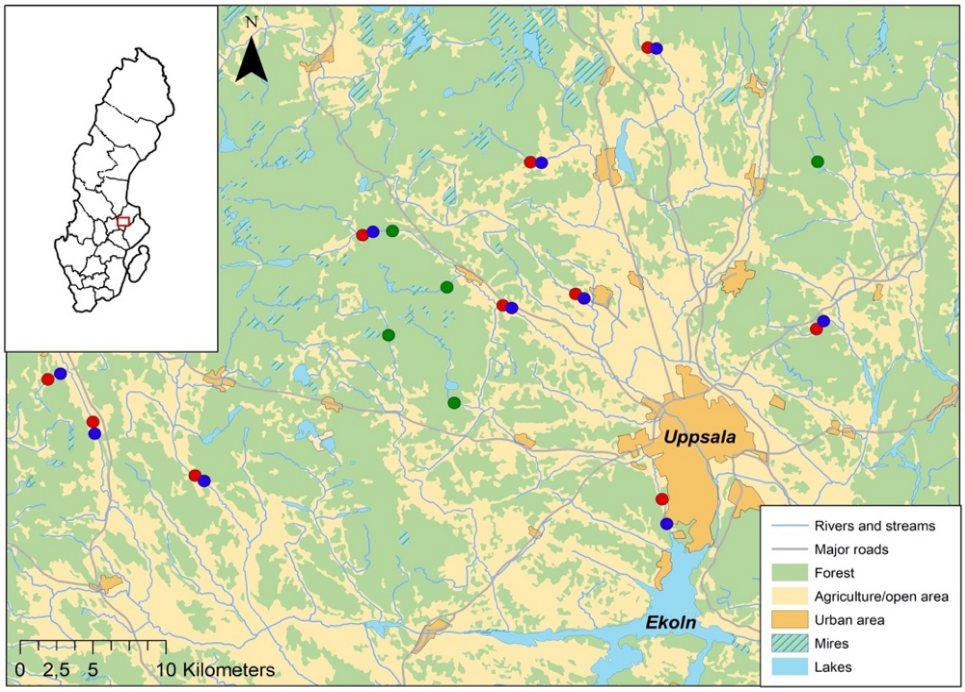

Figure 2. Map of the Ekoln basin with reference forest sites (green), and paired sites: (red) unbuffered sites and (blue) buffered sites. Background map: GSD-General map, vector © Lantmäteriet (2018)

The region has a mean annual precipitation of 544 mm and mean annual temperature of 5.6°C. However, during the year of my study, 2018, the summer months were both warmer and dryer than the average. For example, the long-time mean temperature in July is 16.4°C degrees but in 2018 the mean temperature was 22°C (SMHI 2019).

Terrestrial biota were sampled alongside 25 stream reaches in the study catchment. All stream reaches were relatively similar in size (Summer range: width 1-12 m, depth 0.05-0.50 m, Bank full range: width 2.5-13.5 m, depth 0.30-1.0 m) but differed in the extent of riparian wooded vegetation along the banks. Twenty of the reaches consisted of 10 paired sites on 10 different streams that were affected by agricultural land use (Figure 2). Each pair consisted of one reach with no or sparse riparian wooded vegetation (henceforth unbuffered) and one reach with riparian wooded vegetation (henceforth buffered). The site pairs were a few hundred meters apart, with the buffered sites downstream from the unbuffered sites. The remaining five sites were references sites and consisted of reaches in forest settings (henceforth forest). Site names and type can be found in Appendix 1: Table 7.

3.2 Habitat assessment

The properties of the habitat in the riparian zone could potentially have an effect on the invertebrate communities. Accordingly, a habitat assessment was undertaken at the same time as invertebrate sampling and using the same plot system. Habitat assessment and invertebrate sampling took place during the day in the months of June and July 2018. The riparian zone studied measured 30 x 5 m on each side of a stream channel, in total covering an area of 300m^2 per site (Figure 3). Each side was divided into three plots measuring 10 x 5 m each, resulting in six blocks per site. Three of the habitat characteristics that were assessed were used in this thesis: canopy cover (%), tree species identification and diversity, and the cover of habitat types (%). All six plots per site were sampled for these habitat characteristics. An example of the field protocol used to collect this data can be found in Appendix 1: Table 8.

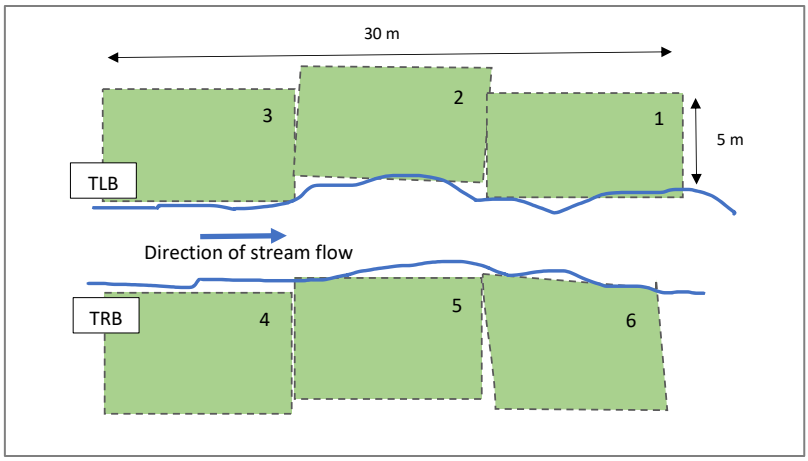

Figure 3. The layout of each study site with the riparian zones on each side of the channel measuring 30 x 5 m, divided into 3 plots measuring 5 x 10 m. In total each site had 6 plots. TLB= True left bank, TRB= True right bank.

Canopy cover was measured at the centre of each plot using CanopyApp (For Apple iOS, Version 1.0.3, University of New Hampshire). A photograph was taken using the application, holding the smartphone horizontally (facing the sky) at breast height (approximately 130 cm), the application then calculated the percentage of area within the photograph the canopy covered.

Trees were identified to species level with the aid of two plant identification applications: British tree identification (For Apple iOS, Version 3.0.1, Woodland trust) and PlantSnap (For Apple iOS, Version 2.01.22, PlantSnap inc.). For all trees with a diameter of 5 cm or more at breast height the diameter was also measured using a measuring tape and recorded.

The percentage cover of different habitat types was assessed visually for each plot. The habitat types were the same ones recorded when collecting invertebrates and can be found in Appendix 1: Table 9. The coverage of each habitat type (e.g. herbs) was assessed as a layer on a horizontal plane (i.e. one can visualize the cover as the shadow of the layer at midday). Similar methods for estimating coverage are widely used, for example in the National Inventory of Landscapes of Sweden program, and though subjective they are fairly robust (Damgaard 2014).

3.3 Invertebrate sampling

Riparian invertebrates sampling was conducted according to the CROSSLINK project protocol (Appendix 1). The sampling method used was a semi-quantitative approach using timed visual searches. This method was used to both get a relative indication of abundances and provide material for analysis. A minimum of four plots were sampled (Figure 3). Each plot was searched by myself and up to two additional

people for a set amount of time (e.g. 10 min) and the area searched was noted if the whole plot had not been covered (See Appendix 1: Table 9 for an example of the field protocol recording the mentioned variables). Each plot was also divided into strips depending on the number of people searching, with each person searching within a set strip e.g. 0 to 2 meters, to allocate the effort evenly across the plot area. The number of people searching and the time taken were used to calculate the duration of the search. For example, two people searching for 10 minutes would amount to 20 minutes in total. The duration of the sampling and area covered, together with the number of invertebrates collected, was used to calculate the catch per unit effort (CPUE). CPUE is a relative measure of abundance, making abundances between sites comparable.

$$\text{CPUE} = \frac{\text{Number of invertebrates}}{\left(\frac{\text{Area sampled}}{\text{Duration of sampling}}\right)}$$

The collected invertebrates were web-building (WS) and free-living spiders (FLS). Some common web-building spider families are Linyphiidae, Araneidae and Tetragnathidae (Figure 4). Lycosidae and Pisauridae are two common free-living spider families (Figure 4). Opiliones (OP, huntsmen), ground beetles (GB, Carabidae) and rove beetles (RB, Staphylinidae) were also collected. For each invertebrate group (FLS, WS, etc) the aim was to collect a minimum of 20 individuals per site to meet the required biomass necessary for fatty acid analysis. We used visual searching to find invertebrates (i.e., looking for webs, turning over stones and wood and riffling through leaf litter). When an invertebrate was found they were gently coaxed into sample tubes and the tube labelled with site name, bank and plot number (see Figure 3), habitat found in and distance from stream. The invertebrates were placed in coolers in the field and then stored in a freezer (-20°C) in the laboratory until they could be identified and pre-processed for fatty acid analysis.

3.4 Invertebrate identification, biomass and fatty acid pre-processing

Identification of the invertebrates took place in September 2018. Frozen samples were studied individually using a microscope (Nikon stereo-zoom microscope) and identified, the tubes were then re-labelled and the samples re-frozen. Spiders (Araneae) were identified to family level using the Araneae key to families (Nentwig *et al.* 2018), and with the aid of Jocqué & Dippenaar-Schoeman (2007) and Kronestedt (2001). Hunstmen (Opiliones) were left at order level. Ground beetles (Carabidae) were identified to genus level using Lindroth (1985) and Hackston (2018a, online key adapted from Lindroth, 1974). Rove beetles (Staphylinidae) were

determined to sub-family level using Hackston (2018b, online key adapted from Tottenham, 1954). In the rest of the thesis Araneae and Opiliones will together be referred to as spiders. The taxa groups identified can be found in Appendix 1: Table 10. Figure 4 and 5 show a selection of the spider families and ground beetle's genus identified.

Figure 4. A selection of the spider families identified. From top right: (A) Lycosidae, (B) Pisauridae, (C) Tetragnathidae, (D) Linyphiidae, (E) Theridiidae, (F) Araneidae, (G) Clubionidae, (H) Thomisidae, (I) Philodromidae.

Figure 5. A selection of the ground beetle genus identified. From top left: (A) Elapharus, (B) Pterostichus, (C) Bembidion, (D) Leistus

In October and November 2018, the invertebrates were pre-processed for fatty acid analysis in the laboratory. The fatty acid content was not analysed for all collected invertebrate taxa. The invertebrates targeted for fatty acid analysis were: all ground beetle genera (Carabidae), Staphylindae, Opiliones, and the spider families, Linyphiidae, Tetragnathidae, Lycosidae and Pisauridae. However, all invertebrates went through the initial preparation stages, including biomass quantification. For each site all invertebrates belonging to the same family or genus were pooled together to one sample resulting in a total of 138 samples. The pooling was done to average individual variations in fatty acid content, and to reach fatty acids analysis mass requirements (ideally 5 mg dry weight per sample). The number of individuals per sample was recorded. The samples were then placed in test tube racks and covered with parafilm to avoid cross contamination during freeze-drying. The samples were freeze-dried (Freeze-dryer: LyoDry compact, Mechatech systems LTD, Bristol, UK) for a minimum of 48 hours at - 45°C. The samples were then weighed, and the mass recorded in grams to four decimal places (e.g. 0.0053 g). All non-target invertebrates for fatty acid analysis were then returned to the freezer.

The invertebrates targeted for fatty acid analysis went through further pre-processing. These target samples were pulverized using a mortar and pestle and then re-weighed to account for loss during grinding. Between each sample the tools used were wiped with 99% ethanol to prevent cross-contamination. The samples were then stored in the freezer (-20°C).

3.5 Fatty acid analysis

A method based on Grieve & Lau (2018) was used for fatty acid analysis and the analysis was done at the Swedish Metabolomics Centre in Umeå (A collaboration between Umeå University, Swedish University of Agriculture and Chalmers University) in December 2018. The process included three main stages: lipid extraction, methylation and gas chromatography-mass spectrometry (GC-MS). Methylation of the fatty acids (FAs) to fatty acid methyl esters (FAMEs) is necessary to increase the volatility of the FAs and decrease their polarity, making them suitable for GC-MS (Wu *et al.* 2017). GC-MS machines separate and detect complex chemical compounds based on their volatility and mass, generating a spectrum that can be interpreted, thus resulting in the fatty acid profiles of the samples (Sparkman *et al.* 2011). The limit of the GC-MS machine used was 98 samples, so the 138 samples were divided into two batches and each of those batches split into two for the extraction process.

First the FAs were extracted. Approximately 5 mg (range of 4.5 to 5.5 mg) of each invertebrate sample was weighed into micro-centrifuge tubes (1.5 ml) and

labelled. Three drops of nano-filtered water were added to re-hydrate the samples. Then 20 µl of internal standard deuterium D29- pentadecanoic acid (conc. 120 ng/µl) and 400 µl hexane-isopropanol (3:2, V:V) extraction solution was added. Two metal beads were added per tube with forceps and the lids closed, to homogenize the samples they were then shaken in a mixer mill (Mixer mill MM 400, Retsch GmbH, Haan, Germany) at 30 s^{-1} for two minutes. The beads were then removed with magnets and 111µl of sodium sulphate (Na_2SO_4 6.67%) added. The samples were vortexed (Vortex Genie 2, Scientific Industries Inc., Bohemia, USA) and left for 30 minutes in the fridge. The samples were centrifuged (Mikro 220R, Hettich GmbH, Tuttlingen, Germany) for five minutes at 4 °C and 14000 rpm to separate the organic from the aqueous phase. 150 µl of the organic phase (supernatant) was extracted to a GC vial (300 µl inset) and the extract dried with an evaporator at room temperature for two hours under vacuum (miVac Quattro concentrator, Genevac, Ipswich, UK). The dried extract was re-dissolved with 50 µl hexane and then 70 µl internal standard deuterium D33-methyl heptadecanoate (conc. 8.5714 ng/µl) was added and the samples vortexed. The 120 µl was then split into two GC vials (60 µl each), capped and stored in a -80 °C freezer until methylation. For each sample one 60 µl was methylated and one stored as a back-up.

Before methylation the samples were removed from the freezer and dried with an evaporator using the same parameters as above. A solution of trimethylsilyldiasomethane and IPA:dichloromethane(1:5) 1:100 was prepared. To each 60 µl sample 200 µl of the trimethylsilyldiasomethane:IPA:dichloromethane methylation solution was added. The vials were caped and vortexed, then uncapped and left to react for 16 hours (overnight) in a fume-hood. The next day 60 µl of heptane with internal standard alkane C13 (10 ng/µl) was added before GC-MS.

The FAMEs were analysed with a GC-MS (7890A GC, Agilent Technologies, California, US & Pegasus HT TOF-MS, LECO, Michigan, US). Standard FAMEs and Bacterial FAMES were also run to identify the specific FAs in the samples. The operational variables of the GC-MS were: the GC-MS was installed with a DB-5 capillary column (30 m length, 250 µm internal diameter, 0.25 µm film thickness), a splitless injection of 1µm was used for each sample, constant flow method was used with helium as the carrier at a rate of 1.0 ml/min, the inlet temperature was 260°C and the oven temperature was set to rise during 30 minutes from 70°C to 320°C, and then to maintain 320°C for 8 minutes. The resulting spectrum was then analysed, using the observed peaks and retention times to identify the individual FAs in each sample. Based on the internal standard D29 and the mass of each sample, the individual concentration of the FAs in each sample was quantified as mg per g dry mass.

3.6 Statistical analyses

All statistical analyses were implemented in RStudio using the R statistical computing language and environment (version 3.5.2, R Core Team 2018). A list of the main packages and important functions used can be found in Appendix 1: Table 11. Where applicable, assumptions of normality and variance were checked with Q-Q plots and square-root or log transformations applied if necessary. For percentages, logit or arcsine square-root transformations were applied. Site pairs were not spatially independent of each other, (a few hundred meters apart along the same stream) which was accounted for by fitting stream identity as a blocking factor (eg TEM for both TEM- FBF and TEM-AGR, see Appendix 1: Table 7 for abbreviation codes) in all hypothesis testing. Habitat assessment data was analysed to determine the differences between site types regarding the cover of different habitats available. As only 124 ground beetles and rove beetles from 23 sites were collected and identified they were excluded from further analysis. Thereby, only spider data was analysed and addressed in the rest of the thesis.

3.6.1 Riparian spider community

Differences in spider abundance and diversity between site types was explored using comparison of abundances, number of taxa, Shannon diversity, Pielou's evenness and Berger-Parker dominance which together give an overview of diversity and overall abundance. Shannon diversity is a commonly used diversity index taking in to account both species richness and evenness (Gardener 2014). It can range from 0 to 5, with a higher value indicating higher diversity. Pielou's evenness is based on Shannon diversity, and simply describes how even the community is in regard to species abundances. Pielou's evenness ranges from 0 to 1, with community evenness increasing closer to 1 (Smith & Wilson 1996). Berger-Parker dominance describes the proportion of the most abundant species, essentially also describing how even the species abundances are, and it ranges between 0 and 1, with higher values indicating higher dominance of one species (Gardener 2014). All equations can be found in Table 2. The differences in abundance and diversity between site types were tested with one-way ANOVAs and Tukey's tests for post hoc comparisons.

Table 2. *The three indices Shannon diversity, Pielous evenness and Berger-Parker dominance and their formulas. Where: S= total number of species, n_{max}=is the number of the most abundant species and P_i =proportion of species belonging to species i.*

Index	Formula
Shannon diversity	$H = \sum_{i=1}^{s} P_i \ln(P_i)$
Pielou's evenness	$J = H/\ln(S)$
Berger-Parker dominance	$d = n_{max}/S$

To visualize the differences in the community composition of spider families among sites types the data was ordinated using the method Non-Metric Multidimensional scaling (NMDS). Hellinger transformation was applied to the raw abundance data. Hellinger transformations give low weights to rare species or zeros in the data and are appropriate for ordinations using Euclidean distances (Legendre & Gallagher 2001). A permutational multivariate analysis of variance, (PERMANOVA: Pairwise.adonis2, Appendix 1: Table 11) was used to test the difference between site types. Assumptions of homogeneity of spread were tested using the functions "betadisper" and "ANOVA" (Appendix 1: Table 11).

Differences in the distribution of spiders within the plots between site types and with increasing distance from the stream was tested using ANOVA, with the model including both main effects and the interaction between site type and distance from the stream. Differences in catch per unit effort between site types were analysed for both abundances and biomass, and tested using ANOVA and Tukey post hoc-tests. Additionally, the differences between site types in relation to two families, Linyphiidae and Lycosidae, was examined and the significance tested using ANOVA and Tukey post hoc-tests.

A redundancy analysis (RDA) was used to examine if any of the habitat variables could explain the variation in spider communities between site types. Tree species were included as habitat variables. The tree species were classified into conifer or deciduous to decrease the number of explanatory variables. As multicollinearity between explanatory variables (habitat) can cause errors in the model and give misleading results (The Pennsylvania State University 2018), the habitat variables were checked both pairwise for linear correlations and with variance inflation factors (VIF). VIF quantifies how much of the variance is inflated due to multicollinearity and literature recommends that an acceptable VIF is below 4 or 10 (Depending on soruce: Quinn & Keough 2002; The Pennsylvania State University 2018). The habitat variables moss & lichen and plant litter had VIF above 4 and were therefore removed from the analysis. VIF factors were re-checked after the removal. Habitat variables were either log or arcsine squareroot (coverage) transformed, and standardised as they had different units. The spider abundance matrix used was the same Hellinger transformed data as described for the NMDS analysis above. Different approaches for scaling of the NMDS triplot were tested, with scaling two selected as it focuses on the correlation between variables (Buttigieg & Ramette 2014), and as it was the least cluttered plot. Constrained (explained) variation was noted and R^2 and adjusted R^2 calculated. Model and axis significance were tested using ANOVA (anova.cca; a version used for ordinations to assess the significance of the constraints). A reduced model was also tried using "ordiR2step" (Appendix 1: Table 12). Additionally, a separate examination of the habitat types in which Linyphiidae and Lycosidae were collected in the field was also done.

3.6.2 Fatty acid profiles

The content of polyunsaturated fatty acids in the spider samples was explored, both in relation to other (i.e. non-polyunsaturated) fatty acids as a proportion of the total fatty acid content, and as concentration in mg PUFA per g dry mass. Differences between site types, spider families and interactions between site type and spider family were tested with a mixed-effects model ANOVA. Tukey post hoc-tests were used to test differences between site types. Similar to the RDA with spider abundances and habitat variables done earlier, a RDA with fatty acid data and habitat variables was tried. It resulted in no significant models and was therefore abandoned.

To visualise overall patterns in specific PUFAs between site types a principal component analysis (PCA) was undertaken based on log-transformed and standardised data. Variance partitioning was then used to assess the independent contribution of site type, spider family and stream identity to the explained variation in PUFA composition. The significance of each fraction was tested by permutation tests using 999 randomisations. A permutational multivariate analysis of variance (PERMANOVA: adonis, Appendix 1, Table 11) was used to test the difference in specific PUFA content between site type, spider families and interactions between them. Differences for each specific PUFA between site types, spider family and interactions were analysed and tested using mixed-effects effect model ANOVA.

4 Results

4.1 Habitat characterisation of the site types

Site types differed in both tree species composition and density (Figure 6 & Appendix 2: Table 12). Forest sites had the highest number of trees. Buffered sites were mainly dominated by deciduous trees, whereas forest sites comprised of coniferous trees to a higher degree. Standing dead wood was most commonly recorded in forest sites (Figure 6).

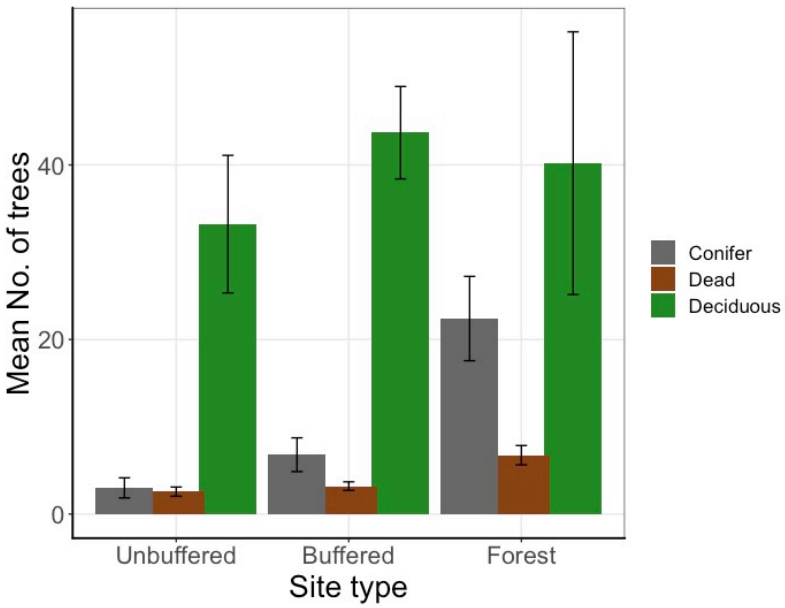

Figure 6. Mean number ± SE (per 300 m^2) of the tree groups dead, deciduous and conifer found per site type.

The recorded coverage of habitat types differed between unbuffered, buffered and forest sites (Figure 7 & Appendix 2: Table 13). The canopy cover was high and similar between buffered and forest sites with a mean around 70% (Figure 7). Unbuffered sites had substantially lower canopy cover with an approximate mean of 40%. The herbaceous vegetation layer also varied between site types. In unbuffered sites the cover of managed and unmanaged grasses was higher, as well as the herb cover. Buffered sites had relatively high cover of herbs but very few grasses, and forest sites had a sparse herbaceous layer (Figure 7). The moss layer in forest sites was prominent, characterised by high cover of moss and lichen, rocks and plant litter. In buffered sites the cover of plant litter was high, but less moss, lichen and rocks were recorded. In unbuffered sites, the coverage of plant litter, moss and lichen and rock was low. Unbuffered sites had the highest (but low) coverage of exposed bare ground.

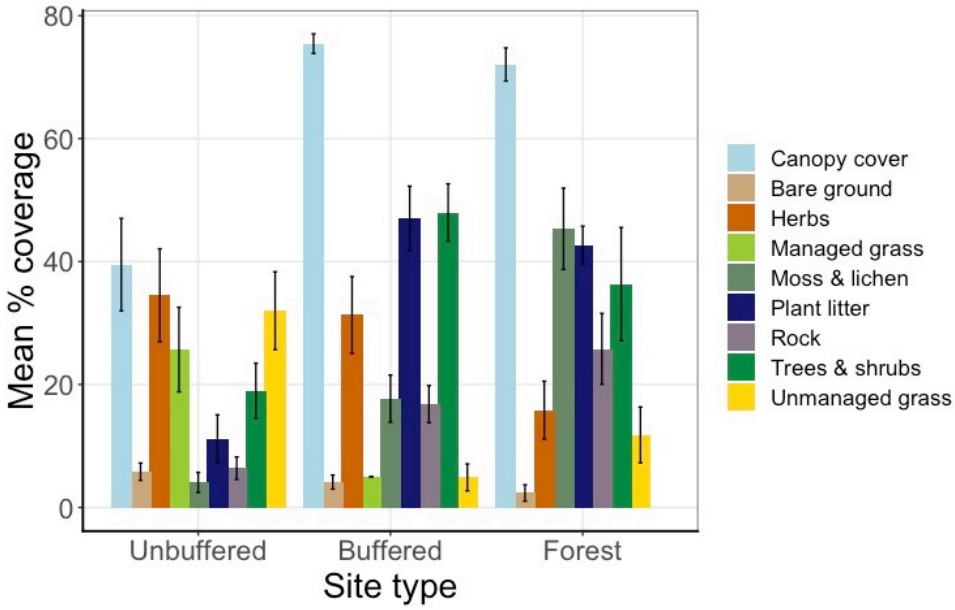

Figure 7. Mean percent coverage ± SE (per 300 m^2) of different habitat types found in unbuffered sites, buffered sites and forest sites.

4.2 Riparian spider diversity and community composition

4.2.1 Diversity and abundance

In total, 1229 spiders were collected and identified (Table 3, Appendix 2: Table 14 & 15), belonging to 15 families (14 Araneae and 1 Opiliones, see Figure 4).

Table 3. *Total number of sites spiders were found in, total abundances of spiders and total taxa according to site type*

Site type	No. of sites spiders found in	Spider abundances	Taxa richness
Unbuffered	10	454	13
Buffered	10	555	12
Forest	5	220	12
Total	25	1229	15

Mean spider abundances and number of taxa found were similar between site types, with no significant differences found between site types (Figure 8). However, biodiversity varied between site types, with unbuffered and forest sites being slightly more diverse and even, and with lower dominance than buffered sites. The differences between site types was significant for the diversity indices, Shannon diversity (ANOVA: $F_{2,22}$=4.80, p=0.02), Pielou's evenness (ANOVA: $F_{2,22}$=5.01, p=0.02) and Berger-Parker dominance (ANOVA: $F_{2,22}$=4.59, p=0.02). Post-hoc testing (Tukey, P<0.05) revealed that the difference in all three of these indices was limited to between unbuffered sites and buffered sites (Figure 8). For abundance and diversity results per site see Appendix 2: Table 14.

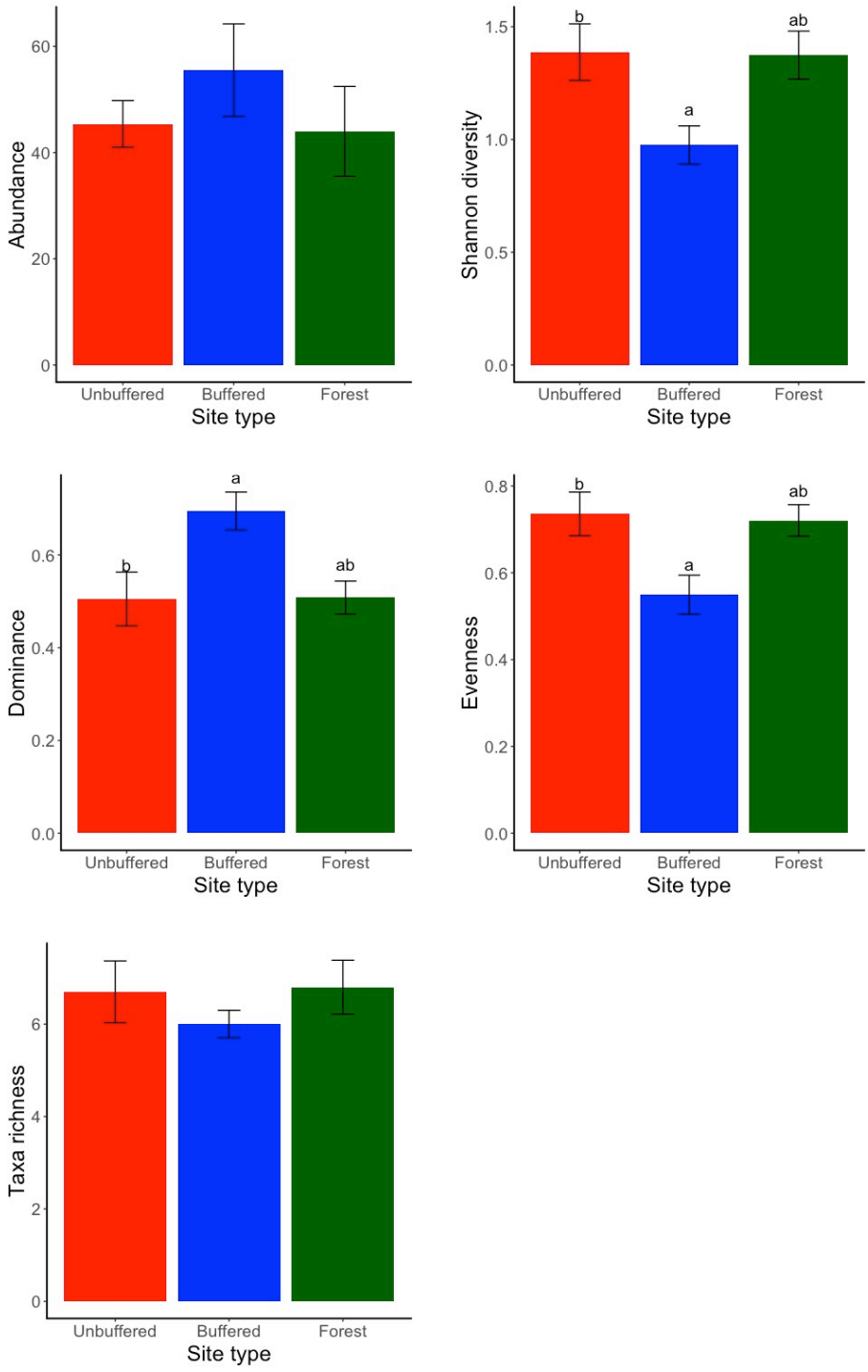

Figure 8. Mean ± SE per site type for spider abundances, Shannon diversity, Dominance, Evenness and Taxa richness. Letters above the bars denote homogenous subsets based on Tukey's post-hoc testing of differences among groups. Note: Bars with the same letter are not significantly different and different scales on the y-axes.

The NMDS plot (Figure 9) indicates differentiation in spider family composition between site types. Community composition shifts from the buffered sites on the right side of the plot, most associated with Linyphiidae and Araneidae, to the more widely dispersed unbuffered sites on the left side of the plot, most associated with Lycosidae, Pisauridae and other free-living families. The forested sites were intermediate between the two groups. PERMANOVA analysis confirmed that spider community composition differed significantly between unbuffered and buffered sites (PERMANOVA: R^2=0.28, $F_{1,18}$=7.05, p=0.002) but not between the forest sites and unbuffered sites (PERMANOVA: R^2=0.17, $F_{1,13}$=2.70, p>0.05) or buffered sites (PERMANOVA: R^2=0.22, $F_{1,13}$=3.65, p>0.05).

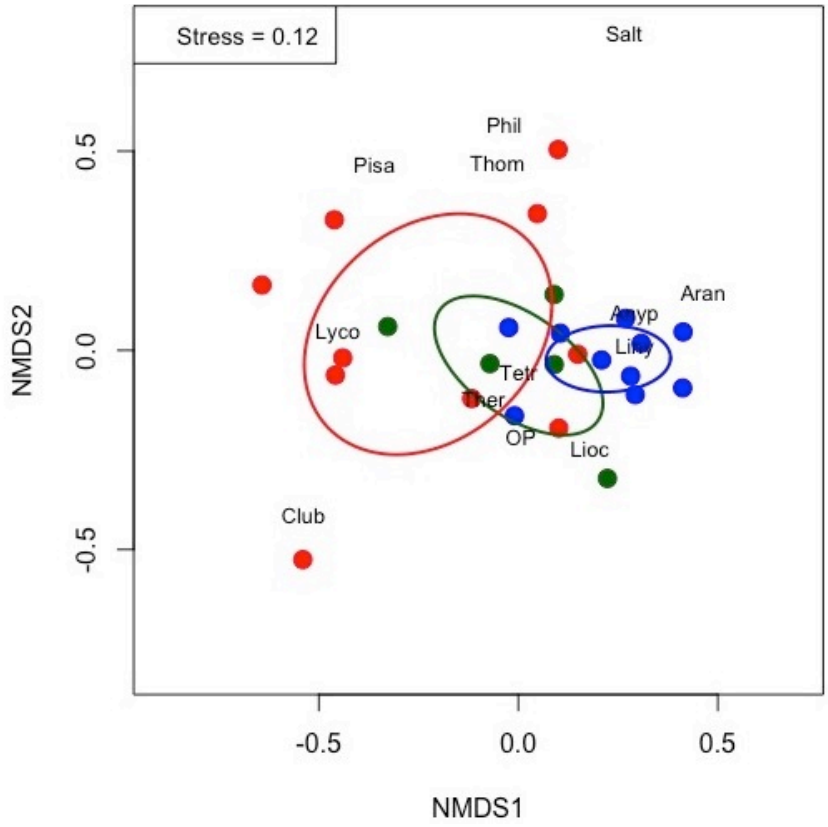

Figure 9. NMDS ordination plot of spider abundances per taxa and site type. Red-unbuffered, Blue-buffered and Green-forest sites. Spider family abbreviations: Anyphaenidae (Anyp), Araneidae (Aran), Clubioniade (Club), Eutichuridae (Euti), Linyphiidae (Liny), Liocranidae (Lioc), Lycosidae (Lyco), Opiliones (OP), Philodromidae (Phil), Pisauridae (Pisa), Salticidae (Salt), Sparassidae (Spar), Tetragnathidae (Tetr), Theridiidae (Ther) and Thomisidae (Thom)

Note that some of these families, although having highest abundances in particular site types (e.g. Linyphiidae in the buffered sites, Lycosidae in the unbuffered sites) nevertheless occurred in almost all sites (see Appendix 1: Table 15). Other families

were instead found almost exclusively in one type of site. For example, Pisauridae were mostly found at unbuffered sites, explaining their position in the NMDS plot. The difference in spread between sites was not significantly different (betadisper & ANOVA), thereby assumptions of homogeneity of spread were met.

4.2.2 Distribution patterns

The majority of spiders were collected at a mean distance of one meter from the stream, regardless of site type (Figure 10). No significant differences were found between the type of site (Anova: $F_{2,24}$= 0.06, p=0.94), distance (Anova: $F_{1,24}$ =0.06, p=0.80) or the interaction between the site type and distance (Anova: $F_{2,24}$ =0.07, p=0.93). However, the buffered sites and forest sites showed slightly more variation in distribution, whilst in unbuffered sites there was a distinct density peak at the one-meter mark which then dropped off and stayed low (Figure 10).

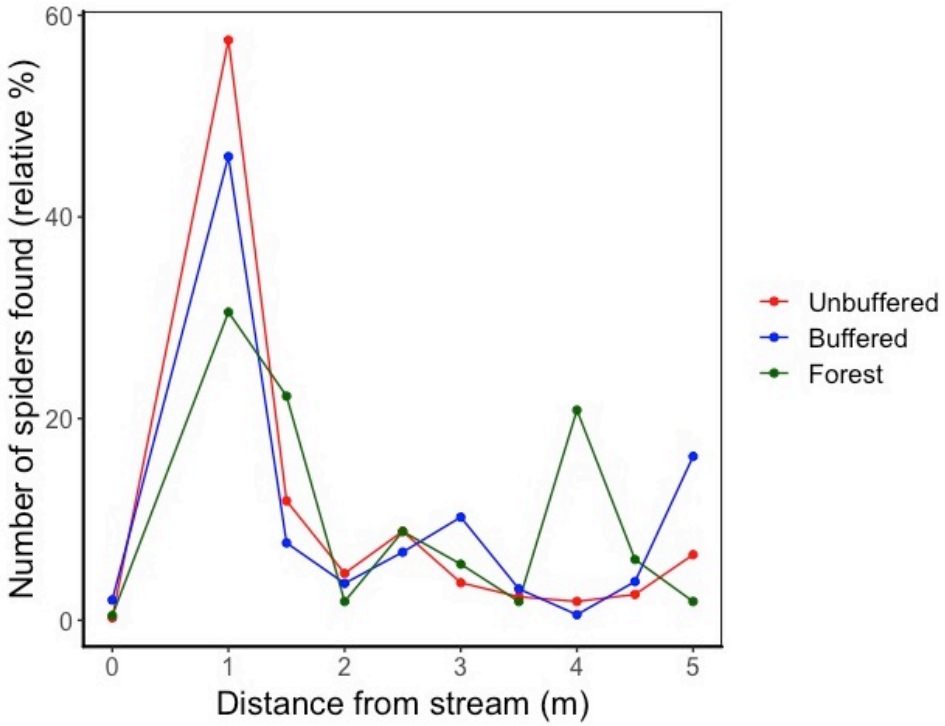

Figure 10. The number of spiders (relative %) collected at different distances from the stream at unbuffered, buffered and forest sites.

4.2.3 Catch per unit effort

The number of spiders collected at each site type varied significantly when effort was taken into account (ANOVA: $F_{2,9}$=7.84, p=0.01), with Post-hoc testing (Tukey) revealing that the difference was significant only between forest sites and buffered sites. The catch per m^2 per hour was highest for buffered sites followed by unbuffered sites and lowest for forest sites (Figure 11).

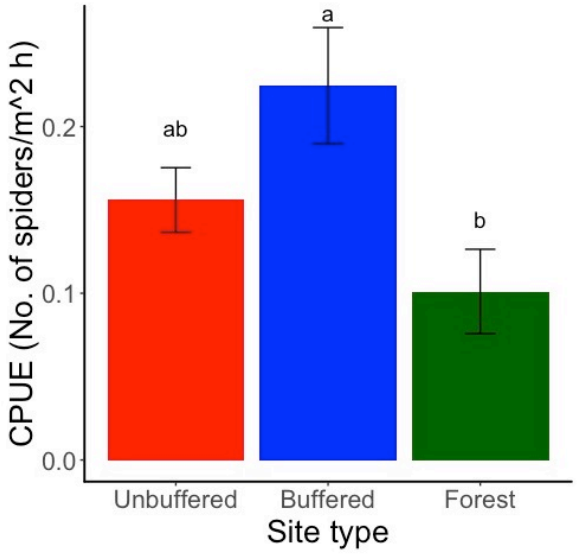

Figure 11. Mean ± SE of catch per unit effort per site type, based on abundance of spiders collected per m^2 h. Letters above the bars denote homogenous subsets based on Tukey's post-hoc testing of differences among groups. Note: Bars with the same letter are not significantly different.

The catch per unit effort calculated for mass revealed a different pattern (Figure 12). Unbuffered sites always support the highest mass per site and hour. The mass was analysed both with and without an extreme forest site outlier Lafsjön, as it had a major effect on the outcome. With the outlier there were no significant differences between sites, however without the outlier the mass per unit effort was significantly different between unbuffered sites and forest sites (ANOVA: $F_{1,8}$= 4.68 p=0.05, Post hoc-test Tukey). In Figure 12, the influence of this outlier can be seen. The forest site mean is halved when the outlier is excluded (Figure 12B) compared to when it is included (Figure 12A).

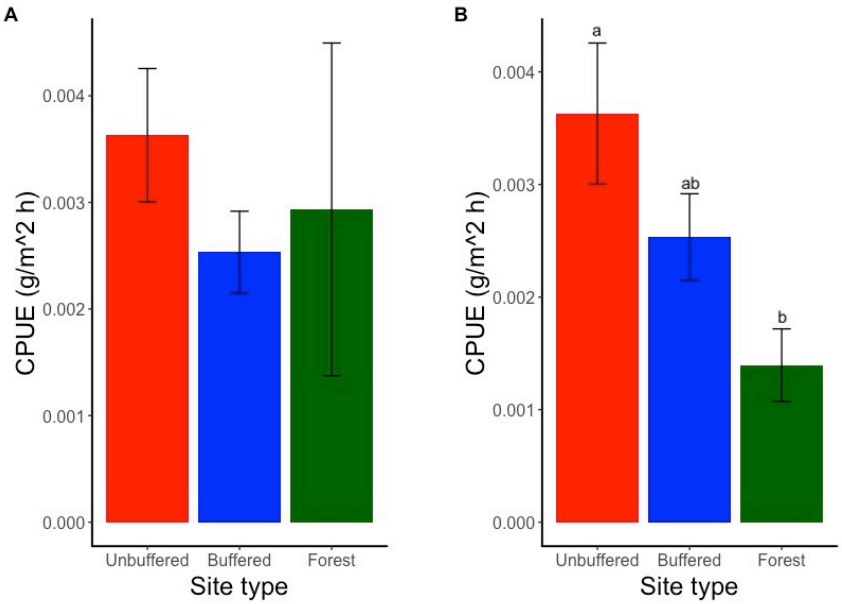

Figure 12. Mean ± SE of catch per unit effort per site type based on mass of spiders collected per m^2 h. (A) Shows all sites including an extreme outlier forest site (Lafsjön) and (B) is without the forest outlier. Letters above the bars denote homogenous subsets based on Tukey's post-hoc testing of differences among groups. Note: Bars with the same letter are not significantly different.

4.2.4 Linyphiidae and Lycosidae

Linyphiidae and Lycoisdae were the two most commonly collected families. In total, 609 Linyphiidae and 267 Lycosidae were collected (Appendix 2: Table 15). The abundances of Linyphiidae varied significantly between site types (ANOVA: $F_{2,9}$=17.22, p<0.001). The post hoc-test (Tukey) revealed the buffered sites differed significantly from the two other site types (Figure 13). Lycosidae abundances instead showed the opposite trend (Figure 13) with the unbuffered sites being significantly different from the buffered sites (ANOVA: $F_{2,9}$=5.21, p=0.03, Post hoc-test Tukey). However, abundances of Lycosidae at the forest sites were not significantly different from either of the other site types, which can be explained by the large standard error caused by the extreme outlier Lafsjön.

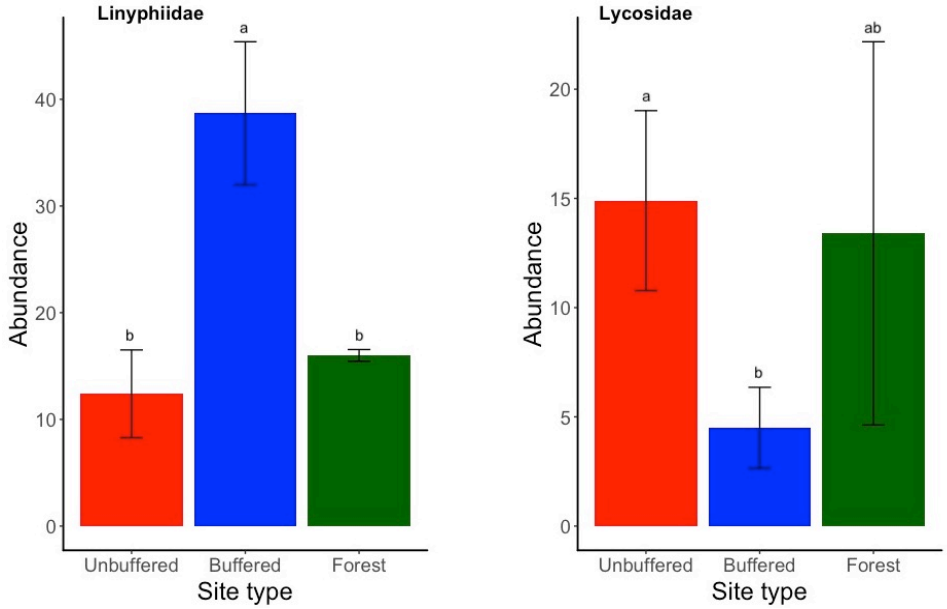

Figure 13. Mean ± SE of abundances of Linyphiidae and Lycosidae per site type. Letters above the bars denote homogenous subsets based on Tukey's post-hoc testing of differences among groups. Note: Bars with the same letter are not significantly different and different scales on the y-axes.

4.3 Relationships between habitat and spider community composition

The differences between the spider community composition at the different site types can partly be explained by variation in available habitat (RDA constrained variation 65.5%, unconstrained variation 34.5%) (Figure 14). The full RDA model was found to be significant (ANOVA: $F_{10,14}$= 2.66, p=0.002), as was the first axis, RDA 1 (ANOVA: $F_{1,14}$= 15.48, p=0.001) but not the second (ANOVA: $F_{1,14}$= 4.61, p=0.12). RDA 1, the first axis, explains 38.1% of the total variation and 58.2% of constrained variation.

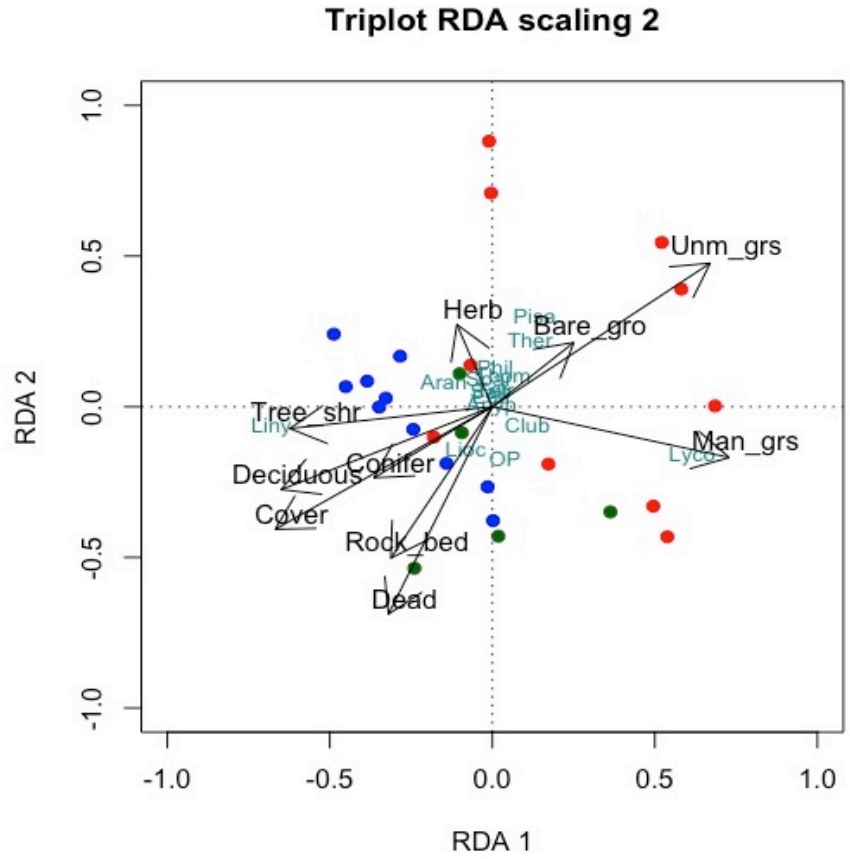

Figure 14. Redundancy analysis (RDA) triplot showing the effect of habitat types on the spider communities. Site types: Red- unbuffered, Blue- buffered and Green- forest. Light blue text is spider family abbreviations. Scaling 2: The cosine of angles between all vectors reflect their linear correlations, e.g. Very little correlation: cos (90)=0, Positive correlation: cos (30)= 0.87, Negative correlation: cos (180)= -1 (Imagined vectors, all lines not drawn to avoid a cluttered plot). R^2= 65.5%, Adjusted R^2=40.9%.

The variance inflation factors (VIF) for the habitat variables varied between 2.18 and 3.48, all below the limits (4 or 10) usually suggested in literature (Quinn &

Keough 2002; The Pennsylvania State University 2018). Both biplot scores for constraining variables (habitat) and a reduced model (produced with ordiR2step) suggest that unmanaged grass and managed grass are major structuring variables, affecting RDA1. The reduced model also pinpointed dead trees as having an effect on RDA2.

The habitat types in which Linyphiidae was collected were relatively consistent between site types (Figure 15). Linyphiidae was most often collected from trees and shrubs, and to a lesser degree from herbs. The habitat type in which Lycosidae was collected varied more between sites, with bare ground and rock being most common in unbuffered sites and buffered sites and plant litter and moss and lichen being more common in forest sites.

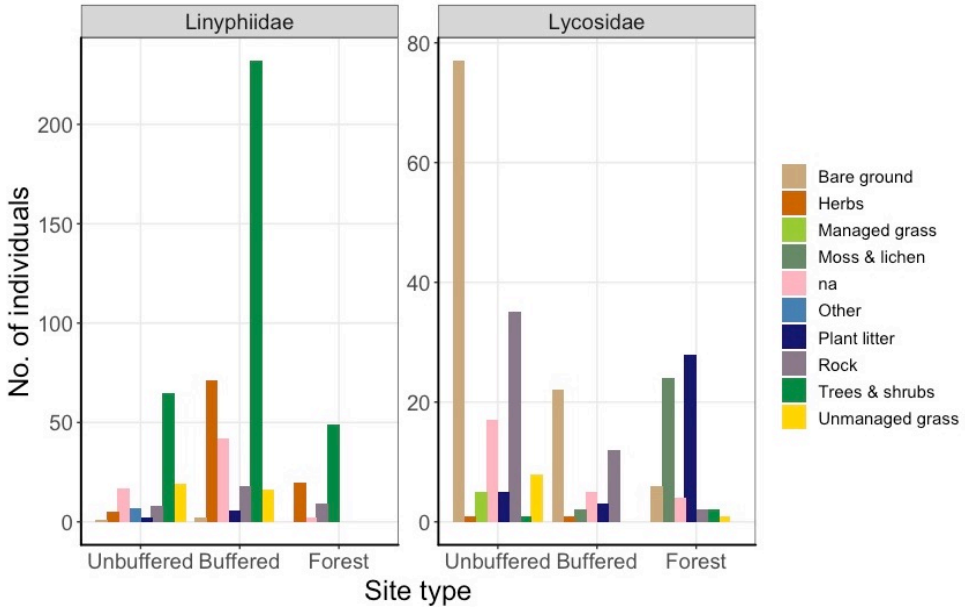

Figure 15. Total number of Linyphiidae and Lycosidae collected in different habitats per site type. Na abbreviation for not available. Note: different scales on the y-axes.

4.4 Fatty acid profiles of riparian spiders

4.4.1 Polyunsaturated fatty acids in relation to total fatty acid content

Of all the spiders collected, the target spiders that were analysed for fatty acid content made up 88.9% of the total abundance of spiders found and 82% of the total dry mass. The total content of fatty acids in spiders varied between site types (ANOVA: $F_{2,22}$=4.45, p=0.02), with forest sites having the highest concentrations, followed by buffered sites, while spiders in unbuffered sites had the lowest fatty acid concentrations (Figure 16). Tukey post hoc-tests revealed that the difference was significant between forest sites and unbuffered sites, with buffered sites intermediate between the two other site types. Mean fatty acid content per site can be found in Appendix 2: Table 16.

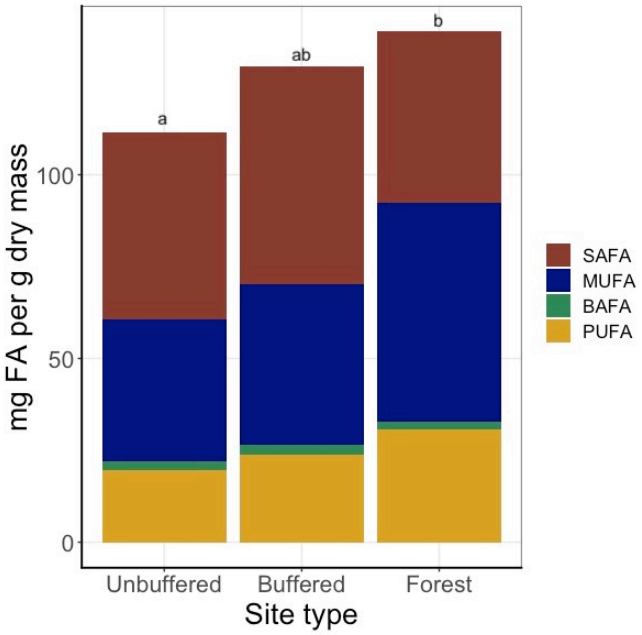

Figure 16. Mean proportion in mg per g dry mass of fatty acids in spiders at the different site types. SAFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, BAFA: Bacterial fatty acids, PUFA: Polyunsaturated fatty acids. Letters above the bars denote homogenous subsets based on Tukey's post-hoc testing of differences among groups. Note: Bars with the same letter are not significantly different.

PUFA content differed between site types (Figure 17 & Table 4), both as a percentage of total fatty acid content (Figure 17A) and as mg PUFA per g dry mass (Figure 17B). Post hoc-testing (Tukey) on PUFA content as a percentage identified forest sites as significantly different to both of the other site types ($P<0.05$). A Tukey test on site type differences in PUFA content as mass indicated a difference between forest sites and unbuffered sites ($P<0.05$) with differences between forest sites and buffered sites close to significant ($P=0.07$).

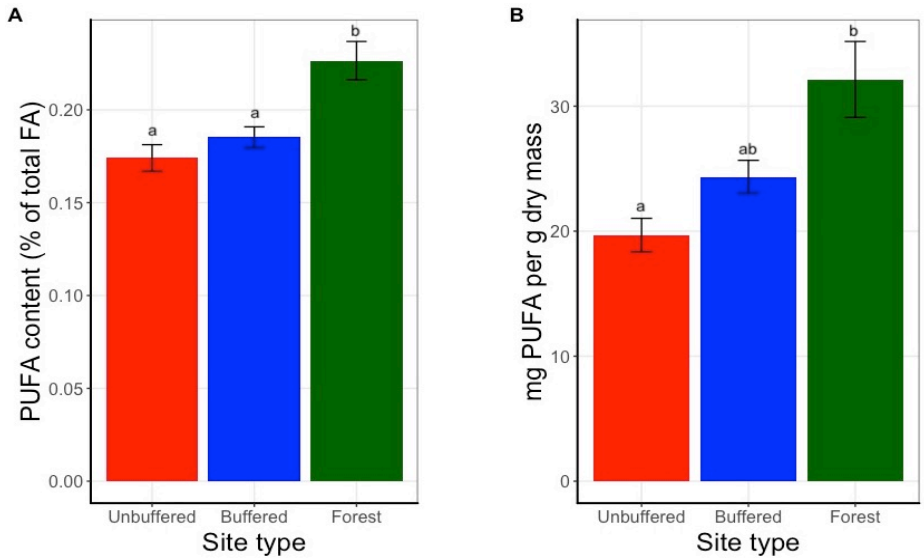

Figure 17. Mean PUFA content ± SE per site type for (A) percentage of total FA and (B) mg per g dry mass. Letters above the bars denote homogenous subsets based on Tukey's post-hoc testing of differences among groups. Note: Bars with the same letter are not significantly different and different scales on y-axes.

There were also significant differences in PUFA content among spider families (Table 4), with the post hoc Tukey tests revealing significant differences ($P<0.05$) between Opiliones and the families Linyiphiidae, Lycosidae and Pisauridae for both mg PUFA per g dry mass and PUFA as a percentage of fatty acid content. For percentage PUFA the difference between Pisauridae and Tetragnathidae was close to being significant at the 5% level ($P=0.06$) and for mg PUFA significant differences were found between Linyiphiidae and Tetragnathidae ($P<0.05$). The interaction between spider family and site type was also significant (Table 4), suggesting that PUFA content of spider families varied across sites, as can be seen in Figure 18.

Table 4. *Results of mixed-effects model ANOVA test of site type and spider family on PUFA % of total FA and mg PUFA per g dry mass.*

Variable	Numerator DF	Denominator DF	F ratio	p value
PUFA % of FA				
Site type	2	20	5.02	0.01
Spider family	4	57	4.73	0.002
Site type: Spider family	8	57	2.51	0.02
mg PUFA per g dry mass				
Site type	2	16	10.43	0.001
Spider family	4	59	9.64	<0.001
Site type: Spider family	8	59	2.33	0.03

Both Tukey post hoc-tests on the interaction between spider family and site type and Figure 18 illustrate that there were variations in PUFA content between spider families, both within site types and across site types. The highest PUFA content was found in Lycosidae spiders in forest sites, which was significantly different to the Lycosidae PUFA content in the two other site types ($P<0.05$). Forest Lycosidae were also characterised by higher PUFA concentration than Opiliones in all site types and Tetragnathidae in unbuffered sites ($P<0.05$). Overall, PUFA content of Pisauridae and Opliones did not vary greatly across sites (Figure 18). Opiliones had the lowest PUFA content compared to the other spider families in buffered and forest sites. However, in unbuffered sites Tetragnathidae had the lowest PUFA content. The PUFA content of Tetragnathidae in unbuffered sites is lower than in both other site types, but only significantly so from Tetragnathidae in forest sites ($P<0.05$).

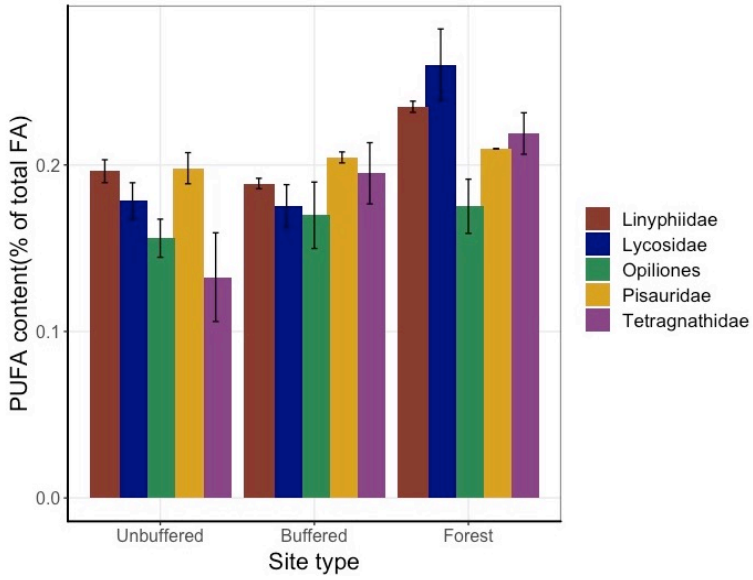

Figure 18. Mean ± SE of PUFA content as a percentage of total FA per spider family and site type.

4.4.2 Specific polyunsaturated fatty acids

In total eight specific PUFAs were identified during analysis: Alpha-linolenic acid (ALA18:3ω3), Linoleic acid (LIN 18:2ω6c), Eicosatrienoic acid (20:3ω3), Eicosadienoic acid (20:2ω6), Docosadienoic acid (22:2ω6), Arachidonic acid (ARA 18:4ω6), Eicosapentaenoic acid (EPA 20:5ω3) and Docosahexaenoic acid (DHA 22:6ω3). There was a difference between site types regarding the spiders specific PUFA profiles as can be seen in the PCA plot (Figure 19). PC1 explains 54.9% and PC2 19.6% of the total variance.

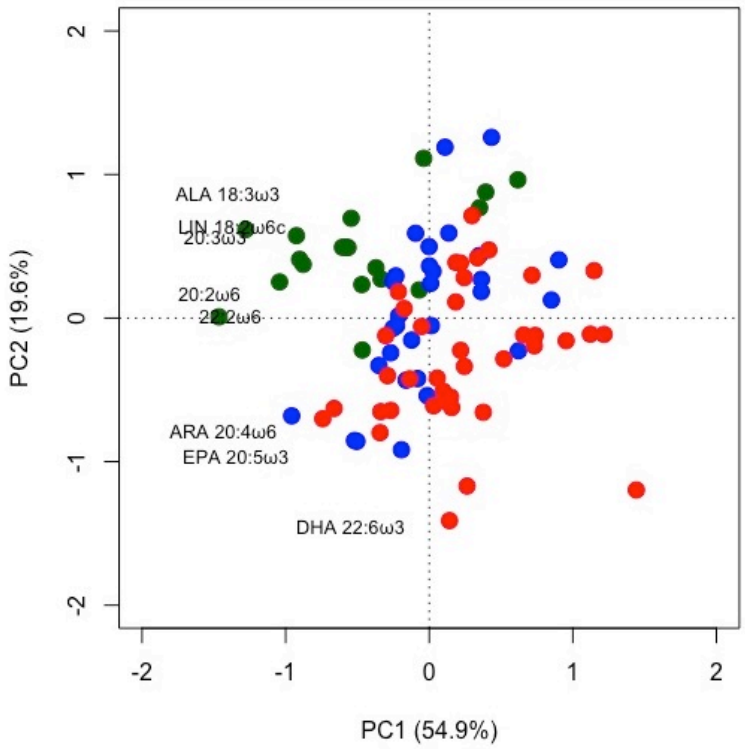

Figure 19. Principal components analysis (PCA) plot with all spider samples, colour coded according to site type (Red- unbuffered, Blue- buffered, Green- forest), and the eight specific PUFAs found. PC1 explains 54.9% of the total variance and PC2 explains 19.6% of the total variance, the cumulative variance explained is 74.5%. Only the first two PC eigenvalues >1

In the ordination space defined by both axes (Figure 19), unbuffered sites were grouped mainly to the bottom right, buffered sites banded across the middle and forest sites clustered predominantly in the top right of the plot. This was associated with a shift in the PUFA composition of the spiders. ARA, EPA and DHA were all most associated with stream sites in the agricultural landscape in the lower half of the ordination plot. Of these, DHA was most clearly associated with the agricultural sites in general, and the unbuffered agricultural sites in particular. In contrast, ARA

and EPA were more linked with sites towards the left side of PC1 (with negative PC values), crossing the transition from unbuffered through buffered to forested. All remaining PUFAs were strongly associated with the forested sites to the upper right of the ordination plot.

Further analysis of the distribution pattern of specific PUFAs seen in Figure 19 revealed that spider family was the main variable explaining shifts in PUFA composition, independently explaining 26% of the variation (Table 5). Stream identity (site pairs) independently explained 10% of the variation and site type accounted for only a small amount with 2%, but still significant (Table 5). The remaining 14% of the variation is shared variation between different combinations of three variable classes.

Table 5. *Results of variance partitioning showing the effect of the full model (all variables) and the independent contribution of spider family, site type and stream identity (site pairs) on the distribution pattern of the specific PUFAS as seen in Figure 19. Residual DF= 68.*

Variable	DF	F ratio	p value	R^2	Adjusted R^2
Full model	19	6.06	0.001	0.63	0.52
Spider family	4	10.72	0.001	0.23	0.26
Site type	1	4.32	0.01	0.02	0.02
Stream identity	13	2.35	0.002	0.17	0.10

Specific PUFA content differed between site types (Figure 20, PERMANOVA: R^2=0.10, $F_{2,73}$=6.87, p=0.002), and between spider families (Figure 21, PERMANOVA: R^2=0.24, $F_{4,73}$=8.09 , p<0.001), with an interaction between site type and spider family (Figure 22, PERMANOVA: R^2=0.10, $F_{8,73}$=1.70 , p=0.03), indicating that spider PUFA composition differed between site types.

Analysis of each specific PUFA in regard to differences between site type, spider family and the interaction between site type and spider family indicated that the specific PUFAs did not all follow the same trend (Table 6). For the PUFAs ALA, LIN, Eicosatrienoic acid (20:3ω3), Eicosadienoic acid (20:2ω6), Docosadienoic acid (22:2ω6) significant differences were found in PUFA content between both site types and spider families (Table 6). The content of ARA, EPA and DHA only differed between spider families (Table 6). At this univariate level no interaction between site type and spider family was evident at the 5% level. However, ALA, LIN and Docosadienoic acid (22:2ω6) were all only slightly above this cut off level (Table 6).

Table 6. *The results of the mixed-effects model ANOVA test for differences between the content of the eight specific PUFAs in regard to site type, spider family and the interaction between site type and spider family. p values in bold are significant.*

Specific PUFA	Numerator DF	Denominator DF	F ratio	p value
ALA 18:3ω3				
Site type	2	37	34.51	**<0.001**
Spider family	4	64	7.15	**<0.001**
Site type: Spider family	8	63	1.99	0.06
LIN 18:2ω6c				
Site type	2	42	6.73	**0.003**
Spider family	4	65	5.93	**<0.001**
Site type: Spider family	8	64	1.83	0.09
ARA 20:4ω6				
Site type	2	33	1.69	0.19
Spider family	4	63	21.74	**<0.001**
Site type: Spider family	8	62	1.25	0.29
EPA 20:5ω3				
Site type	2	33	1.98	0.15
Spider family	4	63	19.61	**<0.001**
Site type: Spider family	8	63	0.85	0.56
20:3ω3				
Site type	2	31	49.54	**<0.001**
Spider family	4	63	22.14	**<0.001**
Site type: Spider family	8	62	1.59	0.14
20:2ω6				
Site type	2	40	10.66	**<0.001**
Spider family	4	65	4.46	**0.003**
Site type: Spider family	8	64	1.89	0.32
DHA 22:6ω3				
Site type	2	48	0.79	0.46
Spider family	4	65	8.10	**<0.001**
Site type: Spider family	8	65	1.07	0.40
22:2ω6				
Site type	2	51	6.18	**0.003**
Spider family	4	66	8.79	**<0.001**
Site type: Spider family	8	65	1.77	0.09

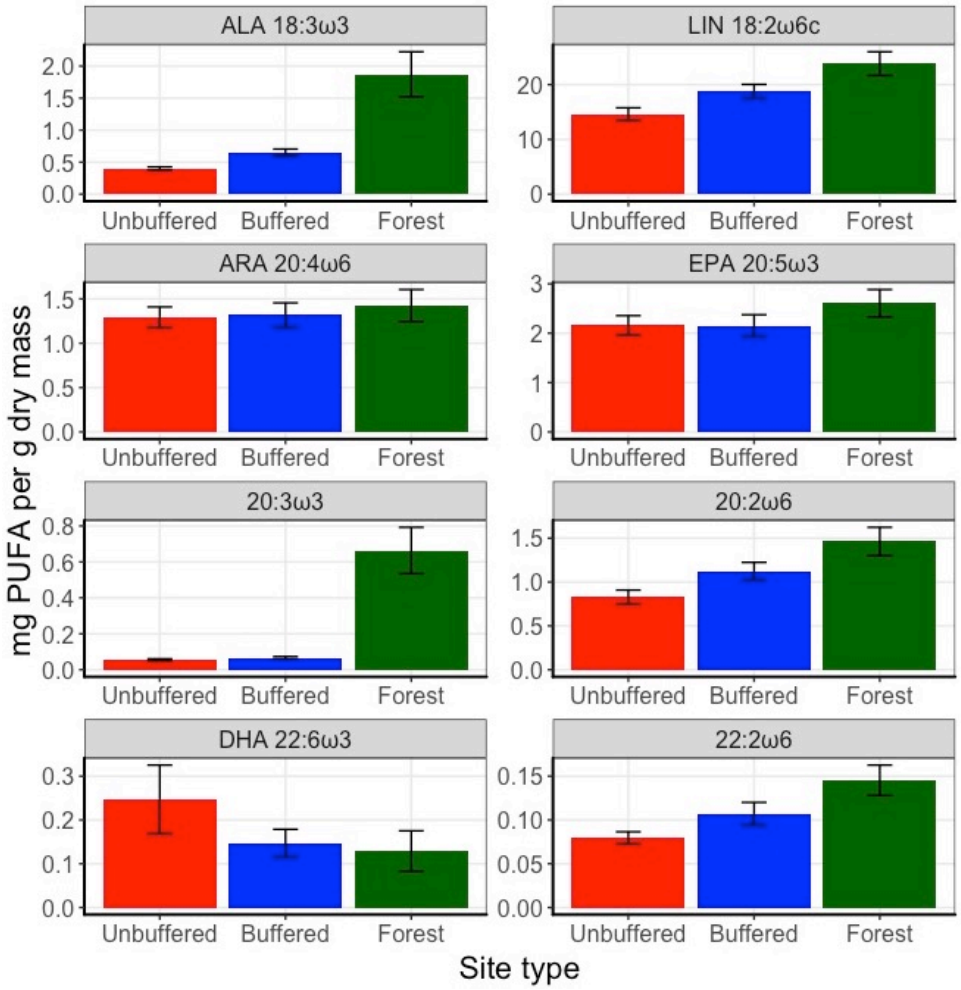

Figure 20. Mean ± SE in mg per g dry mass of the specific PUFAs content per site type. Note: different scales on the y-axis. Means per site can be found in Appendix 2: Table 17.

As seen in Figure 20 the site type differences seen between ALA, LIN, Eicosatrienoic acid (20:3ω3), Eicosadienoic acid (20:2ω6), Docosadienoic acid (22:2ω6) in Table 6 were due to higher content of these five PUFAs in forest sites compared to the other site types. Unbuffered sites had consistently the lowest content of these five PUFAs. That there were negligible differences between site types in the content of ARA and EPA was also clear (Figure 20). Though no significant differences were found for DHA between site types (Table 6), in Figure 20 a trend can be seen with the highest content of DHA found in unbuffered sites, followed by buffered sites, with the lowest content found in forest sites.

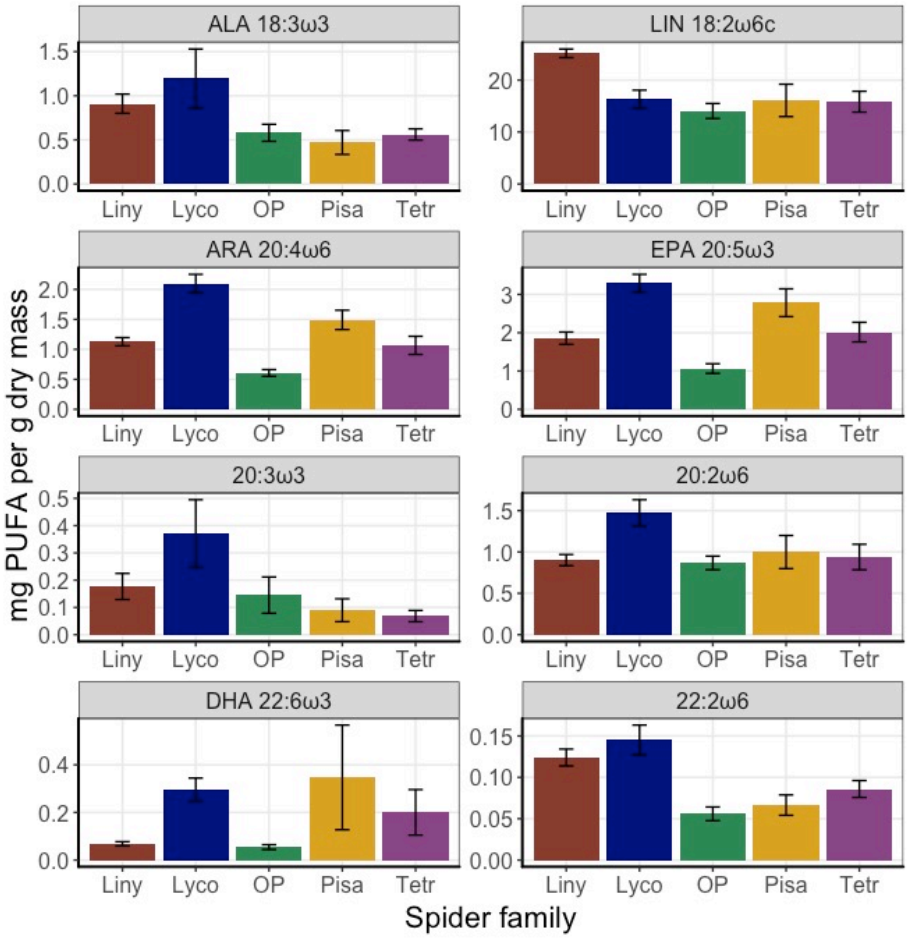

Figure 21. Mean ± SE in mg per g dry mass of the specific PUFAs content per spider family. Spider family abbreviations: Liny (Linyphiidae), Lyco (Lycosidae), OP (Opiliones), Pisa (Pisauridae), Tetr (Tetragnathidae). Note: different scales on the y-axes.

The difference found between spider families in the content of the specific PUFAs, (Table 6) is evident in Figure 21. Lycosidae had the highest content of most of the PUFAs with the exception of LIN and DHA (Figure 21). Linyphiidae instead had the highest LIN content. The content of DHA was relatively high and even between Lycosidae and Pisauridae, intermediate in Tetragnathidae, and lowest in Opiliones and Linyphiidae. The ARA and EPA content of Pisauridae, though lower than Lycosidae, was notably higher than the three other spider families (Figure 21). Opiliones had generally the lowest content of the PUFAs compared to the other spider families (Figure 21).

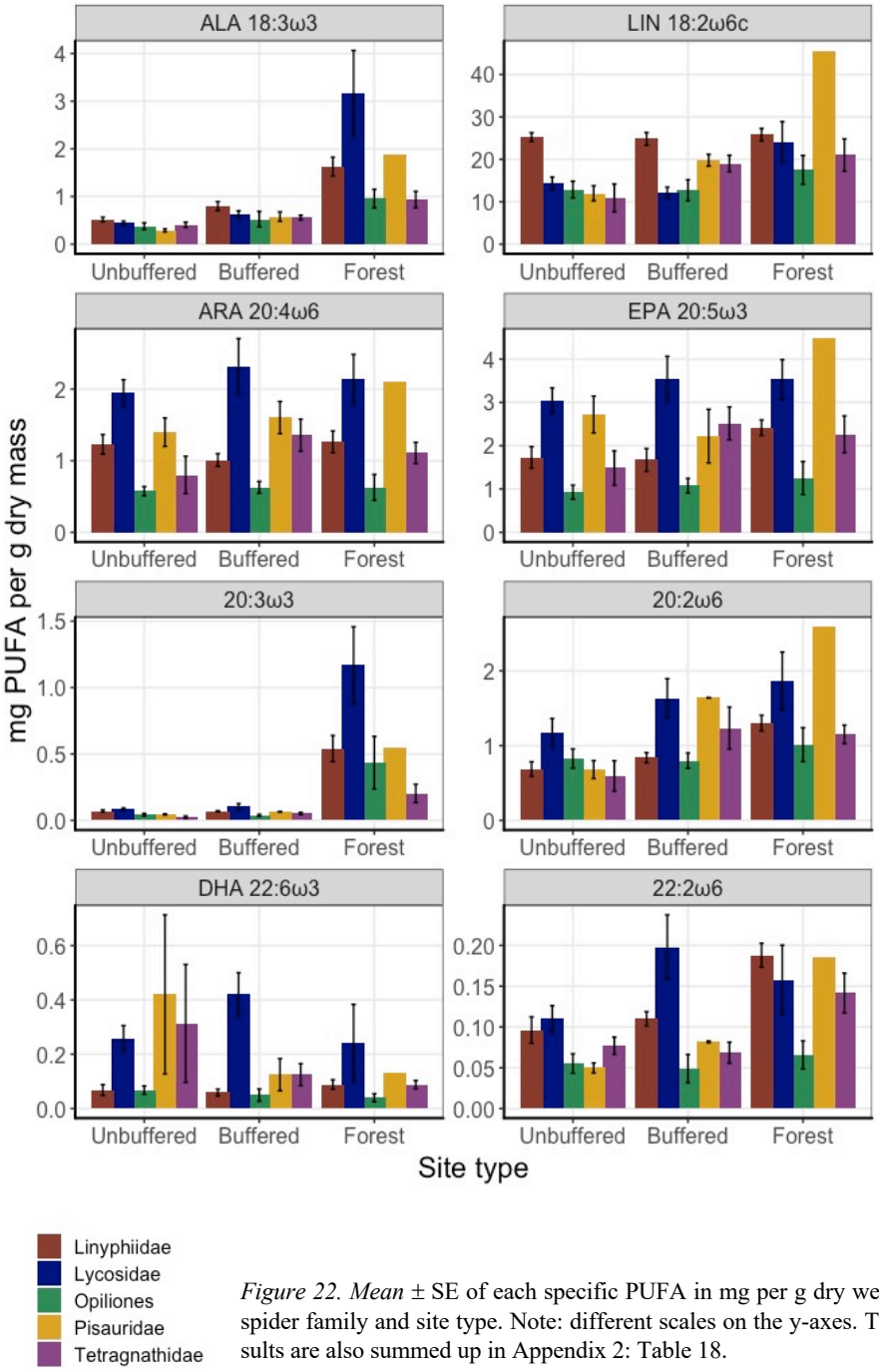

Figure 22. Mean ± SE of each specific PUFA in mg per g dry weight per spider family and site type. Note: different scales on the y-axes. These results are also summed up in Appendix 2: Table 18.

In general, the differences seen in Figure 22 between site types and spider families reflects the results in Figure 20 and Figure 21, with trends in PUFA content driven by either spider family or site type. Evidence of possible interactions between site

type and spider family were mainly driven by the responses of Lycosidae. The content of the PUFAs ALA and Docosadienoic acid (22:2ω6) in Lycosidae found at forest sites was markedly higher in relation to the other families in forest sites and Lycosidae at other site types. DHA content of Lycosidae was also high in buffered sites, relative to not only other families at the buffered sites, but also to Lycosidae sampled from forest and unbuffered sites. Forest Pisauridae also had high content of several PUFAs relative to other families and Pisauridae in other site types, however this was based on one sample only (Fiby forest).

5 Discussion

Vegetation heterogeneity is generally recognized as being important to riparian buffer function in regard to maintaining biodiversity and connectivity, but is often highly simplified in human modified landscapes, including in agricultural landscapes where riparian zones are at best buffers of grass or herbs (Schultz *et al.* 2004; Naiman *et al.* 2005; Clark & Reeder 2007; Degerman & Bergqvist 2008; Bjelke *et al.* 2016). In this study, I examined the difference in biodiversity, abundances and community composition of riparian spiders between sites with sparse or no wooded vegetation (unbuffered sites) and forested buffers (buffered sites) in an agricultural landscape, and reference forest sites. Additionally, I analysed the fatty acid content of the spiders, with a focus on polyunsaturated fatty acids (PUFAs) to ascertain how the trophic connectivity between terrestrial and aquatic systems is affected by the buffer properties of the different sites.

My results suggest that buffer properties have an impact on spider populations, with abundances, diversity and community composition of spider families varying between the site types. There were also clear differences in the PUFA profiles of the spiders, which was largely driven by spider taxonomic identity, but also influenced by site type and an interaction between site type and spider family, as well as stream identity. In the following sections I discuss these results. I start by focusing on the differences in spider communities between site types and potential drivers of this differences. I then discuss the distinction in PUFA content between site types and spider families, possible reasons for these differences and what the results may indicate regarding connectivity. The implication of my results for the transfer of PUFAs into terrestrial food-webs is also addressed. Finally, I conclude by discussing my results in relation to riparian buffer management and future research needs.

5.1 Riparian spider community

My results show that riparian buffer properties have an effect on spider populations, influencing community composition, abundance, diversity and distribution. This is in accordance with previous research that has stressed the impact that riparian buffer characteristics may have on the diversity and abundance of riparian communities (Schultz *et al.* 2004; Naiman *et al.* 2005). The differences in spider communities between site types are largely shaped by a shift in spider functional types, with web-building spiders being more common in buffered sites and free-living spiders dominating in unbuffered sites (Figure 9). The most commonly collected spider family across all sites was the web-building Linyphiidae, the vast majority however were collected in buffered sites (Figure 13). In contrast, free-living Lycosidae, the second most abundant spider family, was most commonly found at unbuffered sites (Figure 13).

There is a difference in community composition between buffered and unbuffered sites, as hypothesised, which is chiefly driven by the abundances of Linyphiidae and Lycosidae. However, the differences in community composition are not limited to Lycosidae and Linyphiidae as the NMDS plot (Figure 9) illustrates. Several taxa were found almost exclusively at one or the other site type, for example, wetlands specialist spiders within the Pisauridae (Artdatabanken, SLU 2019) were mainly found in unbuffered sites. In the reference forest sites, community composition was intermediate between the other two site types, being similar to buffered sites overall but also including families more typical of unbuffered sites (with one site in particular, Lafsjön, having high abundances of Lycosidae), and therefore showed no clear difference to either.

I hypothesised that spider diversity would be higher in buffered sites than in unbuffered sites. The results instead indicate that spider diversity at the family level is higher at unbuffered sites than buffered sites, this in spite of similar mean number of taxa found in each site type (Table 3 & Figure 8). This can be explained by the fact that both taxa richness and evenness affect diversity measures such as Shannon diversity. The apparent lower diversity at the buffered sites is driven by the higher abundances of Linyphiidae, which numerically dominated samples compared to other spider families, reducing taxonomic evenness (Appendix 2: Table 15). However, it is worth noting though that Linyphiidae is the most species rich family in Sweden (approximately 300 species, Kronestedt 2001). Accordingly, diversity at the family level may not be a reflection of diversity at the species level. Further research is required to assess whether an increased abundance of Linyphiidae in the buffered sites is also associated with an increased species richness.

No differences were found between site types in total abundances, which is in opposition to what I hypothesised. Despite this, the clear influence of the high abundances of Linyphiidae can be seen, with the highest overall abundance of spiders found at buffered sites (Figure 8). This is also the case when effort is taken into account (Figure 11). Catch per unit effort can be used as relative measure of abundance and accounts for differences in sampling effort between sites. As sites were variable in how steep the banks were or thick the vegetation was, the effort between sites was also inevitably variable. Catch per unit effort is also an important measure in relation to our sampling method. We used visual searches, which is a semi-quantitative, observational method, requiring diligence and energy from the surveyors. Taking into account the effort is therefore essentially a quality control of the search method. In many invertebrate studies, pitfall traps are used (Moring & Stewart 1994; Perner & Malt 2003; Carlson 2014), which are suitable for surface dwelling invertebrates (Henderson & Southwood 2016) and for nocturnal invertebrates. Using pitfall traps may have increased the abundances of free-living spiders and ground beetles that we collected; however, this method is unsuitable for web-building spiders. Pitfall traps were therefore not optimal for this study, as the aim was to sample a more diverse range of invertebrates.

The highest catch per unit effort for abundances was at buffered sites, again due to the large number of Linyphiidae found at these sites (Figure 11 & Figure 13). The influence of the abundance of Lycosidae found at unbuffered sites is reflected in the overall abundance of the unbuffered sites, which explains why no difference was found in abundance between unbuffered and buffered sites. This pattern is echoed in the catch per unit effort for biomass (Figure 12), with the high biomass at unbuffered sites tied to the abundance of the large sized free-living spiders, and the lower mass in buffered sites tied to the smaller web-building spiders. Forest sites had the lowest total abundances, the lowest catch per unit effort, and the lowest biomass. This may be due to the general lower productivity in forest stream ecosystems resulting in less prey (Carlson *et al.* 2016) but could also be a result of the spiders being more widely dispersed as they are not limited by habitat boundaries to the same degree as they are in the other two site types.

The patterns seen in abundances, diversity and community composition, clearly shaped by the evident shift in spider functional type from the web-building spiders dominating in the buffered sites, to free-living spiders in the unbuffered sites, indicates that site characteristics have an impact in shaping spider communities. Potential explanations could be differences in habitat characteristics and prey availability, both of which been shown to affect spider populations (Kato *et al.* 2004; Baxter *et al.* 2005; Laeser *et al.* 2005; Prieto-Benitez & Mendez 2011).

5.1.1 Habitat effects on spider communities

Previous studies have shown that differences in the community composition of riparian invertebrates between sites can largely be explained by habitat heterogeneity and vegetation structure (Perner & Malt 2003; Sadler *et al.* 2004; Batary *et al.* 2008; Lambeets *et al.* 2008; Prieto-Benitez & Mendez 2011; Stenroth *et al.* 2015). This is consistent with the results of this study with differences in community composition between unbuffered and buffered sites partly explained by variations in available habitat structures (Figure 14). Trees and shrubs have an impact on the micro-climate within the riparian zones, dampening temperature fluctuations, increasing shading and humidity, and reducing wind exposure, which may all favour more environmentally sensitive species (Moore *et al.* 2005; Naiman *et al.* 2005). Vegetation also provides suitable substrate for web-building spiders to build their webs in. Thus, in riparian zones that have been cleared of vegetation the number of web-building spiders is usually low (Laeser *et al.* 2005), as is the case in this study. The high abundances of web-building spiders such as Linyphiidae in the buffered sites can be explained by the availability of suitable trees, shrubs and herbs where they can build their webs (Figure 6 & 7), and further supported by the fact that most Linyphiidae were collected from these habitats (Figure 15). In contrast, Lycosidae are actively hunting predators relying on for example visual cues, this hunting strategy is more adapted to ground surfaces and different species prefer specific surface types, for example gravel or sandbanks near water or leaf litter in forest environments (Moring & Stewart 1994; Lambeets *et al.* 2008). The highest abundances of Lycosidae spiders were found in unbuffered sites on sandbanks and rocks (Figure 15), despite these not being the main ground cover types in unbuffered sites (Figure 7). These open areas probably allow for better access to both aquatic and terrestrial prey explaining the aggregation of Lycosidae in these habitats. However, this result may also have been affected by sampling bias on our part as the spiders were easier to see in these open areas. In Figure 14, positive correlations can be seen between grass, bare-ground and Lycosidae, and trees and shrubs and Linyphidae, representative of the unbuffered and buffered sites. In the forest reference sites Lycosidae were found mainly amongst leaf litter or on moss and lichen and these may have been different species than those found at the unbuffered sites. Moring & Stewart (1994) studied the distribution of Lycosidae species in a range of riparian habitats and found that different species had distinct habitat preferences. The habitat preferences of these two families is one explanation for the differences between site types. In Sweden a large proportion of Linyphiidae species are associated with forest habitats whilst many Lycosidae species are instead found in agricultural systems and wetlands (Artdatabanken, SLU 2019).

However, the riparian buffers in this study were not large connected forest or wetland ecosystems, but small, isolated habitats in a larger disturbed landscape. The riparian zones in the buffered sites are essentially fragments of more or less undisturbed forest habitat in an agricultural landscape and may therefore act as refugia for species requiring the habitat structures that are available in these forest fragments (i.e., trees and shrubs for web-builders) and the micro-climate associated with the vegetation. Furthermore, riparian buffers are an edge habitat both towards the stream and towards the agricultural landscape. A meta-analysis (Prieto-Benitez & Mendez 2011) found that spider abundances at forest edges were generally high, potentially due to the increased prey availability from two habitats meeting. Studies have also found that field edges in agroecosystems harbour higher diversity compared to adjacent cultivated fields, probably due to both an ecotone effect with two habitat types meeting, but also these edges are generally less disturbed and more heterogenous environments than the adjacent agricultural fields (Clark & Reeder 2007; Prieto-Benitez & Mendez 2011). Studies comparing invertebrate communities in unforested riparian zones in agricultural landscapes with different management intensity have also concluded that vegetation heterogeneity and disturbance intensity have an impact on spider communities (Perner & Malt 2003; Batary *et al.* 2008). The unbuffered riparian zones in this study are generally relatively undisturbed (aside from a few exceptions that are grazed by cattle), with high grasses, herbs and shrubs (Figure 7) and therefore may also act as refugia for spiders more adapted to these types of environments than forests.

In this study the majority of spiders were collected at an average of one meter from the stream channel (Figure 10). This result is consistent with previous studies, with spiders often aggregating at riparian edges (Kato *et al.* 2003; Paetzold *et al.* 2005; Burdon & Harding 2007). However, though no significant differences were found, the forest reference sites and buffered sites show more variability in the distribution of spiders. This may be due to a more stable micro-climate in buffered and forest sites compared to unbuffered sites. Carlson *et al.* (2016), studied the dispersal abilities of emerging stream insects and found that small dipterans could disperse further in forested riparian zones compared to exposed sites. Thereby, the need for spider consumers to aggregate close to the stream may not be as important in forest sites. Additionally, edge-effects may play a role as there would be a harsher contrast in both temperatures and humidity levels between the stream side riparian edge and the terrestrial riparian edge in unbuffered sites, whilst in forested sites this gradient would be less severe due to shading by vegetation (Moore *et al.* 2005). The slight difference in spider distribution between site types could also be a function of the availability of suitable substrates. Web building spiders were more prevalent in forest sites and built their webs in the vegetation available which was spread out within the buffer, whilst the sandbanks and rocks that the free-living Lycosidae in

unbuffered sites preferred were always closer to the stream edge. Spiders habitat preferences are largely tied to hunting strategies, which in turn is connected to prey preferences and availability.

5.1.2 Prey: preferences and availability

Several studies have found that emerging aquatic insects from streams in agricultural landscapes are often highly abundant and smaller (e.g. dipteran families such as Chironomidae), compared to larger, less abundant insects from streams in forested landscapes (Carlson 2014; Stenroth *et al.* 2015; McKie *et al.* 2018). Stenroth *et al.* (2015) in fact found that not only was the emergence of small dipterans higher at buffered agricultural sites, but that Linyphiidae spiders also dominated near these streams. Linyphiidae generally feed on small prey (Nyffeler 1999) so the connection between small emerging insects and Linyphidae seems likely. This could be one explanation for the high abundances of Linyphiidae found at buffered sites in this study.

Free living spiders, such as Lycosidae, often have a larger diet span due to their mobile hunting strategy (Nyffeler 1999) and are therefore more opportunistic, probably reacting faster to prey fluxes. As the abundances of emerging insects from streams in agricultural landscapes is often higher than forest systems this could explain the higher number of free-living spiders such as Lycosidae at unbuffered sites compared to forest sites. Prey preferences will be discussed in further detail in the next section in relation to polyunsaturated fatty acids.

5.2 Fatty acid profiles

Fatty acids play several key roles in organisms, with polyunsaturated fatty acids (PUFAs) in particular recognized as essential for heterotrophs, as they are vital to several functions and *de novo* synthesis of them is limited (Gladyshev *et al.* 2009; Parrish 2009; Twining *et al.* 2016). Additionally, PUFAs can be used as biomarkers, useful in identifying possible sources of prey (Iverson 2009). Differences in ratios between different fatty acids can give an indication of possible variations in prey identity and quality at the different sites. In this study the total fatty acid content of the spiders differed between site types, with highest levels found in forest sites, followed by the buffered sites, with unbuffered sites having the lowest content (Figure 16). PUFA content also differed between site types with the highest quantity found in the forest sites, both as a percentage of total fatty acid content and as mass (Figure 17). This difference between site types in PUFA content gives an indication that prey availability or preference may vary between the sites, which potentially could

be due to differences in site properties. Additionally, fatty acid and PUFA content may be an indicator of overall condition of the spider communities, with a diet rich in lipids (Wilder 2011). The higher levels of fatty acids and PUFAs in spiders in forest sites may therefore signify a better overall physiological condition of spiders at these sites. Differences in PUFA content were also found between spider families. For example, Pisauridae spiders had consistently high levels of PUFAs across all sites (Figure 18). This suggests that differences in PUFA content between sites may not only driven by site-typical characteristics but also by the spider taxonomic identity. Additionally, an interaction between site type and spider family was also found, which indicates that though PUFA content varies with spider family identity, the PUFA content of the spider family is also dependent on site type. For example, the PUFA content of Lycosidae spiders in general was relatively high, but forest Lycosidae were characterized by a considerably higher PUFA content than at the two other site types (Figure 18).

This analysis of differences in overall PUFA content between site types and families gives an indication of possible patterns and interactions. However, although aquatic systems are generally recognized as important for the synthesis of PUFAs in general, specific PUFAs have different sources (Torres-Ruiz *et al.* 2007). Therefore, inferences about reliance on aquatic subsidies and linkages between aquatic and terrestrial systems can only be made by examining differences in specific PUFA profiles.

5.3 Specific polyunsaturated fatty acids

Higher plants cannot generally synthesize long-chain PUFAs such as Docosahexaenoic acid (DHA), Eicosapentoenoic acid (EPA) and Arachidonic acid (ARA), all of which are essential fatty acids (Gladyshev *et al.* 2009; Twining *et al.* 2016). Instead, aquatic algae and bryophytes are major sources of these long chain PUFAs (Torres-Ruiz *et al.* 2007; Gladyshev *et al.* 2013; Taipale *et al.* 2013; Twining *et al.* 2016). These aquatically derived PUFAs can be transferred into terrestrial ecosystems by emerging aquatic insects, which are an important subsidy to terrestrial consumers such as spiders (Baxter *et al.* 2005; Gladyshev *et al.* 2009; Schindler & Smits 2017). Therefore, the content of long-chain PUFAs in terrestrial organisms gives an indication of their reliance on aquatic subsidies. PUFA content further gives an indication of the quality of the food available, this includes both possible terrestrially derived Alpha-linolenic acid (ALA) and Linoleic acid (LIN), (as they are precursors to the other essential PUFAs) and the aquatically derived PUFAs (Gladyshev *et al.* 2009; Parrish 2009).

The ordination and variance partitioning (Figure 19 & Table 5), together with the PERMANOVA results indicates that that there are differences in the overall PUFA profiles of the spiders, driven by spider family identity, site type and an interaction between the two, as well as stream identity. Though total PUFA content were highest in forest sites, followed by buffered sites and lastly unbuffered site (Figure 17), the pattern seen in the ordination plot (Figure 19) suggests that there are differences in what type of specific PUFAs dominate in the different site types. Variation partitioning revealed that the pattern seen in the ordination plot was largely driven by spider family with an approximate 26% independent contribution to total variability (Table 5). Site type contributed to a small (2%) but significant part of the distribution (Table 5). Stream identity also contributed to the pattern, independently explaining 10% of the variation seen. PERMANOVA results revealed that the PUFA profiles varied between site types and spider families, and that there was an interaction between the two.

The differences seen in PUFA profiles between site types in the ordination (Figure 19), which is further strengthened by the PERMANOVA and ANOVA results (Table 6), as well as the bar charts in Figure 20, may be driven by a number of on-site properties. Variations in properties (e.g. shading) between site types potentially affects in-stream PUFA production, the transfer of the PUFAs to the riparian zone via emerging aquatic insects and what spider taxa are present, which in turn affects to what degree aquatic PUFAs are found in the spider communities present (Lambeets *et al.* 2008; Hill *et al.* 2011; Guo *et al.* 2016; Schindler & Smits 2017). In contrast, the role of stream identity in affecting the PUFA content of the spiders instead suggests that in some cases other factors than on-site properties affected PUFA production, transfer and assimilation. As buffered and unbuffered sites within pairs were only a few hundred meters apart along the same stream this result can be expected. Although on-site vegetation differed between site pairs, with one having a forested buffered and the other not, in-stream characteristics such as nutrient levels are likely to be relatively similar at such short distances, which may also have an impact on algae communities and PUFA production (Stelzer & Lamberti 2001; Hill *et al.* 2011; Guo *et al.* 2016).

In aquatic systems intraspecific variation in PUFA content has been found in aquatic invertebrates as a result of various factors including differences in season and nutrient levels (Lau *et al.* 2013). However, interspecies variations in PUFA content has been found to be mainly driven by taxonomic identity and not by site variables, with different taxonomic groups having different reliance on and assimilation of PUFAs (Lau *et al.* 2012). In this study the differences between spider families in specific PUFA content (PERMANOVA, Figure 19 & Figure 21), and interactions between spider family and site type (PERMANOVA & Figure 22), indicates that these patterns to some degree also hold true for terrestrial spiders, but are contingent

on the degree to which they are reliant on aquatic prey. Generally, each spider family had a stable PUFA content pattern in relation to other spider families. For example, Lycosidae had generally the highest content of each of the specific PUFAs and Opiliones had low PUFA content (Figure 21). This pattern was largely maintained across site types (Figure 22). For instance, the PUFA content of Lycosidae varied somewhat across sites but were nearly always higher than the levels found in Linyphiidae, Opiliones and Tetragnathidae (Figure 22). However, the interaction between spider family and site type is also evident, as most clearly illustrated by Lycosidae. The concentration of two PUFAs ALA and Eicosatrienoic acid (20:3ω3) in spiders from forest sites was higher compared to the other site types, but markedly so for the Lycosidae (Figure 22), indicating an interaction between this specific family and site type. A similar trend can be seen for Lycosidae in buffered sites for the PUFAs DHA and Docosadienoic acid (22:2ω6). These results suggest that though site typical properties may have an effect on the specific PUFA content in spiders, a major influence on the PUFA content of a spider is what taxa it belongs to, and its prey-capture method, the degree to which the taxa relies on aquatic prey, and its reliance on and assimilation of PUFAs.

5.3.1 The long-chain PUFAs: ARA, EPA and DHA

In the ordination plot (Figure 19) ARA, EPA and especially DHA, though on the same side of the plot as the other PUFAs, are more associated with sites in the agricultural landscape. This is in opposition to my second hypothesis. Algae, such as diatoms, have been pinpointed as major producers of DHA, EPA and ARA in freshwater systems (Torres-Ruiz *et al.* 2007; Gladyshev *et al.* 2013), and as diatoms are often associated with forested rather than agricultural streams (Richardson & Danehy 2007; Law 2011), one would expect that levels of these long-chain fatty acids would be higher in forested systems. Headwater streams, as most of our forest streams were, are small and prone to low flows and partially drying out in summer (Richardson & Danehy 2007). This would put aquatic algae communities under severe stress and potentially lead to fewer diatoms. Shading also limits diatom production and as the canopy cover was high in both forested and buffered sites (Figure 7) this could also potentially be the reason for the even content of EPA and ARA found across site types (Table 6, Figure 20) and the lower content of DHA found in buffered and forest sites (Figure 20). However, ARA and EPA, positioned as they are between unbuffered and buffered sites (Figure 19), are potentially also associated with the transition from unbuffered to buffered, indicating that buffering may increase the transfer of these two PUFAs in particular. DHA content was surprisingly highest in unbuffered sites, (though not significantly different) (Figure 20). This seems to be mainly driven by three spider families, Lycosidae, Pisauridae and

Tetragnathidae (Figure 21). At unbuffered sites, marginal vegetation may favour these families, which could be one explanation for the high DHA content of the spiders at unbuffered sites.

Studies have shown that some spider families are more reliant on aquatic subsidies than others. In general, I found that long-chain PUFA content was higher in spider families that are generally associated with a high reliance on aquatic prey, which is in line with my third hypothesis. Riparian Lycosidae have often been found to be highly reliant on aquatic subsidies (Paetzold *et al.* 2005; Krell *et al.* 2015; Stenroth *et al.* 2015). This study further confirms this as Lycosidae had consistently high content of the aquatic PUFAs regardless of site (Figure 22). Pisauridae are also known for being aquatic specialist and they too have generally high levels of aquatically derived PUFAs. The web-building spider family Tetragnathidae have also often been shown to have a high reliance on aquatic prey (Kato *et al.* 2004; Krell *et al.* 2015), in this study the results were not as conclusive. The mechanism of storing PUFAs may also be different in free-living spiders compared to web-building spiders, which may be an explanation for the inconclusive result in regard to Tetragnathidae.

5.3.2 The long-chain PUFAs: Eicosatrienoic, Eicosadienoic and Docosadienoic acid

Eicosatrienoic acid (20:3ω3), Eicosadienoic acid (20:2ω6) and Docosadienoic acid (22:2ω6) were all mostly associated with the forest sites in the ordination plot (Figure 19). Additionally, the content of these three PUFAs were found to be higher in forest sites than in unbuffered or buffered sites (Table 6 & Figure 20). Eicosatrienoic acid, Eicosadienoic acid and Docosadienoic acid are all long-chain acids and at least the first two are considered essential PUFAs (Cunnane 2000). Little could be found on these PUFAs in the literature compared to the other three commonly studied PUFAs (EPA, DHA and ARA). Eicosatrienoic acid is produced by aquatic bryophytes and freshwater microalgae (Raphidophyceae) (Torres-Ruiz *et al.* 2007; Taipale *et al.* 2013). Eicosadienoic acid has been found in freshwater microalgae of the class Euglenophyceae (Taipale *et al.* 2013). No information could be found on Docosadienoic acid. Higher levels of Eicosatrienoic acid and Eicosadienoic acid in forest sites could therefore be a result of certain aquatic bryophytes and algae present in the stream that are adapted to the shading (Richardson & Danehy 2007) (Figure 7) and lower water temperatures that characterised forest sites. Many of the forest streams had very low flows and some had partially dried out by mid-summer. The algae and bryophytes communities at these sites are thereby probably specialized, adapted to this fluctuation in flow (Richardson & Danehy 2007). This could be one explanation for why these PUFAs were more common in forest sites than the

other sites which didn't dry out. The unreliable flow conditions of forest streams in summer may also lead to adaptions in the timing of aquatic invertebrates' emergence. They may emerge more rapidly than in other systems to avoid being stranded, which could potentially lead to a stronger aquatic signal in terrestrial consumers that utilise this flux. Eicosatrienoic acid in particular is found in much higher concentrations in forest sites, especially in Lycosidae spiders (Figure 22). This could be connected to the PUFA source i.e. bryophytes, being more common in forest sites but also the aquatic invertebrates that feed on bryophytes. Torres-Ruiz *et al.* (2007) found evidence to support that at least some aquatic invertebrates (Isopoda and Oligochaete) utilize bryophytes as a food source. These invertebrates do not have an adult flying stage, and therefore do not emerge. However, exposure of these bryophytes mats during summer low flows may give short-term access to ground hunting spiders, such as Lycosidae, enabling them to feed on the aquatic invertebrates stranded in the bryophyte mats. This mechanism could explain the stronger signal of Eicosatrienoic acid in Lycosidae in forest sites.

5.3.3 The PUFAs ALA and LIN

In the ordination plot (Figure 19) ALA and LIN are strongly associated with forest sites. Further analysis confirmed that the content of these two PUFAs was higher in forest site spiders compared to the two other site types (Table 6 & Figure 20). As both ALA and LIN can be produced by higher plants the source of these two PUFAs is more difficult to infer. However, Torres-Ruiz *et al.* (2007), found that freshwater green algae in the streams they studied had consistently higher levels of ALA and LIN than allochthonous sources. Furthermore, Arce-Funck *et al.* (2015) found that certain aquatic fungi high produced high levels of ALA and LIN. In detritus-based food webs, typical of forested ecosystems where shading often limits primary production and allochthonous input is higher (Allan & Castillo 2007), fungi could thereby be a major source of these two PUFAs. It is therefore not unreasonable to suggest that at least part of the LIN and ALA content found in the spiders is from aquatic sources, especially at forest sites. Alternatively, the higher levels of LIN and ALA in the forested sites could also be due to a higher dependence on terrestrial prey. Linyphiidae have been found to feed on terrestrial prey, for example Collembola, to a large extent (Nyffeler 1999), as well as aquatic prey (Stenroth *et al.* 2015). In this study they most notable for their high and level content of LIN (Figure 21 & Figure 22), which may be aquatically derived but could also be from terrestrial sources. Opiliones have generally very broad diets and often scavenge for food (Shear 2009). This is evident in this study, with the low levels of PUFAs found consistently in Opiliones (Figure 22). There could, however, also be a taxonomic difference in how they assimilate PUFAs, compared to the Araneae.

5.3.4 The transfer of aquatic PUFAs into terrestrial food webs

The PUFA profile of the spider families can not only be used to infer dietary preferences but may also be indicative of the role they have in linking aquatic-terrestrial food-webs. In this study there is evidence to suggest that the large-bodied free-living spiders Lycosidae and Pisauridae may potentially play a significant role in transporting aquatically-derived essential PUFAs into terrestrial ecosystems. These spiders have a high reliance on aquatic prey which results in a higher PUFA content. Additionally, they are large, so they are both high quality and quantity prey to higher consumers such as birds. However, I found most Lycosidae and Pisauridae in unbuffered sites. The unbuffered sites with their sparse vegetation are probably not optimal habitats or foraging grounds for birds or bats as they lack structures suitable for nests and are more exposed to predators (Naiman & Décamps 1997), therefore though Lycosidae and Pisauridae are potentially important links to terrestrial food webs, the habitat they are found is probably equally as important to the efficiency of the link. It is also important to note that though the aquatically-derived PUFA content of Linyphiidae was not high this may not necessarily mean that they do not play an important role in connecting aquatic and terrestrial food webs. Linyphiidae prey is limited to what flies into their web, which sets the boundaries both for the size and what type of prey they get access to. Additionally, the webs intercept flying insects on a vertical plane. The small, aquatically-derived Dipteran families (e.g. Chironomidae) that Linyphiidae are likely to catch in their webs are generally filter/collector feeders (Allan & Castillo 2007), meaning the long-chain PUFAs that aquatic algae produce are unlikely to be found in high concentrations in Dipteran families. Thus, though Linyphiidae may not be important for the transfer aquatic PUFAs, they may still be a vital connection between aquatic and terrestrial food-webs.

5.4 Conclusions: Implications for buffer management and future research

In most agroecosystems riparian buffers are currently either absent or dominated by grasses and herbs, even though the importance of structurally complex and heterogenous vegetation is emphasized for maintaining biodiversity and ecological linkages. My results suggest that unforested riparian zones and forested buffers play different roles, harboring different functional types of spiders in an otherwise impacted landscape. Linkages between aquatic and terrestrial were relatively similar regardless of buffer type, as demonstrated by the similarity of aquatically-derived PUFAs across sites. Instead spider taxonomic identity, which in turn is affected by buffer properties, seems to be a main driver of variations in PUFA content. Thereby, it seems that the transfer of aquatic PUFAs from spiders further into terrestrial food-webs may be primarily through particular families, such as Lycosidae. At the ecosystem level this transfer is potentially most efficient in buffered and forested sites where the vegetation is more favourable towards a wider range of terrestrial predators, including birds and bats. Additionally, in buffered sites Linyphiidae abundances were high. Though the aquatic PUFA content of Linyphiidae was comparatively low in relation to Lycosidae, Linyphiidae webs probably targets a different functional group of emerging insects which in themselves have low aquatic PUFA content. Thus, the high abundances of Linyphiidae at buffered sites are potentially indicative of trophic connectivity independent from PUFA transfer. Therefore, though grass and herb buffers may be important refugia for some species in agricultural systems, linkages are probably maintained to higher degree in the presence of forested buffers. Ultimately allowing grass/herb buffers to naturally re-shrub and reforest themselves may be beneficial to stream-riparian systems function at longer time-scales. These 'new' riparian buffers could also ideally be managed to some degree to benefit a variety of different taxa. For example, Schultz *et al.* (2004) describe a buffer system which includes several zones, including a grass/herb zone. A version of this could be implemented with more varied vegetation structure overall in riparian buffers, which would benefit both species that need more open areas and others that benefit from complex vegetation structure. Additionally, the few pockets of riparian forest that are left should be protected, as they are important habitats to many sensitive species and may also serve as source habitats for surrounding newly restored riparian buffers.

In this study I have attempted to use PUFA biomarkers in terrestrial systems to infer diet, as a relatively novel approach to better understand coupled food-webs. To date, the majority of studies have used stable isotope analysis to the establish the reliance of spiders on aquatic prey, whilst PUFAs have mainly been used in aquatic systems (Kato *et al.* 2004; Torres-Ruiz *et al.* 2007; Lau *et al.* 2013; Krell *et al.*

2015). As the results of the PUFA analysis in this study show similar trends to the results of previous stable isotope analysis in regard to spider family's aquatic subsidy reliance, this indicates that PUFAs could also be useful tools for inferring prey origin in terrestrial systems. Ultimately, PUFA analysis at each trophic level, from possible sources to emerging insects and further into the terrestrial food-web, within the same ecosystem would answer many questions regarding the transfer and assimilation of PUFAs, constituting a challenging yet fascinating exercise. To confirm the use of PUFAs as tool in terrestrial ecosystems, however, it may be useful to perform stable isotope analysis and/or molecular gut content analysis on the same samples used in this PUFA analysis to validate the results attained and provide further details. Additionally, as trophic connectivity may go beyond PUFA content, as potentially illustrated by Linyphiidae in this study, it may be necessary to use a wide array of approaches to better understand the complex interactions between aquatic and terrestrial food webs.

References

Allan, J.D. (2004). Landscapes and Riverscapes: The Influence of Land Use on Stream Ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, vol. 35 (1), pp. 257–284

Allan, J.D. & Castillo, M.M.I. (2007). *Stream ecology: structure and function of running waters*. 2. ed., reprinted. Dordrecht: Springer.

Arce-Funck, J., Bec, A., Perrière, F., Felten, V. & Danger, M. (2015). Aquatic hyphomycetes: a potential source of polyunsaturated fatty acids in detritus-based stream food webs. *Fungal Ecology*, vol. 13, pp. 205–210

Artdatabanken, SLU (2019). *Artfakta spindlar*. Available at: https://artfakta.artdatabanken.se/?&s=spindlar&v=1 [2019-04-21]

Batary, P., Baldi, A., Samu, F., Szuts, T. & Erdos, S. (2008). Are spiders reacting to local or landscape scale effects in Hungarian pastures? *Biological Conservation*, vol. 141 (8), pp. 2062–2070

Baxter, C.V., Fausch, K.D. & Saunders, C.W. (2005). Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones: Prey subsidies link stream and riparian food webs. *Freshwater Biology*, vol. 50 (2), pp. 201–220

Bell, M.V., Henderson, R.J. & Sargent, J.R. (1986). The role of polyunsaturated fatty acids in fish. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, vol. 83 (4), pp. 711–719

Biggs, J., von Fumetti, S. & Kelly-Quinn, M. (2017). The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia*, vol. 793 (1), pp. 3–39

Bjelke, U., Boberg, J., Oliva, J., Tattersdill, K. & McKie, B.G. (2016). Dieback of riparian alder caused by the *Phytophthora alni* complex: projected consequences for stream ecosystems. *Freshwater Biology*, vol. 61 (5), pp. 565–579

Brett, M.T. & Muller-Navarra, D.C. (1997). The role of highly unsaturated fatty acids in aquatic food web processes. *Freshwater Biology*, vol. 38 (3), pp. 483–499

Burdon, F.J. & Harding, J.S. (2007). The linkage between riparian predators and aquatic insects across a stream-resource spectrum. *Freshwater Biology*, vol. 0 (0), pp. 330–346

Burdon, F.J., McIntosh, A.R. & Harding, J.S. (2013). Habitat loss drives threshold response of benthic invertebrate communities to deposited sediment in agricultural streams. *Ecological Applications*, vol. 23 (5), pp. 1036–1047

Burr, G. & Burr, M. (1930). On the nature and role of the fatty acids essential in nutrition. vol. 86, pp. 587–621

Buttigieg, P.L. & Ramette, A. (2014). A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses. *FEMS Microbiology Ecology*, vol. 90 (3), pp. 543–550

Carlson, P. (2014). *Land Use Effects on Ecological Linkages between Small Streams and their Surrounding Terrestrial Habitats*. (Diss.). Swedish University of Agricultural Sciences.

Carlson, P.E., McKie, B.G., Sandin, L. & Johnson, R.K. (2016). Strong land-use effects on the dispersal patterns of adult stream insects: implications for transfers of aquatic subsidies to terrestrial consumers. *Freshwater Biology*, vol. 61 (6), pp. 848–861

Clark, W.R. & Reeder, K.F. (2007). Agricultural Buffers and Wildlife Conservation: A Summary About Linear Practices. *Fish and Wildlife Response to Farmland Conservation Practices*. Bethesda: The wildlife society, pp. 45–55.

Corbacho, C., Sánchez, J.M. & Costillo, E. (2003). Patterns of structural complexity and human disturbance of riparian vegetation in agricultural landscapes of a Mediterranean area. *Agriculture, Ecosystems & Environment*, vol. 95 (2), pp. 495–507

Cunnane, S.C. (2000). The conditional nature of the dietary need for polyunsaturates: a proposal to reclassify 'essential fatty acids' as 'conditionally-indispensable' or 'conditionally-dispensable' fatty acids. *British Journal of Nutrition*, vol. 84 (6), pp. 803–812

Damgaard, C. (2014). Estimating mean plant cover from different types of cover data: a coherent statistical framework. *Ecosphere*, vol. 5 (2), pp. 1–7

Degerman, E. & Bergqvist, B. (2008). Ekologiskt funktionella kantzoner. In: Degerman, E. (ed.) *Ekologisk restaurering av vattendrag*. Stockholm, Göteborg: Naturvårdsverket och Fiskeriverket, pp. 33–52.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.-H., Soto, D., Stiassny, M.L.J. & Sullivan, C.A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, vol. 81 (02), p. 163

Fausch, K.D., Power, M.E. & Murakami, M. (2002). Linkages between stream and forest food webs: Shigeru Nakano's legacy for ecology in Japan. *Trends in Ecology & Evolution*, vol. 17 (9), pp. 429–434

Fritz, K.A., Kirschman, L.J., McCay, S.D., Trushenski, J.T., Warne, R.W. & Whiles, M.R. (2017). Subsidies of essential nutrients from aquatic environments correlate with immune function in terrestrial consumers. *Freshwater Science*, vol. 36 (4), pp. 893–900

Gardener, M. (2014). *Community Ecology: Analytical Methods Using R and Excel*. Exeter: Pelagic Publishing Ltd.

Gladyshev, M.I., Arts, M.T. & Sushchik, N.N. (2009). Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA+DHA) from aquatic to terrestrial ecosystems. In: Kainz, M., Brett, M.T., & Arts, M.T. (eds.) *Lipids in Aquatic Ecosystems*. New York, NY: Springer New York, pp. 179–210.

Gladyshev, M.I., Sushchik, N.N. & Makhutova, O.N. (2013). Production of EPA and DHA in aquatic ecosystems and their transfer to the land. *Prostaglandins & Other Lipid Mediators*, vol. 107, pp. 117–126

Greenwood, M.J. & McIntosh, A.R. (2008). Flooding impacts on responses of a riparian consumer to cross-ecosystem subsidies. *Ecology*, vol. 89 (6), pp. 1489–1496

Grieve, A. & Lau, D.C.P. (2018). Do autochthonous resources enhance trophic transfer of allochthonous organic matter to aquatic consumers, or vice versa? *Ecosphere*, vol. 9 (6), p. e02307

Guo, F., Kainz, M.J., Sheldon, F. & Bunn, S.E. (2016). The importance of high-quality algal food sources in stream food webs - current status and future perspectives. *Freshwater Biology*, vol. 61 (6), pp. 815–831

Guschina, I.A. & Harwood, J.L. (2009). Algal lipids and effect of the environment on their biochemistry. In: Kainz, M., Brett, M.T., & Arts, M.T. (eds.) *Lipids in Aquatic Ecosystems*. New York, NY: Springer New York, pp. 1–24.

Hackston, M. (2018a). *Family Carabidae illustrated key to genus.pdf*. Available at: https://docs.google.com/viewer?a=v&pid=sites&srcid=ZGVmYXVsdGRvbWFpbnxtaWtlc2luc2VjdGtleXN8Z3g6M2VlY2IzMmViNjY3YzNjOQ [2018-10-10]

Hackston, M. (2018b). *Family Staphylinidae key to UK subfamilies.pdf*. Available at: https://docs.google.com/viewer?a=v&pid=sites&srcid=ZGVmYXVsdGRvbWFpbnxtaWtlc2luc2VjdGtleXN8Z3g6NGRhMDU3YWMzYjE2MmNkMg [2018-10-10]

Henderson, P.A. & Southwood, T.R.E. (2016). *Ecological Methods*. Hoboken, United Kingdom: John Wiley & Sons, Incorporated. Available at: http://ebookcentral.proquest.com/lib/uu/detail.action?docID=4356618 [2019-04-23]

Hickey, M.B.C. & Doran, B. (2004). A Review of the Efficiency of Buffer Strips for the Maintenance and Enhancement of Riparian Ecosystems. *Water Quality Research Journal*, vol. 39 (3), pp. 311–317

Hill, W.R., Rinchard, J. & Czesny, S. (2011). Light, nutrients and the fatty acid composition of stream periphyton: Periphyton stoichiometry and fatty acids. *Freshwater Biology*, vol. 56 (9), pp. 1825–1836

Hill, W.R., Ryon, M.G. & Schilling, E.M. (1995). Light Limitation in a Stream Ecosystem: Responses by Primary Producers and Consumers. *Ecology*, vol. 76 (4), pp. 1297–1309

Hixson, S.M. & Arts, M.T. (2016). Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. *Global Change Biology*, vol. 22 (8), pp. 2744–2755

Iverson, S.J. (2009). Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Kainz, M., Brett, M.T., & Arts, M.T. (eds.) *Lipids in Aquatic Ecosystems*. New York, NY: Springer New York, pp. 281–308.

Jocqué, R. & Dippenaar-Schoeman, A.S. (2007). *Spider families of the world*. 2nd ed. Tervuren, Belgium : [Pretoria, South Africa]: Musée royal de l'Afrique centrale ; ARC-PPRI.

Johnson, R.K. & Almlöf, K. (2016). Adapting boreal streams to climate change: effects of riparian vegetation on water temperature and biological assemblages. *Freshwater Science*, vol. 35 (3), pp. 984–997

Jordbruksverket (2019-01-24). *Villkor för miljöersättning för skyddszoner*. [text]. Available at: http://www.jordbruksverket.se/amnesomraden/stod/jordbrukarstod/stodochersattningar/miljoersattningar/skyddszoner/villkor.4.6c64aa881525004b53bdcd1f.html [2019-05-10]

Kato, C., Iwata, T., Nakano, S. & Kishi, D. (2003). Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. *Oikos*, vol. 103 (1), pp. 113–120

Kato, C., Iwata, T. & Wada, E. (2004). Prey use by web-building spiders: stable isotope analyses of trophic flow at a forest-stream ecotone: Stream subsidies to riparian spiders. *Ecological Research*, vol. 19 (6), pp. 633–643

Keeler, B.L., Polasky, S., Brauman, K.A., Johnson, K.A., Finlay, J.C., O'Neill, A., Kovacs, K. & Dalzell, B. (2012). Linking water quality and well-being for improved assessment and valuation of ecosystem services. *Proceedings of the National Academy of Sciences*, vol. 109 (45), pp. 18619–18624

Krell, B., Röder, N., Link, M., Gergs, R., Entling, M.H. & Schäfer, R.B. (2015). Aquatic prey subsidies to riparian spiders in a stream with different land use types. *Limnologica*, vol. 51, pp. 1–7

Kronestedt, T. (2001). *Checklist of Swedish Spiders (Araneae) in Sweden*. Available at: http://www2.nrm.se/en/svenska_spindlar/spindlar.html [2019-04-15]

Kuglerová, L., Ågren, A., Jansson, R. & Laudon, H. (2014). Towards optimizing riparian buffer zones: Ecological and biogeochemical implications for forest management. *Forest Ecology and Management*, vol. 334, pp. 74–84

Laeser, S.R., Baxter, C.V. & Fausch, K.D. (2005). Riparian vegetation loss, stream channelization, and web-weaving spiders in northern Japan. *Ecological Research*, vol. 20 (6), pp. 646–651

Lambeets, K., Hendrickx, F., Vanacker, S., Van Looy, K., Maelfait, J.-P. & Bonte, D. (2008). Assemblage structure and conservation value of spiders and carabid beetles from restored lowland river banks. *Biodiversity and Conservation*, vol. 17 (13), pp. 3133–3148

Larned, S.T. (2010). A prospectus for periphyton: recent and future ecological research. *Journal of the North American Benthological Society*, vol. 29 (1), pp. 182–206

Lau, D.C.P., Goedkoop, W. & Vrede, T. (2013). Cross-ecosystem differences in lipid composition and growth limitation of a benthic generalist consumer. *Limnology and Oceanography*, vol. 58 (4), pp. 1149–1164

Lau, D.C.P., Vrede, T., Pickova, J. & Goedkoop, W. (2012). Fatty acid composition of consumers in boreal lakes - variation across species, space and time: Aquatic consumer fatty acids. *Freshwater Biology*, vol. 57 (1), pp. 24–38

Law, R.J. (2011). A Review of the Function and uses of, and Factors Affecting, Stream Phytobenthos. *Freshwater Reviews*, vol. 4 (2), pp. 135–166

Legendre, P. & Gallagher, E.D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia*, vol. 129 (2), pp. 271–280

Likens, G.E. & Bormann, F.H. (1974). Linkages between Terrestrial and Aquatic Ecosystems. *BioScience*, vol. 24 (8), pp. 447–456

Lindroth, C.H. (1985). *The carabidae (coleoptera) of Fennoscandia and Denmark*. Leiden, Copenhagen: E.J. Brill/Scandinavian Science Press Ltd.

Malmqvist, B. & Rundle, S. (2002). Threats to the running water ecosystems of the world. *Environmental Conservation*, vol. 29 (2), pp. 134–153

Marczak, L.B. & Richardson, J.S. (2008). Growth and development rates in a riparian spider are altered by asynchrony between the timing and amount of a resource subsidy. *Oecologia*, vol. 156 (2), pp. 249–258

Martin-Creuzburg, D., Kowarik, C. & Straile, D. (2017). Cross-ecosystem fluxes: Export of polyunsaturated fatty acids from aquatic to terrestrial ecosystems via emerging insects. *Science of The Total Environment*, vol. 577, pp. 174–182

McKie, B.G., Sandin, L., Carlson, P.E. & Johnson, R.K. (2018). Species traits reveal effects of land use, season and habitat on the potential subsidy of stream invertebrates to terrestrial food webs. *Aquatic Sciences*, vol. 80 (2). DOI: https://doi.org/10.1007/s00027-018-0565-4

Moore, R.D., Spittlehouse, D.L. & Story, A. (2005). Riparian microclimate and stream temperature response to forest harvesting: A review. *Journal of the American Water Resources Association*, vol. 41 (4), pp. 813–834

Moring, J.B. & Stewart, K.W. (1994). Habitat Partitioning by the Wolf Spider (Araneae, Lycosidae) Guild in Streamside and Riparian Vegetation Zones of the Conejos River, Colorado. *The Journal of Arachnology*, vol. 22 (3), pp. 205–217

Moyo, S., Chari, L.D., Villet, M.H. & Richoux, N.B. (2017). Decoupled reciprocal subsidies of biomass and fatty acids in fluxes of invertebrates between a temperate river and the adjacent land. *Aquatic Sciences*, vol. 79 (3), pp. 689–703

Muehlbauer, J.D., Collins, S.F., Doyle, M.W. & Tockner, K. (2014). How wide is a stream? Spatial extent of the potential "stream signature" in terrestrial food webs using meta-analysis. *Ecology*, vol. 95 (1), pp. 44–55

Muller-Navarra, D.C., Brett, M.T., Liston, A.M. & Goldman, C.R. (2000). A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, vol. 403 (6765), pp. 74–77

Naiman, R.J. & Décamps, H. (1997). The ecology of interfaces:Riparian Zones. *Annual Review of Ecology and Systematics*, vol. 28 (1), pp. 621–658

Naiman, R.J., Décamps, H. & McClain, M.E. (2005). *Riparia: Ecology, Conservation, and Management of Streamside Communities*. Burlington, US: Elsevier Science & Technology. Available at: http://ebookcentral.proquest.com/lib/uu/detail.action?docID=286739 [2019-04-22]

Nakano, S., Miyasaka, H. & Kuhara, N. (1999). Terrestrial-aquatic linkages: Riparian arthropod inputs alter cascades in a stream food wen. *Ecology*, vol. 80 (7), pp. 2435–2441

Nakano, S. & Murakami, M. (2001). Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98 (1), pp. 166–170

Nentwig, W., Blick, T., Gloor, D., Hänggi, A. & Kropf, C. (2018). *Version (9) 2018.* Available at: https://araneae.nmbe.ch/key [2018-09-15]

Nyffeler, M. (1999). Prey Selection of Spiders in the Field. *The Journal of Arachnology*, vol. 27 (1), pp. 317–324

Ollero, A. (2007). Channel Adjustments, Floodplain Changes and Riparian Ecosystems of the Middle Ebro River: Assessment and Management. *International Journal of Water Resources Development*, vol. 23 (1), pp. 73–90

Paetzold, A., Schubert, C.J. & Tockner, K. (2005). Aquatic Terrestrial Linkages Along a Braided-River: Riparian Arthropods Feeding on Aquatic Insects. *Ecosystems*, vol. 8 (7), pp. 748–759

Parrish, C.C. (2009). Essential fatty acids in aquatic food webs. In: Kainz, M., Brett, M.T., & Arts, M.T. (eds.) *Lipids in Aquatic Ecosystems*. New York, NY: Springer New York, pp. 309–326.

Perner, J. & Malt, S. (2003). Assessment of changing agricultural land use: response of vegetation, ground-dwelling spiders and beetles to the conversion of arable land into grassland. *Agriculture, Ecosystems & Environment*, vol. 98 (1), pp. 169–181 (Biotic Indicators for Biodiversity and Sustainable Agriculture)

Polis, G.A., Anderson, W.B. & Holt, R.D. (1997). Toward an intergration of landscape and food web ecology: The Dynamics of Spatially Subsidized Food Webs. *Annual Review of Ecology and Systematics*, vol. 28 (1), pp. 289–316

Pollierer, M.M., Scheu, S. & Haubert, D. (2010). Taking it to the next level: Trophic transfer of marker fatty acids from basal resource to predators. *Soil Biology and Biochemistry*, vol. 42 (6), pp. 919–925

Prieto-Benitez, S. & Mendez, M. (2011). Effects of land management on the abundance and richness of spiders (Araneae): A meta-analysis. *Biological Conservation*, vol. 144 (2), pp. 683–691

Quinn, G. & Keough, M. (2002). *Experimental design and data analysis for biologists*. First. Cambridge: Cambride University Press.

R Core Team (2018). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. Available at: https://www.R-project.org/

Renouf, K. & Harding, J.S. (2015). Characterising riparian buffer zones of an agriculturally modified landscape. *New Zealand Journal of Marine and Freshwater Research*, vol. 49 (3), pp. 323–332

Richardson, J.S. & Danehy, R.J. (2007). A Synthesis of the Ecology of Headwater Streams and their Riparian Zones in Temperate Forests. *Forest Science*, vol. 53 (2), pp. 131–147

Richardson, J.S., Naiman, R.J. & Bisson, P.A. (2012). How did fixed-width buffers become standard practice for protecting freshwaters and their riparian areas from forest harvest practices? *Freshwater Science*, vol. 31 (1), pp. 232–238

Richardson, J.S., Zhang, Y. & Marczak, L.B. (2010). Resource subsidies across the land-freshwater interface and responses in recipient communities. *River Research and Applications*, vol. 26 (1), pp. 55–66

Rustan, A.C. & Drevon, C.A. (2005). Fatty Acids: Structures and Properties. In: John Wiley & Sons, Ltd (ed.) *Encyclopedia of Life Sciences*. Chichester: John Wiley & Sons, Ltd,

Sabo, J.L. & Power, M.E. (2002). River-watershed exchange: Effects of riverine subsidies on riparian lizards and their terrestrial prey. *Ecology*, vol. 83 (7), pp. 1860–1869

Sadler, J.P., Bell, D. & Fowles, A. (2004). The hydroecological controls and conservation value of beetles on exposed riverine sediments in England and Wales. *Biological Conservation*, vol. 118 (1), pp. 41–56

Schindler, D.E. & Smits, A.P. (2017). Subsidies of Aquatic Resources in Terrestrial Ecosystems. *Ecosystems*, vol. 20 (1), pp. 78–93

Schultz, R.C., Isenhart, T.M., Simpkins, W.W. & Colletti, J.P. (2004). Riparian forest buffers in agroecosystems – lessons learned from the Bear Creek Watershed, central Iowa, USA. *Agroforestry Systems*, vol. 61, pp. 35–50

Shear, W.A. (2009). Harvestmen: Opiliones—which include daddy-long-legs—are as exotic as they are familiar. *American Scientist*, vol. 97 (6), pp. 468–475

Sibley, P.K., Kreutzweiser, D.P., Naylor, B.J., Richardson, J.S. & Gordon, A.M. (2012). Emulation of natural disturbance (END) for riparian forest management: synthesis and recommendations. *Freshwater Science*, vol. 31 (1), pp. 258–264

SMHI (2019). *Månads-, årstids- och årskartor | SMHI*. Available at: https://www.smhi.se/klimatdata/meteorologi/kartor/monYrTable.php?par=tmpAvv [2019-04-15]

Smiley, P.C., King, K.W. & Fausey, N.R. (2011). Influence of herbaceous riparian buffers on physical habitat, water chemistry, and stream communities within channelized agricultural headwater streams. *Ecological Engineering*, vol. 37 (9), pp. 1314–1323

Smith, B. & Wilson, J.B. (1996). A Consumer's Guide to Evenness Indices. *Oikos*, vol. 76 (1), p. 70

Sparkman, O.D., Penton, Z. & Kitson, F.G. (2011). *Gas Chromatography and Mass Spectrometry: A Practical Guide*. Cambridge: Academic Press.

Stelzer, R.S. & Lamberti, G.A. (2001). Effects of N: P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. *Limnology and Oceanography*, vol. 46 (2), pp. 356–367

Stenroth, K., Polvi, L.E., Fältström, E. & Jonsson, M. (2015). Land-use effects on terrestrial consumers through changed size structure of aquatic insects. *Freshwater Biology*, vol. 60 (1), pp. 136–149

Stutter, M.I., Chardon, W.J. & Kronvang, B. (2012). Riparian buffer strips as a multifunctional management tool in agricultural landscapes: introduction. *Journal of environmental quality*, vol. 41 (2), pp. 297–303

Taipale, S., Strandberg, U., Peltomaa, E., Galloway, A., Ojala, A. & Brett, M. (2013). Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquatic Microbial Ecology*, vol. 71 (2), pp. 165–178

Taipale, S., Vuorio, K., Strandberg, U., Kahilainen, K.K., Järvinen, M., Hiltunen, M., Peltomaa, E. & Kankaala, P. (2016). Lake eutrophication and brownification downgrade availability and transfer of essential fatty acids for human consumption. *Environment International*, vol. 96, pp. 156–166

The Pennsylvania State University (2018). *Lesson 12: Multicollinearity & Other Regression Pitfalls | STAT 501*. Available at: https://newonlinecourses.science.psu.edu/stat501/node/343/ [2019-03-22]

Torres-Ruiz, M., Wehr, J.D. & Perrone, A.A. (2007). Trophic relations in a stream food web: importance of fatty acids for macroinvertebrate consumers. *Journal of the North American Benthological Society*, vol. 26 (3), pp. 509–522

Truchy, A., Angeler, D.G., Sponseller, R.A., Johnson, R.K. & McKie, B.G. (2015). Linking Biodiversity, Ecosystem Functioning and Services, and Ecological Resilience. *Advances in Ecological Research*. pp. 55–96.

Twining, C.W., Brenna, J.T., Hairston, N.G. & Flecker, A.S. (2016). Highly unsaturated fatty acids in nature: what we know and what we need to learn. *Oikos*, vol. 125 (6), pp. 749–760

Uppsala Kommun (2018). *Statistik om Uppsala Kommun 2018*. Uppsala: Analysenheten. Available at: https://www.uppsala.se/contentassets/f09f9e6b994f41408c66064a2da8470b/statistikfolder_2018.pdf [2019-04-18]

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R. & Davies, P.M. (2010). Global threats to human water security and river biodiversity. *Nature*, vol. 467 (7315), pp. 555–561

Wenger, S. (1999). *A review of the scientific literature on riparian buffer width, extent and vegetation*. University of Georgia, Athens, USA.: Institute of Ecology.

Wilder S. M. (2011). Spider Nutrition: An Integrative Perspective. In: Casas J. (ed.). *Advances in Insect Physiology* pp 87-136. Academic Press, Cambridge, USA.

Wu, Z., Zhang, Q., Li, N., Pu, Y., Wang, B. & Zhang, T. (2017). Comparison of critical methods developed for fatty acid analysis: A review: Other Techniques. *Journal of Separation Science*, vol. 40 (1), pp. 288–298

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Popular science summary: Riparian zones- links and webs

If you've ever flown across an agricultural landscape, you may have noticed the blue-green veins that wind through the yellow fields of cultivated crops. The blue is off course streams and rivers. The green is the riparian zone and it does not always look the same. In fact, often this zone is merely a grass border towards the stream, whilst other sections are essentially pockets of forest. The question is does it matter?

Riparian zones that consist of varied vegetation are considered valuable for maintaining biodiversity. They provided habitat, access to water and shelter from the impact of human activities for a number of species. Riparian zones are also links between aquatic and terrestrial ecosystems, regulating flows of nutrients and organisms. Despite this knowledge, riparian forests have often been cleared in agricultural landscapes.

In an agricultural landscape I studied the effect of riparian zones with and without trees on spider populations. Spiders are sensitive to changes in habitat. Spiders are also known to feed on emerging aquatic insects. Some essential fatty acids are produced exclusively by aquatic algae and can be transferred into terrestrial food-webs by emerging aquatic insects. When the insects become prey, these essential fatty acids are consumed and stored by the spiders. As the fatty acids source is known to be aquatic, analysis of the spider's fatty acid content provides information on the degree to which they feed on these aquatic insects. This gives an indication of the strength of the trophic linkage between aquatic and terrestrial systems.

Spiders can be divided into two functional groups: web-building and free-living spiders. I found that in riparian zones with forest fragments web-building spiders dominated. These spiders need structures to build their webs on, such as trees. In the more open riparian zones free-living spiders were instead more common than web-builders. These grass buffers may be open enough for these spiders to hunt efficiently. They are also relatively undisturbed habitats compared to the bordering cultivated crops. The aquatic fatty acid content in spider populations was relatively similar between the forested and the open riparian zones. Instead, the fatty acid content differed between spider taxa. Free-living spiders generally had high levels of aquatic fatty acids which suggests that they are important links between aquatic and terrestrial food-webs. However, despite free-living spiders being more common in open riparian zones, birds and other predators of spiders often prefer forested habitats. Therefore, though not as abundant, free-living spiders in forested riparian zones are probably contributing more to connectivity as a whole. To benefit both diversity and connectivity, the answer may not be sharp borders between grass and forest zones. Instead, blurred borders, allowing freedom of movement may be the solution.

Appendix 1: Methods

Site information

Table 7. *Site names, type of site (buffer characteristics: Forest, unbuffered and buffered) and code used for each site. Note: AGR is short for agriculture, unbuffered sites.*

Name of site	Type of site	Code used
Dalkarlsbo	Forest	DAL_FOR
Granlunda	Forest	GRA_FOR
Lafssjon	Forest	LAF_FOR
Fibyån	Forest	FIB_FOR
Overbo	Forest	OVE_FOR
Örsundaån	Unbuffered	ÖRS_AGR
Örsundaån	Buffered	ÖRS_FBF
Temybacken	Unbuffered	TEM_AGR
Temybacken	Buffered	TEM_FBF
Jumkil	Unbuffered	JUM_AGR
Jumkil	Buffered	JUM_FBF
Lissan	Unbuffered	LIS_AGR
Lissan	Buffered	LIS_FBF
Burunge	Unbuffered	BUR_AGR
Burunge	Buffered	BUR_FBF
Skattmansöån	Unbuffered	SKA_AGR
Skattmansöån	Buffered	SKA_FBF
Hågaån	Unbuffered	HÅG_AGR
Hågaån	Buffered	HÅG_FBF
Närlinge	Unbuffered	NÄR_AGR
Närlinge	Buffered	NÄR_FBF
Långhällar	Unbuffered	LÅN_AGR
Långhällar	Buffered	LÅN_FBF
Åloppbäcken	Unbuffered	ÅLO_AGR
Åloppbäcken	Buffered	ÅLO_FBF

Example of habitat assessment field protocol

Table 8. *Example of the field protocol for habitat assessment. TLB: abbreviation for true left bank.*

Longitudinal transect		**0-10 m**	
Plot		**1**	**2**
Measurement	**Category**	**TLB**	**TLB**
A: Cover	% of canopy cover	75%	
B: % Area	Managed (e.g. mown or grazed) short grasses	-	
	Unmanaged grasses, rushes, and sedges	≤0.1%	
	Herbs	≤5%	
	Mosses and lichens growing on the ground	≤20%	
	Small trees and shrubs DBH < 5 cm	≤70%	
	Rocks and bedrock	≤5%	
	Bare ground	≤1%	
	Plant litter	≤10%	
C: #, x, y, % area	Fallen logs Ø > 10 cm (large wood)	2, (10, 24), (356,720), 7%	
D: Identity and Diameter at breast height	Trees DBH > 5 cm	Populus tremula; 45, 25, 35 Alnus glutinosa; 25, 24, 36, 30, 19, 45	

Example of invertebrate field sampling protocol

Table 9. *Example of the invertebrate field protocol, recording the time, area and number of people sampling and the habitats in which the invertebrates were collected from. TLB: abbreviation for true left bank. Category abbreviations. Web-building spiders WS, Free-living spiders FLS, Ground beetles GB.*

Plot	**1**			**2**			**3**		
Bank	**TLB**			**TLB**			**TLB**		
Time	10 min								
Area	25 m^2								
No. of people	2								
Category	# WS	# FLS	# GB	# WS	# FLS	# GB	# WS	# FLS	# GB
Managed short grasses	0	1	0						
Unmanaged grasses, rushes, and sedges	1	2	0						
Herbs	3	1	0						
Mosses and li-chens	0	0	0						
Small trees and shrubs DBH < 5 cm	15	0	0						
Rocks and bed-rock	0	3	1						
Bare ground	0	3	2						
Plant litter	2	1	1						
Other (e.g., gravel bars)	0	1	3						

Crosslink terrestrial invertebrate protocol.

Note: references to tables and figures in this section are not valid.

CROSSLINK Spring/Summer sampling 2018: Aquatic-terrestrial linkages

1. Sampling strategy

Overall area to sample

Both banks will be targeted over the effective sampling reach (0-30 m) using a maximum of 6 plots (5 x 10 m = 50 m^2). These are the same plots as used for the terrestrial vegetation surveys (see Figure 1). There is no requirement to sample all six plots, but a **minimum of four** should be sampled. There should be an emphasis on getting enough individuals for biomarkers analysis (i.e., PUFAs, and *potentially* SIA). This means you may need to sample all 6 plots to get the requisite number of spiders and beetles, and collecting more than is required of one group (e.g., >20 individual beetles) is a good idea if you need the extra effort to find enough individuals from the other remaining groups. Flexibility may be important for differing stream sizes and sites where habitat may vary greatly between the two banks (e.g., a road on one bank).

Achieving a semi-quantitative estimate of abundance

We want to quantify the "catch per unit effort" (CPUE) which requires recording two components in combination with the number of invertebrates collected:

1) Area sampled (m^2)
2) Time sampled (h)

These will help to derive an estimated CPUE:

$$CPUE = \frac{\text{No. of invertebrates}}{(\text{Area sampled}/\text{Duration of sampling})}$$

Why? CPUE is often used as a relative measure of abundance.
Definitions

1) Area: the area searched should equal the area of the sample plot (i.e., 50 m^2). This will then be summed as the total area of plots sampled (see Table 1).
2) Time: The exact time taken for the search needs to be recorded (see Table 1), as this is a major component of sampling effort. It is

important that the allocation of effort reflect the proportion of different habitat types present. Whilst it is logical to investigate important habitat types thoroughly (e.g., exposed gravel bars for carabid beetles), the effort should be proportional to the distribution of habitat types (see recorded in the Vegetation Assessment).

As a guideline, you should not spend more than 15 minutes per plot (this may also depend on the number of people collecting; e.g., with 2 people the max. would be 7.5 minutes). Another way to interpret this guideline is if you easily find 10 individuals in a plot (e.g., 5 spiders and 5 beetles), then you can move to the next plot. It should be recognized that you can return to a plot to sample again if searching the other plots proves to be unsuccessful. However, you need to record the total time sampled, in addition to the area sampled (i.e., if less that the entire plot).
These guideline should help keep the fieldwork manageable. If you are unable to find any consumers despite following the sampling procedure as described, then this is still an important result.

2. Sampling methods

Sampling techniques to be used for collection

1) Visual searching and collection by hand (preferred method)
2) Sweep-netting

These sampling techniques should be used in the same way across all countries.

- It is preferable if the same sampling methods are applied consistently by partners to certain habitat types (e.g., always sampling "soft vegetation" by sweep netting).
- Also, it is preferable if personnel are delegated to search certain habitat types, and consistently sample these habitats across all plots/sites.

If you do use different methods (e.g., sampling "soft vegetation" with sweep netting and visual searching other habitat types), then you will need to separately record data from the different methods. This information needs to be noted so that we can account for variation in catch efficiencies with the different sampling methods using random effects in our statistical models. Likewise, different personnel should separately record data, and their identities noted so that we can account for individual variation in catch efficiencies using random effects. Finally, the overarching variation across countries will also be accounted for as a random effect in our statistical models.

Specific details on sampling methods

1. Visual searching and collection by hand

Visual searching should be used for all habitat types other than “soft vegetation” (see below). Search visually for spiders and beetles by investigating habitat types in each sampling plot. Look for webs or retreats (curled leaves, silken cases) on vegetation or other structures. Check under loose bark, fallen wood, debris, rocks etc. for free-living spiders and ground beetles. Ground-dwelling beetles often inhabit the interstices of exposed gravel bars. This means it is necessary to search between and underneath stones. Water can be poured over the area to help drive beetles out of their interstitial refugium.
You will need to capture invertebrates using forceps or a small aspirator. It may be possible to guide the spiders or beetles into a larger sample container, before transferring them to a smaller sample container without needing to handle them.

2. Sweep-netting

   This may be a more effective method for sampling “soft vegetation” such as unmanaged grass habitat (e.g., long grasses, sedges, and reeds) and some herbs/forbs. Thus, sweep-netting should be the primary sampling method used on long grasses, sedges, reeds, herbs (i.e., “soft vegetation”). Visual searching should be used for all other habitat types. It needs to be emphasized that these methods should be consistently applied across sites so as to not confound the sampling effort.
   The general “sweep-netting” method involves the use of a heavy insect net (which can be bespoke; e.g., a white pillow-case on a wire frame attached to a wooden pole/handle could be an effective alternative). The net should be vigorously swept through the surface of the vegetation. After repeated sweeps (e.g., a standardized level of effort such as 5 passes) put the contents onto a flat white sheet and remove spiders and beetles. Note: this method doesn´t work well with thorny or wet vegetation.

3. Collection details

Allocation of effort

Efforts should be made to start searching by the shoreline (i.e., near the water’s edge), and systematically moving further and further into the plot by moving parallel to streams edge (e.g., Fig. 2). This means that we increase the chances of finding invertebrates closer to the stream channel, and thus more likely to be using aquatic-derived subsidies.

However, it is important that we do not overly bias our sampling effort to one or two habitat types (e.g., exposed gravel bars). Thus, using the data (i.e., % cover) collected for Vegetation Assessment can help guide the allocation of effort (i.e., so that the sampling effort does not disproportionately focus on one or two habitat types, but more accurately represents the overall habitat diversity within each plot).

Sample Collection

Preferably, individual specimens should be kept in separate containers. However, if there are many individuals collected, then it may be sufficient to keep similar individuals from a habitat type in the same container. The important thing is that key information (e.g., plot number, habitat type) about individuals is available prior to taxa identification and sample preparation for biomarker analyses.

The samples should be **kept on ice** in the field, and **frozen at -20°C** as soon as possible prior to preparation for extraction (PUFAs) and/or encapsulation (SIA). It is important to note that we don´t want these individuals preserved in a solvent (i.e., ethanol).

We **will not** attempt to keep animals alive for gut clearance, but will use other strategies to avoid the potentially confounding influence of stomach contents.

Labelling requirements

There are key requirements for labelling, so that individual invertebrates can be related back to their habitat type and spatial location. Key information for the label include:

1. Site
2. Bank
3. Plot (and bank)
4. Habitat type (i.e., exact details about habitat patch within plot; see Table 2)
5. Exact **lateral** and longitudinal position (relative to stream edge/downstream end of the site). The longitudinal information is optional since this component is implicit in ´Plot´ at a coarse scale.

Minimum requirements for biomarker analyses

Since we aim to collect enough specimens for i) PUFAs and *potentially* ii) SIA, we ideally need a minimum of 20 individuals of each invertebrate group (web-building spiders, free-living spiders, ground beetles) per site. If it is possible to get more (within the time constraints), **then by all means do** (since these groups may be further split by family/sub-family/tribe following taxonomic identification).

It is recommended that each sample weigh between 5 mg (PUFAs) and 4 mg (SIA) dry mass for biomarker analyses. This means that very small spiders and beetles may have insufficient biomass for a sample and will need to be pooled. This should be kept in mind when collecting animals (i.e., you may need an excess of individuals if they need to be pooled together for a viable sample).

Taxonomic identification: done in the lab prior to preparation for biomarkers analysis

We aim to identify spiders to the family level using https://araneae.unibe.ch/key

And carabid beetles to the sub-family/tribe using http://www.coleo-net.de/coleo/texte/carabidae.htm

Preparation for biomarkers analysis

Following identification, and where possible, we should try and use the cephalothoraces and legs of individual spiders and the head and legs of beetles, so as to avoid potential contamination with stomach material. IF there is insufficient material for biomarkers analysis with an individual, then it may be necessary to pool material (e.g., cephalothoraces and legs of three spiders) to get enough for a viable sample. It is recommended that this is done with common sense (e.g., you can combine specimens from different plots if from the same family or tribe). If there is insufficient material for these taxonomic groups, then it is possible to aggregate at a coarser level (e.g., web and free-living spiders, ground beetles). It is desirable that we have a data point for every site.

If there is not enough material with just the cephalothoraces, heads, and legs, then we could use all of the spider (a liquid-feeder), but degut the carabid beetles (this is fairly easily achieved with two pairs of hard forceps). This should be seen as a step of last resort, because we would like to have a data point for every site, but do not want samples contaminated with recent feeding.

Parts of animals or whole animals should be freeze-dried before homogenization (e.g., grinding into a fine powder) and further preparations for biomarkers analysis (Note: protocol pending).

4. Additional information to collect at the time of sampling

General information to record that might influence sampling effectiveness/catch efficiency:

1. Date
2. Time of day
3. Air temperature
4. Wind (e.g., light breeze, strong breeze, etc.)
5. Overall weather conditions (e.g., sunny, overcast)
6. Water levels (e.g., low, normal, high) – this may particularly affect the amount of exposed gravel bar habitat available (although summer sampling means that flows should be low-normal).
7. Light levels (optional)

Riparian invertebrates identified

Table 10. *To the left: Spider (Araneae) families identified, abbreviations used and classified into WS (web-building) or FLS (free-living) spiders. To the right: Ground beetles (Carabidae) identified, and abbreviations used.*

Spider family	Abbreviation	Group	**Ground beetle** Genus	Abbreviation
Anyphaenidae	Anyp	FLS	Elaphrus	Elap
Clubioniade	Club	FLS	Cychrus	Cych
Eutichuridae	Euti	FLS	Agonum	Agon
Liocranidae	Lioc	FLS	Oxypselaphus	Oxyp
Lycosidae	Lyco	FLS	Trechus	Trec
Philodromidae	Phil	FLS	Pterostichus	Pter
Pisauridae	Pisa	FLS	Stomis	Stom
Salticidae	Salt	FLS	Leistus	Leis
Sparassidae	Spar	FLS	Nebria	Nebr
Thomisidae	Thom	FLS	Bembidion	Bemb
Araneidae	Aran	WS	Loricera	Lori
Linyphidae	Liny	WS	Carabus	Cara
Tetragnathidae	Tetr	WS	Staphylininae**	Stap
Theridiidae	Ther	WS	Aleocharinae**	Aleo
Opiliones*	OP	OP		

* Opiliones is a separate order under the class arachnida.
**Staphylinidae is a separate family under the order coleoptera

R statistical packages and functions

Table 11. *R statistical packages used, some functions and references.*

R-package	Some functions used	Reference
agricolae	HSD.test (Tukey)	Felipe de Mendiburu (2019). agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-0. https://CRAN.R-project.org/package=dplyr
BiodiveristyR	diversityresult	Kindt, R. & Coe, R. (2005) Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi. ISBN 92-9059-179-X.
Car	ANOVA	John Fox and Sanford Weisberg (2011). An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion
dplyr	mutate, arrange	Hadley Wickham, Romain François, Lionel Henry and Kirill Müller (2019). dplyr: A Grammar of Data Manipulation. R package version 0.8.0.1. https://CRAN.R-project.org/package=dplyr
faraway	VIF	Julian Faraway (2016). faraway: Functions and Datasets for Books by Julian Faraway. R package version 1.0.7. https://CRAN.R-project.org/package=faraway
ggplot2	geom_bar, geom_boxplot	H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.
ggpubr	ggarrange	Alboukadel Kassambara (2018). ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.2. https://CRAN.R-project.org/package=ggpubr
gridExtra	gridarrange	Baptiste Auguie (2017). gridExtra: Miscellaneous Functions for "Grid" Graphics. R package version 2.3. https://CRAN.R-project.org/package=gridExtra
lmerTest	lmer (linear mixed effects model)	Kuznetsova A, Brockhoff PB, Christensen RHB (2017). “lmerTest Package: Tests in Linear Mixed Effects Models.” _Journal of Statistical Software_, *82*(13), 1-26. doi: 10.18637/jss.v082.i13
lsmeans/mmeans	lsmeans (Tukey)	Russell V. Lenth (2016). Least-Squares Means: The R Package lsmeans. Journal of Statistical Software, 69(1), 1-33. doi:10.18637/jss.v069.i01
Magrittr	pipes	Stefan Milton Bache and Hadley Wickham (2014). magrittr: A Forward-Pipe Operator for R. R package version 1.5. https://CRAN.R-project.org/package=magrittr
openxlsx	write.xlsx	Alexander Walker (2018). openxlsx: Read, Write and Edit XLSX Files. R package version 4.1.0. https://CRAN.R-project.org/package=openxlsx
pairwiseAdonis	pairwise.adonis2	Pedro Martinez Arbizu (2017). pairwiseAdonis: Pairwise Multilevel Comparison usingAdonis. R package version 0.0.1.

R-package	Some functions used	Reference
Plyr	ddply	Hadley Wickham, Romain François, Lionel Henry and Kirill Müller (2019). dplyr: A Grammar of Data Manipulation. R package version 0.8.0.1.https://CRAN.R-project.org/package=dplyr
reshape2	melt	Hadley Wickham (2007). Reshaping Data with the reshape Package. Journal of Statistical Software, 21(12), 1-20. URL http://www.jstatsoft.org/v21/i12/.
stats	lm (linear model)	R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
tidyr	spread	Hadley Wickham and Lionel Henry (2018). tidyr: Easily Tidy Data with 'spread()' and 'gather()' Functions. R package version 0.8.2. https://CRAN.R-project.org/package=tidyr
vegan	betadisper, envfit, metaMDS, adonis, permutest, rda, decostand, ordiR2step	Jari Oksanen, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry, H. Stevens, Eduard Szoecs and Helene Wagner (2019). vegan: Community Ecology Package. R package version 2.5-4. https://CRAN.R-project.org/package=vegan

Appendix 2: Results

Tree data per site type

Table 12. *Mean, standard deviation and standard error of the number of trees per tree class and per site type.*

Site	Property	No. of sites	Mean	SD	SE
Unbuffered	Decidious	9	33.22	23.64	7.88
Buffered	Decidious	10	43.70	16.75	5.30
Forest	Decidious	5	40.20	33.65	15.05
Unbuffered	Conifer	3	3.00	2.00	1.15
Buffered	Conifer	5	6.80	4.32	1.93
Forest	Conifer	5	22.40	10.81	4.83
Unbuffered	Dead	7	2.57	1.40	0.53
Buffered	Dead	10	3.20	1.55	0.49
Forest	Dead	4	6.75	2.22	1.11

Habitat assessment result

Table 13. *Habitat assessment: mean percentage coverage (%), standard deviation and standard error of canopy cover and different habitats found per site type*

Site	Property	No. of sites	Mean	SD	SE
Unbuffered	Canopy cover	10	39.50	23.82	7.53
Buffered	Canopy cover	10	75.45	5.04	1.59
Forest	Canopy cover	5	72.06	6.04	2.70
Unbuffered	Bare ground	10	5.81	4.45	1.41
Buffered	Bare ground	10	4.13	3.61	1.14
Forest	Bare ground	3	2.36	2.31	1.33
Unbuffered	Herb	10	34.52	23.91	7.56
Buffered	Herb	10	31.32	19.74	6.24
Forest	Herb	5	15.83	10.53	4.71
Unbuffered	Managed grass	6	25.68	16.86	6.88
Buffered	Managed grass	1	5.00	0.00	0.00
Unbuffered	Moss & lichen	9	4.08	4.88	1.63
Buffered	Moss & lichen	10	17.70	12.12	3.83
Forest	Moss & lichen	5	45.33	14.79	6.61
Unbuffered	Plant litter	10	11.17	12.32	3.90
Buffered	Plant litter	10	47.00	16.64	5.26
Forest	Plant litter	5	42.67	6.93	3.10
Unbuffered	Rock	10	6.39	5.74	1.81
Buffered	Rock	10	16.80	9.55	3.02
Forest	Rock	5	25.80	12.89	5.77
Unbuffered	Tree & shrubs	10	19.01	14.12	4.46
Buffered	Tree & shrubs	10	48.00	14.74	4.66
Forest	Tree & shrubs	5	36.33	20.60	9.21
Unbuffered	Unmanaged grass	10	32.00	20.04	6.34
Buffered	Unmanaged grass	10	4.90	6.94	2.19
Forest	Unmanaged grass	5	11.80	10.15	4.54

Spider diversity and abundance

Table 14. *Number of spider individuals, number of spider families, Shannon diversity, Pielou's evenness and Berger-Parker dominance for each site. In total 1229 spiders were collected.*

Site name	Site type	No. of individuals	No. of taxa	Diversity	Evenness	Dominance
ALO_AGR	AGR	29	5	1.45	0.90	0.34
BUR_AGR	AGR	56	6	1.28	0.71	0.59
HAG_AGR	AGR	20	6	1.62	0.91	0.25
JUM_AGR	AGR	42	5	0.60	0.37	0.86
LAN_AGR	AGR	42	7	1.58	0.81	0.43
LIS_AGR	AGR	51	7	1.49	0.76	0.53
NAR_AGR	AGR	53	8	1.68	0.81	0.40
ORS_AGR	AGR	68	5	1.03	0.64	0.65
SKA_AGR	AGR	40	6	1.13	0.63	0.65
TEM_AGR	AGR	53	12	2.01	0.81	0.36
ALO_FBF	FBF	25	7	1.56	0.80	0.44
BUR_FBF	FBF	51	5	1.07	0.67	0.55
HAG_FBF	FBF	22	5	0.92	0.57	0.73
JUM_FBF	FBF	23	6	1.06	0.59	0.70
LAN_FBF	FBF	71	7	1.04	0.54	0.72
LIS_FBF	FBF	59	7	0.74	0.38	0.83
NAR_FBF	FBF	61	7	0.96	0.49	0.75
ORS_FBF	FBF	102	5	1.06	0.66	0.60
SKA_FBF	FBF	50	5	0.72	0.45	0.80
TEM_FBF	FBF	91	6	0.60	0.34	0.84
DAL_FOR	FOR	29	5	1.17	0.73	0.52
FIB_FOR	FOR	38	8	1.46	0.70	0.50
GRA_FOR	FOR	36	8	1.57	0.75	0.50
LAF_FOR	FOR	77	6	1.07	0.60	0.62
OVE_FOR	FOR	40	7	1.60	0.82	0.40
TOTAL	-	1229	15	-	-	-

Spider family abundances per site

Table 15. *Spider family abundances per site and total collected. Abbreviations: Ay (Anyphaenidae), Ar(Araneidae), Cl(Clubioniade), Eu(Eutichuridae), Li(Linyphidae), Lo(Liocranidae), Ly(Lycosidae),OP(Opiliones), Ph(Philodromidae), Pi(Pisauridae), Sa(Salticidae), Sp(Sparassidae), Tr(Tetragnathidae), Th(Theridiidae), To(Thomisidae)*

Site name	Site type	Ay	Ar	Cl	Eu	Li	Lo	Ly	OP	Ph	Pi	Sa	Sp	Tr	Th	To
ALO_AGR	AGR	0	0	0	0	10	0	9	5	0	0	0	0	2	3	0
BUR_AGR	AGR	0	0	2	0	7	0	33	8	0	2	0	0	4	0	0
HAG_AGR	AGR	0	0	1	0	0	0	3	5	0	1	0	0	5	5	0
JUM_AGR	AGR	0	0	0	0	2	0	36	0	0	1	0	0	1	2	0
LAN_AGR	AGR	0	1	1	0	18	0	3	5	0	0	0	0	6	8	0
LIS_AGR	AGR	0	0	0	0	5	0	27	0	2	7	0	0	5	2	3
NAR_AGR	AGR	0	3	0	0	21	0	6	1	2	12	0	0	6	2	0
ORS_AGR	AGR	0	0	0	0	44	0	7	3	0	0	0	0	13	1	0
SKA_AGR	AGR	0	0	1	0	4	0	26	6	0	0	0	0	2	1	0
TEM_AGR	AGR	0	2	0	1	19	0	4	2	1	10	2	5	1	4	2
ALO_FBF	FBF	0	0	1	0	11	1	4	5	0	0	0	0	2	1	0
BUR_FBF	FBF	0	1	0	0	28	0	16	1	0	0	0	0	5	0	0
HAG_FBF	FBF	0	1	0	0	16	0	0	3	0	0	0	0	1	1	0
JUM_FBF	FBF	0	1	0	0	16	0	1	1	0	0	0	0	1	3	0
LAN_FBF	FBF	0	0	0	0	51	3	1	4	0	0	0	0	7	4	1
LIS_FBF	FBF	0	2	0	0	49	0	2	1	1	0	0	0	3	1	0
NAR_FBF	FBF	0	2	1	0	46	0	6	2	0	0	0	0	2	2	0
ORS_FBF	FBF	1	0	0	0	61	0	14	3	0	0	0	0	23	0	0
SKA_FBF	FBF	0	0	0	0	40	0	2	1	0	1	0	0	6	0	0
TEM_FBF	FBF	0	1	0	0	76	0	0	1	0	1	0	0	11	1	0
DAL_FOR	FOR	0	0	0	0	15	3	1	9	0	0	0	0	1	0	0
FIB_FOR	FOR	0	0	0	0	19	1	4	1	1	0	0	0	9	2	1
GRA_FOR	FOR	0	0	0	0	18	1	4	5	0	1	1	0	4	0	2
LAF_FOR	FOR	0	0	0	0	17	2	48	1	0	0	0	0	8	1	0
OVE_FOR	FOR	0	2	1	0	16	2	10	4	0	0	0	0	5	0	0
TOTAL		1	16	8	1	609	13	267	77	7	36	3	5	133	44	9

Fatty acid content per site

Table 16. *The mean of each fatty acid (FA) group in mg per g dry mass per site. SAFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, BAFA: Bacterial fatty acids, PUFA: Polyunsaturated fatty acids*

Site name	Site type	SAFA	MUFA	BAFA	PUFA	Total FA	PUFA % of FA
ALO_AGR	AGR	55.152	40.218	2.454	23.033	120.857	0.176
ALO_FBF	FBF	60.064	51.782	3.570	24.398	139.814	0.176
BUR_AGR	AGR	52.834	37.377	2.852	19.291	112.355	0.169
BUR_FBF	FBF	80.522	54.228	3.600	30.049	168.398	0.178
DAL_FOR	FOR	41.362	58.788	1.435	23.123	124.708	0.229
FIB_FOR	FOR	42.970	49.364	2.255	24.371	118.961	0.203
GRA_FOR	FOR	61.760	74.946	2.848	43.006	182.560	0.238
HAG_AGR	AGR	42.660	33.276	1.764	10.560	88.260	0.116
HAG_FBF	FBF	49.773	30.930	1.187	21.747	103.637	0.214
JUM_AGR	AGR	49.065	41.215	2.866	20.857	114.003	0.187
JUM_FBF	FBF	55.590	44.533	3.259	16.204	119.586	0.135
LAF_FOR	FOR	45.093	59.287	2.150	32.084	138.614	0.224
LAN_AGR	AGR	51.112	44.886	1.792	21.215	119.004	0.184
LAN_FBF	FBF	52.252	37.704	1.591	24.561	116.109	0.215
LIS_AGR	AGR	38.050	29.326	1.455	11.373	80.203	0.153
LIS_FBF	FBF	54.148	48.506	1.580	21.842	126.075	0.166
NAR_AGR	AGR	50.985	40.197	1.892	19.870	112.943	0.173
NAR_FBF	FBF	45.782	34.918	4.508	19.279	104.487	0.185
ORS_AGR	AGR	69.254	46.246	2.720	28.687	146.907	0.194
ORS_FBF	FBF	57.302	37.599	2.753	22.636	120.290	0.189
OVE_FOR	FOR	42.207	55.178	2.016	30.890	130.291	0.232
SKA_AGR	AGR	49.601	39.223	1.801	21.399	112.024	0.192
SKA_FBF	FBF	68.730	47.352	3.144	29.460	148.685	0.201
TEM_AGR	AGR	48.362	36.227	2.160	21.199	107.948	0.203
TEM_FBF	FBF	67.488	49.282	2.566	27.722	147.057	0.189

Specific PUFAs per site

Table 17. *The mean content of specific PUFAs in mg per g dry mass per site.*

Site name	Site type	ALA 18:3ω3	LIN 18:2ω6c	ARA 20:4ω6	EPA 20:5ω3	20:3ω3	20:2ω6	DHA 22:6ω3	22:2ω6
ALO_AGR	AGR	0.51	18.07	1.16	1.83	0.06	1.14	0.16	0.10
ALO_FBF	FBF	0.51	19.48	1.26	1.91	0.06	0.85	0.22	0.11
BUR_AGR	AGR	0.38	12.01	1.51	3.49	0.07	0.95	0.81	0.08
BUR_FBF	FBF	0.56	22.85	1.43	3.53	0.08	1.18	0.28	0.13
DAL_FOR	FOR	1.40	17.66	0.90	1.55	0.57	0.89	0.04	0.11
FIB_FOR	FOR	1.14	17.91	1.14	2.25	0.34	1.28	0.17	0.15
GRA_FOR	FOR	2.48	31.88	1.93	3.36	0.99	1.91	0.27	0.18
HAG_AGR	AGR	0.27	7.85	0.57	1.23	0.03	0.46	0.10	0.05
HAG_FBF	FBF	0.64	18.27	0.64	1.14	0.05	0.90	0.04	0.07
JUM_AGR	AGR	0.42	13.93	2.00	3.21	0.07	0.99	0.13	0.11
JUM_FBF	FBF	0.70	12.18	0.74	1.14	0.06	1.11	0.14	0.15
LAF_FOR	FOR	2.01	23.37	1.48	2.79	0.61	1.61	0.06	0.16
LAN_AGR	AGR	0.46	16.12	1.60	2.19	0.07	0.63	0.08	0.07
LAN_FBF	FBF	1.02	19.25	1.11	1.96	0.06	0.97	0.13	0.06
LIS_AGR	AGR	0.25	7.61	0.86	1.84	0.04	0.47	0.23	0.06
LIS_FBF	FBF	0.52	18.57	0.89	1.26	0.04	0.46	0.05	0.06
NAR_AGR	AGR	0.34	14.68	1.52	2.31	0.05	0.77	0.10	0.09
NAR_FBF	FBF	0.41	14.35	1.34	2.12	0.06	0.82	0.09	0.10
ORS_AGR	AGR	0.67	22.36	1.51	2.20	0.08	1.54	0.19	0.13
ORS_FBF	FBF	0.63	16.13	1.59	2.67	0.07	1.21	0.22	0.10
OVE_FOR	FOR	1.84	23.27	1.30	2.47	0.63	1.23	0.05	0.10
SKA_AGR	AGR	0.36	17.00	1.07	1.88	0.04	0.53	0.47	0.05
SKA_FBF	FBF	0.77	21.03	2.10	3.21	0.10	1.93	0.14	0.16
TEM_AGR	AGR	0.37	16.32	1.44	1.88	0.06	0.89	0.16	0.08
TEM_FBF	FBF	0.62	22.80	1.34	1.61	0.06	1.11	0.08	0.09

Specific PUFAS per taxa and site type

Table 18. *Mean of specific PUFAs in mg per g dry mass per site type and spider family. Liny (Linyphiidae), Lyco (Lycosidae), OP (Opiliones), Pisa (Pisauridae), Tetr (Tetragnathidae).*

Type	Taxa	ALA 18:3ω3	LIN 18:2ω6	ARA 20:4ω6	EPA 20:5ω3	20:3ω3	20:2ω6	DHA 22:6ω3	22:2ω6
Unbuffered	Liny	0.52	25.23	1.23	1.73	0.07	0.69	0.07	0.10
Unbuffered	Lyco	0.44	14.35	1.95	3.03	0.08	1.17	0.26	0.11
Unbuffered	OP	0.38	12.86	0.58	0.93	0.04	0.83	0.07	0.06
Unbuffered	Pisa	0.29	11.99	1.40	2.72	0.04	0.68	0.42	0.05
Unbuffered	Tetr	0.40	10.90	0.80	1.48	0.02	0.59	0.31	0.08
Buffered	Liny	0.80	24.82	1.01	1.67	0.07	0.84	0.06	0.11
Buffered	Lyco	0.62	12.21	2.32	3.55	0.10	1.64	0.42	0.20
Buffered	OP	0.52	12.70	0.63	1.08	0.04	0.80	0.05	0.05
Buffered	Pisa	0.58	19.79	1.60	2.22	0.06	1.64	0.12	0.08
Buffered	Tetr	0.56	19.03	1.36	2.52	0.05	1.23	0.12	0.07
Forest	Liny	1.63	25.82	1.26	2.42	0.54	1.30	0.09	0.19
Forest	Lyco	3.16	24.17	2.14	3.53	1.17	1.86	0.24	0.16
Forest	OP	0.96	17.51	0.63	1.25	0.43	1.01	0.04	0.07
Forest	Pisa	1.89	45.66	2.10	4.47	0.55	2.59	0.13	0.19
Forest	Tetr	0.93	21.00	1.11	2.26	0.20	1.15	0.09	0.14