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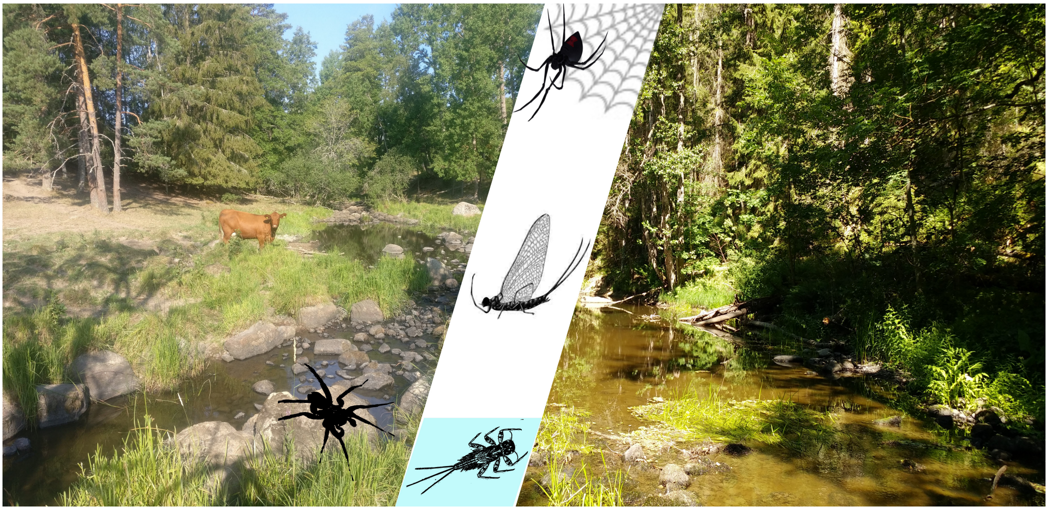
Forested buffers in agricultural landscapes

Mitigation effects on stream–riparian meta-ecosystems

Jasmina Sargac



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Cover: Where water meets the land is where the magic happens
(photo: Jasmina Sargac)

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Abstract

Stream–riparian meta-ecosystems are strongly connected through exchanges of energy, material and organisms. Land use can disrupt ecological connectivity by affecting community composition directly and/or indirectly by altering the instream and riparian habitats that support biological structure and function. Although forested riparian buffers are increasingly used as a management intervention, our understanding of their effects on the functioning of stream–riparian meta-ecosystems is limited. This study assessed patterns in the longitudinal and lateral profiles of streams in modified landscapes across Europe and Sweden using a paired-reach approach, with upstream unbuffered reaches lacking woody riparian vegetation and with downstream reaches having well-developed forested buffers. The presence of buffers was positively associated with stream ecological status as well as important attributes, which included instream shading and the provision of suitable habitats for instream and riparian communities, thus supporting more aquatic insects (especially EPT taxa). Emergence of aquatic insects is particularly important because they mediate reciprocal flows of subsidies into terrestrial systems. Results of fatty acid analysis and prey DNA from spiders further supported the importance of buffers in providing more aquatic-derived quality food (i.e. essential fatty acids) for riparian spiders. Findings presented in this thesis show that buffers contribute to the strengthening of cross-ecosystem connectivity and have the potential to affect a wide range of consumers in modified landscapes.

Keywords: riparian buffers, meta-ecosystems, trophic linkages, polyunsaturated fatty acids (PUFA), molecular gut analysis

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Skogsbevuxna buffertar i jordbrukslandskap - Begränsande effekter på meta-ekosystem i strömmar

Sammanfattning

Meta-ekosystem längs vattendragens stränder är starkt sammankopplade genom utbyte av energi, material och organismer. Markanvändning kan störa sådana ekologiska kopplingar genom att påverka samhällssammansättningen direkt och/eller indirekt genom att förändra de miljöer i vattendragen och längs stränderna som stöder biologisk struktur och funktion. Även om skogbevuxna strandbuffertar i allt högre grad används som en förvaltningsåtgärd, är vår förståelse för deras effekter på funktionen hos meta-ekosystems i vattendrag begränsade. Den här studien analyserade mönster i längsgående och laterala profiler i vattendrag i modifierade landskap i Europa och Sverige genom att använda parade strandsträckor, med uppströms obuffrade sträckor som saknar träig strandvegetation och nedströms sträckor med välutvecklade skogklädda buffertar. Förekomsten av buffertar var positivt associerad med vattendragets ekologiska status och med viktiga attribut, till exempel skuggning i vattendragen och tillhandahållande av lämpliga livsmiljöer i vattendragen och längs stränderna, vilket stöder fler vattenlevande insekter (särskilt EPT-taxa). Kläckningen av vattenlevande insekter som kläcker från akvatiska larver till terrestra adulter är särskilt viktig eftersom de möjliggör ömsesidiga flöden av energi och material in i terrestra system. Resultaten från fettsyraanalys och DNA-analys på spindlars bytesdjur förstärkte ytterligare betydelsen av buffertar för att förse strandspindlar med mer högkvalitativ föda från vattendragen (dvs essentiella fettsyror). Resultaten som presenteras i den här avhandlingen visar att buffertar bidrar till att stärka sammankopplingen mellan ekosystem och har potential att påverka ett brett spektrum av konsumenter i modifierade landskap.

Nyckelord: strandbuffertar, meta-ekosystem, trofiska kopplingar, fleromättade fettsyror (PUFA), molekylär tarmanalys

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Šumski obalni pojasevi u poljoprivrednim područjima - mitigacijski utjecaj na meta-ekosustave potoka

Sažetak

Potoci i njihove obale čine meta-ekosustave koji su snažno povezani preko izmjene energije, materijala i organizama. Korištenje zemljišta ('land use') može poremetiti ekološku povezanost i utjecati na sastav zajednica, izravno i/ili neizravno, promjenom vodenih ili obalnih staništa koja podržavaju biološku strukturu i funkciju. Iako se šumski obalni pojasevi ('buferi') sve više koriste kao praksa u upravljanju vodama, naše razumijevanje njihovih učinaka na funkcioniranje vodeno-obalnih meta-ekosustava je ograničeno. Ova studija procijenila je stanje uzdužnih i bočnih profila potoka u modificiranim krajolicima diljem Europe i Švedske. Pristup je uključivao dvije lokacije na svakom potoku, uzvodna degradirana lokacija bez drvenaste obalne vegetacije, i nizvodna lokacija s dobro razvijenim šumskim pojasom. Prisutnost šumskog pojasa bila je pozitivno povezana s ekološkim statusom potoka, kao i važnim ekološkim atributima, koji su uključivali zasjenjenje u koritu i osiguravanje prikladnih staništa za vodene i obalne zajednice, čime se osiguravaju povoljna staništa za razne vodene kukce (osobito EPT vrste). Izlijetanje vodenih kukaca osobito je važno jer sudjeluju u recipročnim tokovima tvari i energije natrag u kopnene sustave. Rezultati analize masnih kiselina i DNK konzumiranog plijena u obalnim paucima dodatno su poduprli važnost šumskih pojaseva u pružanju kvalitetnijeg izvora hrane (esencijalnih masnih kiselina) dobivene iz vodenih sustava za obalne organizme. Rezultati prikazani u ovom doktorskom radu pokazuju da obalni šumski pojasevi pridonose jačanju povezanosti među ekosustavima i imaju potencijal utjecati na širok raspon organizama u modificiranim krajolicima.

Ključne riječi: obalni pojasevi, meta-ekosustavi, hranidbene mreže, polinezasićene masne kiseline (PUFA), molekularna analiza

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Dedication

To one special turkey and all the wonderful people that enrich my life.

> *"No man ever steps in the same river twice,*
> *for it's not the same river and he's not the same man."*
>
> *Heraclitus*

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Burdon, F.J., Ramberg, E., **Sargac, J.,** Forio, M.A.E., de Saeyer, N., Mutinova, P.T., Moe, T.F., Pavelescu, M.O., Dinu, V., Cazacu, C., Witing, F., Kupilas, B., Grandin, U., Volk, M., Rîşnoveanu, G., Goethals, P., Friberg, N., Johnson, R.K., McKie, B.G. (2020). Assessing the Benefits of Forested Riparian Zones: A Qualitative Index of Riparian Integrity Is Positively Associated with Ecological Status in European Streams. *Water*, 12 (1178).

II. **Sargac, J.**, Johnson, R.K., Burdon, F.J., Truchy, A., Rîşnoveanu, G., Goethals, P., McKie, B.G. (2021). Forested Riparian Buffers Change the Taxonomic and Functional Composition of Stream Invertebrate Communities in Agricultural Catchments. *Water*, 13 (1028).

III. **Sargac, J.**, Johnson, R.K., Burdon, F.J., Taylor, A.R., McKie, B.G. Effects of forested riparian buffers and seasonal variations on stream–riparian meta-ecosystems. (manuscript)

IV. **Sargac, J.**, Johnson, R.K., Burdon, F.J., Hambäck, P., Brett, M.T., Taylor, A.R., Grudzinska-Sterno M., McKie, B.G. Assessing the role of forested buffers on trophic linkages between aquatic and terrestrial ecosystems using fatty acids and prey DNA. (manuscript)

V. Burdon, F.J., **Sargac, J.**, Ramberg, E., Forio, M.A.E., Witing, F., Kupilas, B., Lau, D.C.P., Volk, M., Rîşnoveanu, G., Goethals, P., Friberg, N., Johnson, R.K., McKie, B.G. Novel biomarkers reveal landscape influences on linkages between aquatic and terrestrial food webs. (manuscript)

Papers I-II are reproduced with the permission of the publishers.

The contribution of Jasmina Sargac to the papers included in this thesis was as follows:

I. JS contributed to idea development and writing. Carried out field work for Swedish sites and part of the laboratory work.

II. JS planned the study together with the co-authors. Carried out field work, sampling and part of the laboratory work. Had the main responsibility for data handling, data analyses, interpretation and writing.

III. JS planned the study design with co-authors. Had the main responsibility for planning and conducting field study and laboratory work, data handling, data analyses, interpretation and writing.

IV. JS planned the study design with co-authors. Had the main responsibility for planning and conducting field study and laboratory work, data handling, data analyses, interpretation and writing.

V. JS contributed to idea development and writing. Carried out field work for Swedish sites and part of the laboratory work.

Additional papers

In addition to the papers included in the thesis, the author has contributed to the following peer-reviewed publications:

Ramberg, E., Burdon, F.J., **Sargac, J.**, Kupilas, B., Rîşnoveanu, G., Lau, D.C.P., Johnson, R.K., McKie, B.G. (2020). The Structure of Riparian Vegetation in Agricultural Landscapes Influences Spider Communities and Aquatic-Terrestrial Linkages. *Water*, 12, 2855.

Hamback, P., **Sargac, J.**, Grudzinska-Sterno, M. (2022). Comment on Bohmann et al. Strategies for sample labelling and library preparation in DNA metabarcoding studies. *Authorea Preprints*

Abbreviations

ASPT	Average Score Per Taxon
BMWP	Biological Monitoring Working Party
CPOM	Coarse particulate organic matter
CWM	Community weighted mean
EPA	Eicosapentaenoic acid
EPT	Ephemeroptera, Plecoptera, Trichoptera
ESR	Effective sampling reach
FA	Fatty acid
FPOM	Fine particulate organic matter
HAR	Habitat assessment reach
PUFA	Polyunsaturated fatty acid
RCI	Riparian Condition Index
OTU	Operational Taxonomic Unit

1. Introduction

Throughout history people have been closely associated with freshwater ecosystems. Human settlements are often built in close proximity of freshwater waterbodies (e.g. lakes and rivers), which provide many ecosystem services (Millennium Ecosystem Assessment (MEA), 2005, Hanna et al., 2017). As the human population grows so does the need for space, food and drinking water. Combined, the effects of pollution and climate change result in freshwaters being amongst the most threatened ecosystems globally. The ecological status of European freshwaters has declined by 50% in the last few decades, and in 2015 only about half of the rivers and lakes had achieved good ecological status, as defined in Water Framework Directive (WFD) (EEA, 2015, IPBES, 2018, Gozlan et al., 2019). Compared to large rivers, streams are more directly coupled with the surrounding area (Vanotte et al., 1980, Allan and Castillo, 2007), which makes them more sensitive to anthropogenic pressures (Tolkkinen et al., 2021). **Streams** are open systems that constantly change through space and time, and exchange their energy, material and organisms with the landscape they flow through (Vanotte, 1980, Cushing et al., 1995). Headwater streams in particular can be quite abundant in the landscape, representing up to 80% of the total length in a stream network (Bishop et al., 2008, Leopold et al., 1994). Streams play a large role in the longitudinal connectivity and delivery of subsidies for downstream ecosystems, which underpins their importance for the whole catchment.

Interactions within a stream are present not only in longitudinal direction but also laterally, between the stream channel and its adjacent **riparian zone** (Ward, 1989). This transition zone extends from the edge of water to the edge of terrestrial components of the landscape, forming the boundary between the two systems (Gregory, 1991, Naiman et al., 2005).

One of the primary characteristics that defines any riparian zone is the composition, abundance, and diversity of vegetation, while spatial position enables the connection between aquatic and terrestrial habitats in the form of cross-habitat movement of energy, nutrients and species. Complexity of biophysical processes in riparian zones creates diverse and unique habitats for many terrestrial and aquatic organisms (Hauer et al., 2016), that help sustain regional flora and fauna (Douglas et al., 2009, McCracken et al., 2012, Cole et al., 2015). Corridors of riparian habitats also connect and facilitate the dispersal of populations that could otherwise become isolated (Gregory et al., 1991). Riparian habitats generally support a greater variety of species compared to the surrounding areas and are recognized as hotspots of biodiversity (Naiman et al., 1993).

It is well established that riparian zones play an important role in bank stabilization, water storage and recharge of subsurface aquifers, physical filtration of water, such as sediment and heavy metal removal (Cooper et al., 1987) and geochemical processes, such as C, N and P cycling (Lowrance et al., 1984). Furthermore, the ecological function of the riparian zone is visible through multiple physical, chemical and biological interactions (Swanson et al., 1982). More recently, streams and riparian zones have been described as **meta-ecosystems**, i.e. a set of ecosystems connected across their boundaries by spatial flows of organisms, materials and energy (Gounand et al., 2018). This exchange across the habitats creates a diverse nexus of interactions within food webs and is the key process contributing to ecological functioning and resilience of stream–riparian and meta-ecosystems (Baxter et al., 2005; Richardson and Sato, 2015). For example, streams receive allochthonous organic matter in the form of dissolved (DOM), fine (FPOM), and coarse particulate organic matter (CPOM) from the adjacent terrestrial land (Wallace et al., 1999). Similarly, terrestrial invertebrates that fall into streams provide food source for fish (Wipfli, 1997, Allan, 2003, Eberle and Stanford, 2010, Richardson et al., 2010), while excretes from large mammals also bring substantial quantities of nutrients (Subalusky et al., 2015). In the opposite direction, riparian food webs are subsidized by emerged aquatic insects that are an important energy source for terrestrial arthropods, and this energy is then transferred to higher trophic levels through predation by amphibians, lizards, birds and bats (Collier et al., 2002, Baxter et al., 2005, Regester et al., 2006, Burdon and Harding, 2008, Subalusky et al., 2015).

In natural conditions, stream–riparian meta-ecosystems undergo occasional disturbances, such as altered flow regimes and droughts. These extreme events are periodical and mostly temporary, and therefore do not affect the normal functioning of a system over a long period. Such disturbances can even be beneficial, serving as a revitalizing agent for the native communities (Townsend, 1989). On the other hand, anthropogenic driven disturbances vary greatly in the duration, frequency, spatial extent and intensity, which makes it difficult for the communities to adapt to new and unpredictable conditions. Next to pollution and flow regulation, land use and climate change are widespread anthropogenic pressures affecting the integrity of stream–riparian meta-ecosystems. Agricultural and urban land use intensification, in combination with climate change and hydro-morphological alterations, is creating fragmented and patchy riparian zones. This has been recognized as the main cause of lost connectivity in stream–riparian meta-ecosystems (Ward et al., 1999, Allan, 2004, Fonseca et al., 2021) due to strong impact on the structure and function of riparian habitats. Moreover, removal of riparian vegetation can have instream effects on temperature regimes, nutrients cycling, sediment deposition, habitat alterations and subsequently on the community response. As land use intensifies in the 21st century, there is a pressing need for protection and preservation of stream–riparian ecosystems. Buffering streams from adjacent land-use impacts is increasingly considered as the first step towards their restoration, rehabilitation and conservation, as part of the nature-based solution concept (European Commission, 2015).

1.1 Riparian buffers

Riparian buffer zones (also named in the literature as ‘strips’ or ‘corridors’, hereafter referred to as ‘buffers’) is a common management practice used to potentially mitigate adverse anthropogenic effects in heavily modified catchments (Stutter et al., 2012). Management interventions are many, from simple solutions such as fencing to prevent livestock from entering the stream edge, to grass or herb corridors and more complex forested buffers (Figure 1).

Figure 1. Buffer implemented in an agricultural landscape.

The primary function of buffers is the control of diffuse pollution, hydrological and ecological connectivity, stream shading, carbon sequestration, biomass production and cultural services (Stutter et al., 2012). Accordingly, implementation and restoration of buffers combines biodiversity conservation strategies, as well as socio-economic aspects (ecosystem services). In Europe, the goal is to achieve good ecological status in 60 % of the streams by 2027 (EEA, 2018), which, among other requirements, includes preservation and good management of riparian zones. In practice, it usually involves the implementation of buffers, which can be a binding legal requirement for stakeholders, but in many cases is only a recommendation and therefore voluntary (Schou, 2019). Few countries have regulations on uniform riparian buffer strip widths (e.g. 5 m in Germany and Switzerland), while most countries still lack national regulations for buffer properties (Lind et al., 2019). In Sweden, there is no legally binding requirement for the implementation of buffers, however within the forestry sector leaving the zone 0-10 meters from the stream edge undisturbed is relatively common. In agricultural areas the situation is more complicated. Farmers are following regulations that specify implementation of grass buffers on arable land in nitrate-sensitive areas. A few scattered shrubs and trees are allowed, but no forest or 'forest-like' areas are permitted to be included within these buffers, due to definition of arable land as open areas which are required by law to be kept as such (Degerman and Bergqvist, 2008; Jordbruksverket, 2019). Nevertheless, there is a growing recognition of the importance of riparian zones in supporting biodiversity, function and multiple ecosystem services in streams and adjacent terrestrial systems, especially in heavily modified landscapes. For example, the second pillar of the 2030 EU Biodiversity strategy outlines an ambitious plan to restore at least 25,000 km of rivers to a free-flowing state, as well as to provide more space for nature and high diversity landscape features in agricultural areas, including buffers. These requirements put immense pressure on policy makers and stakeholders in planning cost-effective and efficient restoration projects as well as implementing the appropriate measures for individual water bodies.

The effectiveness of a buffer greatly depends on its inherent features, i.e. vegetation composition, density, width, length and buffer age (Feld et al., 2018), as well as the properties of the stream and of the adjacent upland areas (Kreutzweiser et al., 2010, Lidman et al., 2017, Richardson et al., 2012). Grass buffers, as mechanical filters, are very efficient in sediment trapping (especially sand-size particles) and reduction of phosphorous and nitrogen concentrations, thus improving the runoff water-quality (Mankin et al., 2007). However, forested buffers provide more physical and biogeochemical attributes (Sweeney and Newbold, 2014). For example, canopy cover provided by forested vegetation at the land–water interface enables shading and regulation of water temperature (Davies and Nelson, 1994, DeWalle, 2008), which can have direct effects on instream primary production by changing photosynthetic activity of periphyton, and subsequent effects on the secondary production (Kiffney et al., 2004, Finlay et al., 2011). Additionally, inputs of allochthonous material such as woody debris and leaf litter have direct effects on fluxes of carbon and nutrients in riparian soils and instream, while the energy and nutrients constitute important basal resources for instream food webs (Abelho, 2001, Kominoski et al., 2013). Moreover, large woody debris can increase spatial heterogeneity of instream habitats (Buffington and Montgomery, 1999), supporting more diverse instream communities. Changes in instream habitat that alter the abundance and diversity of emerged aquatic insects can result in trophic cascade effects in stream–riparian meta-ecosystems. Namely, aquatic insects are important subsidies that enter terrestrial food webs through lateral dispersion via emergence in their adult stage (Marczak et al., 2007, Richardson et al., 2010, Marcarelli et al., 2011, Stenroth et al., 2015, Schindler and Smits, 2017). They transform the energy contained in allochthonous organic matter into higher-quality food that is then returned to riparian zones (Paetzold et al., 2005). More importantly, aquatic systems supply terrestrial food webs with high-quality fatty acids that are produced almost exclusively within aquatic environments and therefore limited in terrestrial environments (Arts et al., 2001). Lastly, forested buffers have shown positive effects on biodiversity and improvement of ecological status, especially for small and medium-sized agricultural streams (Tolkkinen et al., 2021).

It is well known that local management interventions are often more effective in small streams (Craig et al., 2008, Greenwood et al., 2012) and might be most suitable in stream systems that are affected by moderate agricultural land use (Turunen et al., 2019). However, the empirical evidence on the effectiveness of forested buffers in reducing adverse effects on stream ecosystems is still insufficient, and our understanding of whether management actions should aim at local (reach) or catchment scales is still inadequate at present. Similarly, relatively little is known of how land use affects the trophic linkages in aquatic - terrestrial food webs (Larsen et al., 2016). Focus of this thesis was therefore on studying the role of forested buffers on structural and functional aspects of stream–riparian meta-ecosystems, in response to perturbations caused by agricultural land use.

2. Objective and research questions

Work in this thesis included measurements of a wide range of environmental, biodiversity, and ecosystem functioning attributes in order to quantify the effects of anthropogenic disturbance on stream–riparian meta-ecosystems in modified landscapes. Emphasis was on the role of forested buffers in mitigating the adverse effects of urban and agricultural land use. **Papers I** and **V** place the thesis in a broader context within Europe, with the main findings from the field studies across four case-study catchments in different countries, while **Papers II – IV** present results from studies on a subset of sites situated in Lake Mälaren catchment in central Sweden.

The thesis addressed following research questions and overall objectives:

1. Which environmental variables are important for explaining biological responses to land use in modified catchments? Are instream responses moderated by local environmental drivers related to the quality and quantity of the riparian buffer or are responses more related to upstream, catchment-level effects? (**Papers I and II**)
2. How does the presence of forested buffers affect the composition of instream and terrestrial communities? Can we detect the effects of spatiotemporal variations on the aquatic-terrestrial connectivity through the availability of aquatic prey for the terrestrial consumers? (**Paper III**)
3. Are spiders relying on aquatic-derived food resources in their diet? What proportion of spider diets is comprised of aquatic and terrestrial food resources and does seasonal variability affect consumption? What are the effects of forested buffers on these trophic interactions? (**Papers IV and V**)

3. Materials and methods

The general study design, habitat assessments and main sampling methods were uniform for all of the papers. In **Papers III** and **IV** additional sampling methods were used. For detailed description of the methods please see the supplementary material of **Paper I**, as well as **Papers III** and **IV.**

3.1 Study design and sampling sites

Case-study catchments were situated in four European countries: Sweden (Lake Mälaren catchment, forested and agricultural stream reaches), Norway (Oslo Fjord catchment, forested and urban stream reaches), Belgium (Zwalm river catchment, forested, agricultural and urban reaches) and Romania (Argeş river basin forested and agricultural reaches). (Figure 2).

The study was designed to obtain a longitudinal profile of the stream network. In each catchment 10-12 streams flowing through an impacted landscape (urban, agricultural or mixed agricultural and urban) were selected for a "paired-reach approach", for assessing longitudinal and lateral patterns in aquatic and terrestrial biodiversity and function. Each stream had an upstream site with no riparian buffer ("unbuffered"), and a downstream site with a riparian buffer ("buffered"), in total 20–24 sites. For addressing longitudinal patterns, we used a "network approach" consisting of an additional 10–12 sites situated upstream and downstream of the site pairs throughout the river network. Upstream (headwater) sites represented least impacted "reference" sites, while downstream or "matrix" sites were located in the matrices of the heterogeneous landscape in which the stream–riparian segments were "embedded". In **Papers II-IV** focus of the research was only on paired sites from streams in Sweden (Figure 2)**:** 20 paired sites from 10 streams in Sweden in **Paper II** and 12 sites from six streams in **Papers III** and **IV**.

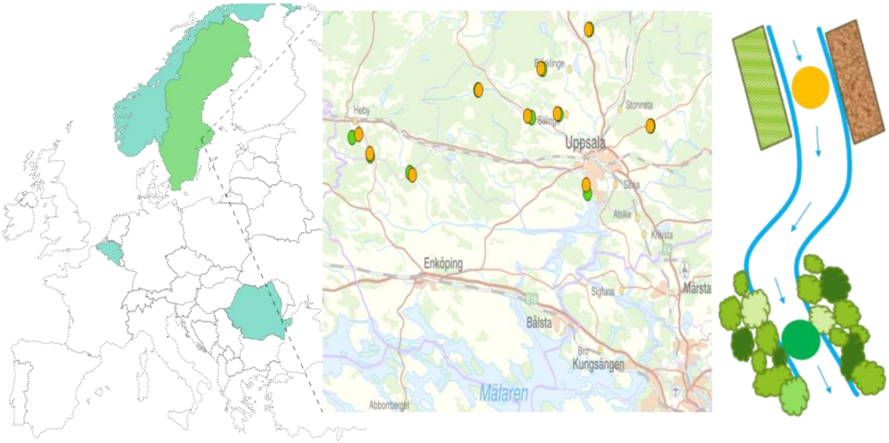


Figure 2. Spatial position of investigated catchments in Europe (left), subset of streams in Sweden (middle) with schematic representation (right) of paired reaches (orange dots -upstream unbuffered reaches, green dots - downstream forest buffered reaches). (Figure adapted from Paper II)

Streams were chosen to be as similar as possible in key environmental variables, i.e. 1st-3rd order, usually 2-5 m wide, with a stable streambed dominated by gravels and cobbles. Reference (headwater) sites were surrounded by intact forest all the way to catchment boundaries upstream. Unbuffered and buffered site pairs (Figure 2 and 3) were placed in a modified, human-impacted landscape. Unbuffered sites were typically dominated by grasses and herbaceous vegetation, with only a few isolated trees within the riparian zone. Buffered sites were selected based on key criteria that characterized well-developed “riparian forest buffer”, described in detail in **Paper I**: extent (buffer on both banks of the stream segment) composition (dominated by small and large trees), length (a woody buffer extending >50 m on both sides of the stream over the sampling reach), and width (>2–3 x wetted stream width). Matrix sites were placed further down in the catchment where the level of human impact was more pronounced, but they typically lacked an extensive riparian buffer as defined above.

Figure 3. Examples of unbuffered (left) and buffered (right) sites.

3.2 Sampling and habitat characterisation

At each site multiple environmental, biodiversity, and ecosystem functioning variables were sampled (Table 1) during three different seasons: **Paper I, II** and **V** in autumn 2017, spring and summer 2018; **Paper III** and **IV** in autumn 2019, spring and summer 2020. Two different components of sampling were conducted at each stream site. Two reaches, differing in length, were sampled in each stream: a longer habitat assessment reach (HAR, 50 m long) and a shorter effective sampling reach (ESR, 30 m long). The start of both reaches was set at the same point at the downstream end, and for buffered sites the end was located as far downstream as possible. HAR was used for the survey of aquatic and terrestrial habitat properties, while shorter ESR was used for biological sampling (i.e. benthic invertebrates and riparian spiders). Additional spatial data for longitude/latitude, catchment properties (boundaries, size, elevation), land use data (% of land cover within the catchments) and buffer properties (buffer size, width and length) were extracted from various online databases (e.g. Google Earth, CORINE Land Cover, Geographical Information System, Swedish meteorological and hydrological institute etc.).

Only a short description of measured variables is presented in the thesis. More details and sampling protocols are available in **Paper I** (Supplementary materials) and **Papers III** and **IV**.

Table 1. Overview of measured variables and approaches used in the thesis to describe the multiple ecological benefits of forested riparian buffers in modified landscapes. Protocols with detailed description are noted in the last column. (Table modified from Paper I).

Variable group	**Response**	**Protocol**
Environmental	Water quality Thermal dynamics Instream habitat Riparian habitat Land use	S2
Biodiversity	Macroinvertebrates Riparian invertebrates Trees	S3
	Emerged aquatic insects	Paper III
Ecosystem functions	Algal biomass accrual	S4
Food webs	Trophic connectivity	S5, Paper IV

3.2.1 Environmental characteristics

Hydromorphological stream properties were quantified within HAR for each study site, and included wetted channel widths, water depths and flow at the time of sampling. Flow was measured along 5-6 randomly selected transects using a flow meter (MiniAir20 Flowmeter, Schiltknecht Messtechnik AG, Gossau, Switzerland). Water temperature was measured using on spot continuous logging throughout sampling season (Eureka Water Probes, Austin, TX, USA). Water quality samples were collected just below the water surface at the downstream end of each site and analysed within 24 h. Water quality assessment included analysis of total nitrogen, inorganic nitrogen (NH_4-N, NO_2+NO_3-N), total phosphorus, total organic carbon, absorbance, pH, conductivity and alkalinity. For further details, see Protocol S2 in Supplementary materials of **Paper I.**

Instream habitat assessment

Instream substrate types were estimated subjectively over the HAR and ESR as percentage (%) of inorganic and organic substrates. Assessment included coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), fine sediment, macrophytes, large woody debris, gravel/pebbles/cobbles, boulders, and bedrock. Inorganic substrate classes were estimated using the Wentworth scale (Wenthwort, 1922), while the percentage cover of filamentous algae and bryophytes was estimated separately as the amount on the underlying substrate. Instream shading was calculated as the average percentage of canopy cover from six measurements obtained in the middle of the stream channel for each study reach. Canopy cover was measured using the CanopyApp for smartphones (University of New Hampshire, Durham, NH, USA).

Riparian habitat assessment

Riparian habitats were surveyed in summer during fully developed leaf coverage on trees and shrubs. Surveys were conducted on six plots (10 x 5 m) placed on both stream banks (3 plots at each side), in the riparian zones adjacent to the HAR at each study site. Canopy cover in the riparian zone was estimated using the same method as described above, from the middle of each plot. Different vegetation/habitat categories were assessed within each plot, as percentage (%) area of cover: managed, short grasses; unmanaged and long grasses including rushes and sedges; herbs and herbaceous vegetation; mosses and lichens growing on the ground; rocks and bedrock; bare ground; small trees and shrubs (DBH < 5 cm); plant litter including leaves; and other (e.g. roads, fences, embankments). Trees on each plot were counted and identified using local identification guides and the smart phone app “PlantSnap” (PlantSnap Inc., Telluride, CO, USA). The circumference of trees was measured when a diameter at breast height (DBH: 130 cm) was ≥ 5 cm.

Condition of stream banks along the riparian HAR (50 m^2) was assessed using the qualitative index of riparian integrity (the Riparian Condition Index—RCI). This index originally developed for New Zealand conditions (Harding et al., 2009) was adapted for European conditions for the purpose of this study. The RCI grades define the status of riparian zone from poor (1) to excellent (5) using the average of 13 different attributes for both banks. The summed total of the attributes provides an overall index that has been associated with stream ecological responses (Burdon et al., 2013). For further details, see **Paper I** (Table 3 in the main text and Protocol S2 in the Supplementary materials).

3.2.2 Biological samples

Macroinvertebrates, emerged aquatic insects and riparian spiders were used in this study for assessing land use effects on biodiversity and as attributes of ecosystem functioning through food web analyses. The same sampling methods were used in all of the papers. For further details about macroinvertebrate and spider sampling, see Protocol S3 in Supplementary materials of **Paper I.**

Macroinvertebrates

Macroinvertebrates were collected using a Surber sampler with a metal frame (dimensions 0.25 x 0.25 m = 0.0625 m^2) and 500 µm mesh netting. Six replicates were sampled at each site, three from erosional run-riffle and three from depositional run-pool habitats. Samples were sieved to remove excess water, pooled together and preserved in 70% ethanol. All individuals were counted and identified to the lowest taxonomic level possible (e.g. species or genus).

Aquatic emerged insects

Emerged insects were collected for **Paper III** and **IV**. At each stream site, two pyramid- shaped floating emergence traps were deployed, with the opening at the top of the net and collector bottle for insect capture (Figure 4). Emergence traps had a base of 60 cm x 60 cm and were placed over different instream habitat types (i.e. riffles and pools). Insects were collected weekly, for three weeks in total during autumn, spring and summer season. To achieve randomization and coverage of different areas within the stream reach, traps were moved to different location each week during the three-week period. Identification of insects was done to the family level, with a small number of animals identified only to higher taxonomic level due to difficulties during identification.

Figure 4. Emergence trap.

Spiders

Riparian spiders were used for **Papers III**, **IV** and **V.** A semi-quantitative method was used to collect different web-building and free-living riparian spiders (order Araneae) and individuals belonging to harvestmen (Arachnida: Opiliones, hereafter referred to as spiders). Spiders were collected methodically along transects parallel to the stream channel within the same 50 m^2 riparian plots adjacent to each reach, as described for riparian habitat assessment. Timed visual searches were used to obtain a relative measure of abundances (catch per unit effort, CPUE). Sampling consisted of 2–3 people that would survey various habitats (e.g. ground, herbs, tree branches, stones, leaf litter) and collected spiders into individual sample tubes. A minimum of two plots were surveyed for a standard amount of time (e.g. 10 min) to achieve sufficient number of animals for different analyses. For each plot the total area that was surveyed was noted and the number of people involved multiplied by the time taken to calculate the duration of sampling. CPUE was then calculated as a relative measure of abundance, thus making abundances between sites comparable as (Eq. 1):

$$\mathrm{CPUE} = \frac{\text{Number of invertebtates}}{\left(\frac{\text{Area sampled}}{\text{Duration of sampling}}\right)} \qquad (1)$$

Spiders and harvestmen were stored on ice during transport from the field and kept in a freezer (–20 ˚C) until identification. All spiders were identified to the family level, while harvestmen were kept at the order level (Opiliones).

3.3 Ecological response

Species response to land use disturbances and the presence/absence of buffers at reach paired sites was assessed using structural and functional composition of the communities (**Papers I-III,** and **Paper V**).

Community composition and biological indices of macroinvertebrates, emerged aquatic insects and spiders were calculated using different biodiversity metrics (e.g. taxa richness, EPT taxa richness, Simpson index, Shannon–Wiener index, evenness and dominance). Total abundance of macroinvertebrates and emerged aquatic insects was expressed as number of individuals per m^2, while spiders' abundance was calculated as CPUE. EPT taxa (E=Ephemeroptera, P=Plecoptera, T=Trichoptera) were typically presented as the %EPT of total taxa abundance.

In **Paper I** the Average Score Per Taxon (ASPT) index was calculated using family-level macroinvertebrate data, as the ratio of the score obtained in the Biological Monitoring Working Party (BMWP) index to the number of taxa scored in the sample, following the equation (Eq. 2):

$$\mathrm{ASPT} = \frac{\text{BMPW Index}}{\sum \text{Taxa}} \qquad (2)$$

Based on their sensitivity to organic pollution, the BMWP index assigns scores from 1 (tolerant) to 10 (sensitive) to each macroinvertebrate taxa, which makes ASPT index suitable for assessing the impact of organic pollution (Armitage et al., 1983). The BMWP index was calculated as the sum of scores for all taxa present in a sample. Index values greater than 100 are associated with unpolluted ("clean") streams. Similarly, high ASPT scores are indicative of unpolluted sites, but the ASPT index is less affected by seasonal variability of the scoring taxa. BMWP index scores <10 are associated with heavily polluted streams. ASPT index in **Paper I** was used in combination with the Riparian Condition Index (RCI) described above to assess the utility of RCI as a predictor of stream ecological status.

Functional traits were used to quantify differences in functional structure of macroinvertebrate and spider communities. In **Paper II,** biological and ecological functional traits were used to quantify differences in functional response between unbuffered and buffered reaches. Selected traits were indicative of changes in the aquatic environment attributable to differences in instream habitat, riparian vegetation and changes in the lateral connectivity of streams (Table 2). Likewise, in **Paper III** and **V** dispersal related trait modalities for macroinvertebrates (Table 2) and functional trait modalities for terrestrial arachnids (**Paper V**) were used to quantify the effects of land use/buffers in stream–riparian meta-ecosystems. Macroinvertebrates trait data for **Paper II** were extracted from database of Tachet et al. (2010) and an online database (https://www.freshwaterecology.info), while **Paper III** and **V** used the DISPERSE database (Sarremejane et al., 2020). Spider traits for **Paper V** were extracted from databases of Gossner et al., (2015) and Entling et al., (2007), supplemented with information from Blandenier (2009) and Macías-Hernández et al. (2020).

Table 2. List of macroinvertebrate traits used in Paper II, III and V.

Trait modalities	**Categories**
Body size (in cm)	≤ 0.25, 0.25-0.5, 0.5-1, 1-2, 2-4, 4-8, > 8
Life cycle duration	≤ 1 year, > 1 year
Potential number of cycles per year	< 1 (semivoltine), 1 (univoltine), > 1 (polyvoltine)
Adult life span	< 1 week, ≥ 1 week – 1 month, 1month - 1year, > 1year
Dispersal	aquatic passive, aquatic active, aerial passive, aerial active
Respiration	tegument, gill, plastron, spiracle
Feeding	deposit feeder, shredder, scraper, filter feeder, predator, parasite
Wing pair type	1 pair + halters, 1 pair + 1 pair of small hind wings, 2 similar-sized pairs
Substratum preferences	boulders/cobbles/pebbles, gravel, sand, silt, mud, macrophytes, microphytes, twigs/roots, organic detritus/litter (hereafter CPOM)
Flow preferences	null (< 5 cm/s), slow (5-25 cm/s), medium (25-50 cm/s), fast (> 50 cm/s)

Traits scores were based on fuzzy coding approach, where a given value indicates if the taxon has no (0), weak (1), moderate (2) or strong (3) affinity with the category (Chevene et al., 1994). In fuzzy coding trait scores are weighted individually for each species, which allows membership of a given species in more than one trait state simultaneously to account for life history and trait plasticity. Trait abundances were calculated as community weighted means (CWM) using the equation of Lavorel et al. (2008) (Eq. 3 for a species *i*):

$$\sum_{i=1}^{n} \text{relative abundance}_i \text{ x trait}_i \qquad (3)$$

CWM trait values show a high sensitivity to disturbance and are therefore good indicators in species' response to change in the environment (Vandewalle et al., 2010, Carreño-Rocabado et al., 2012)

3.4 Trophic connectivity

Paper IV and **V** investigated effects of land use and factors contributing to trophic connectivity in stream–riparian meta-ecosystems, based on the quality and quantity of aquatic-derived prey subsidies for terrestrial consumers (spiders). In **Paper IV** fatty acid concentrations were used as biomarkers for the consumers' diet proportions, with molecular analysis of prey composition from spiders' gut used as a complimentary method. Likewise, **Paper V** used analysis of fatty acids as biomarkers, but together with functional trait data analysis (described in previous chapter) and algal biomass accrual.

Algal biomass accrual (primary production)

Algal growth was used as a predictor for trophic connectivity in **Paper V**. Four unglazed tiles (16 x 16 cm in size) were placed within a 30 m ESR, with comparable habitat conditions (i.e. moderate to fast flowing reaches with rocky substrate). The tiles were deployed during spring-summer for approximately 30 days to assure algal colonization and growth. Algal biomass accrual (chl-*a* mg m^{-2} day^{-1}) was assessed using two complementary methods: (1) in situ measurements using the "Benthotorch" (BBE Moldaenke, Schwentinental, Schleswig-Holstein, Germany) which quantifies the fluorescence of chlorophyll *a* and converts it to chlorophyll biomass (Kahlert and McKie, 2014); (2) pigment extraction and spectrophotometry in the laboratory (Biggs and Kilroy, 2000).

Fatty acid analysis

Fatty acid (FA) content was analysed for larval and adult aquatic insects and terrestrial arachnids (harvestmen and spiders). At each site invertebrates belonging to the same group were pooled together to average individual variations in FA content and achieve adequate sample sizes for the analyses (≈5 mg DM). Samples were freeze-dried, homogenized using mortar and pestle, weighed, and then stored at -20 °C. Fatty acid analysis was conducted at the Swedish Metabolomics Centre in Umeå. Processing involved three steps: lipid extraction, methylation, and gas chromatography-mass spectrometry (GC-MS). Detailed description is available in supplementary material of **Paper IV** and **V**.

Molecular methods

Molecular gut content analysis on Linyphiidae spiders was used as a measure of consumed prey. Each spider was dissected prior to the extraction and only the abdomen (opisthosoma) was used, except in a few cases for very small individuals where the whole body of the spider was used. DNA was extracted from four to 10 individuals at each of the paired sites, using the extraction kit for small size samples (QIAamp DNA Micro Kit, Qiagen, Germany). Next steps in the laboratory procedure followed Hambäck et al. (2021). Primer pair with general forward primer LCO and reverse primer NoAran2 (Hambäck et al., 2021) were used to amplify DNA fragments in COI region (317 bp) for general prey groups, but to reduce amplification of spiders. Positive amplifications were confirmed by visual inspection of PCR products in 1.5% agarose gel. DNA libraries were prepared using SMARTer ThruPLEX DNA-seq at the Science for Life laboratory, Sweden (www.SciLifeLab.se), and sequenced in 1 flow cell on Illumina MiSeq v3, PE 2x300.

Sequencing data was processed using OBITOOLS (Boyer et al., 2016) within the Galaxy portal (usegalaxy.eu, Jalil et al., 2020). Unique sequences were clustered into Operational Taxonomic Unit (OTUs) with a 97% similarity threshold, and a representative sequence from each of the 1,344 OTUs where identified through DNA barcodes in the Barcode of Life Data System (BOLD, www.boldsystems.org, Ratnasingham & Hebert, 2007), with the majority identified to species or genus level. OTUs that indicated contamination or that were irrelevant (i.e. bacteria, fungi, amoebozoans, mammals) were excluded from further analysis. The remaining OTUs were then combined at the same identification level. To correct for the variability in number of sequences between samples, a relative abundance of each prey taxa (in %) was calculated based on the sequence counts (i.e. dividing the number of sequence counts of individual prey by total sequence count in the spider). Prey abundance was averaged across all spider individuals for each site and based on the habitat preferences during their larval development and/or adult stage taxa were divided into three ecological groups (aquatic, semi-aquatic and terrestrial). Detailed description is available in **Paper IV.**

3.5 Data analysis

A number of different statistical tools were used in the data analysis, but only the most important ones are presented and briefly described here. For detailed description of statistical analyses, see individual papers presented in this thesis (**Papers I-V**).

- Principal Component Analysis (PCA) on transformed and standardized (mean of 0 and a standard deviation of 1) variables was used to visualize the difference between the study sites and reduce the dimensionality of environmental variables (**Papers I-III, V**).
- Non-Metric Multidimensional Scaling (NMDS) was used to visualize (dis)similarities and test the differences amongst multiple variables (**Papers I-IV**), usually followed by PERmutational Multivariate ANalysis Of Variance (PERMANOVA) tested with 999 permutations, to investigate the contribution of selected variables to differences between e.g. countries, site types, seasons or taxa (**Papers I, II, IV, V**)
- Linear mixed models (LMM) were used to test overall differences in the response variables (**Papers I, III, IV**). Country, site type, seasons and taxa and their interaction were set as the fixed effects. Reach pairs were not spatially independent, so stream identity was fitted as a blocking factor (random effect).
- Log Response Ratio LRR (**Paper I**) was used to calculate the effect size of riparian attributes (proportional change in the means of treatment and control group). This method is particularly useful for quantifying simple

two-group experimental designs in meta-analyses of ecological research.

- Redundancy analysis (RDA) was used to relate variation in the composition of response variables with explanatory variables (**Papers II, III** and **V**). The significance of the variables was tested with 999 Monte Carlo permutations. Forward selection was carried out with the stopping criteria, and the adjusted coefficient of multiple determination (R^2adj) calculated. Variance inflation factors (VIF) were checked and predictor variables were excluded if they had VIF score > 4. To investigate the independent effects, RDA was followed by partial RDA (pRDA) and variation partitioning (VP) was additionally used to test separate variation amongst variables using the strongest predictors from RDA analysis.
- Structural equation modeling (SEM) was used to understand potential mechanistic pathways indicated by the exploratory analyses (**Paper V**).

Analyses were performed in one of the following statistical programs: JMP Pro 15.0.0 (SAS Institute, Cary, North Carolina), Canoco software (version 5.12, ter Braak and Šmilauer 2012; Šmilauer and Leps 2014) and R (R Core Team, R: A Language and Environment for Statistical Computing, Vienna, Austria).

4. Results and discussion

Key findings of the research are presented and discussed in the following section, summarizing the most important details of this thesis (**Papers I-V**). Discussion for **Papers I** and **V** (that were part of a larger European project), will emphasize the results from streams in Sweden, which are the core of this thesis.

4.1 Riparian integrity and ecological status of streams - European context (Paper I)

The Riparian Condition Index (RCI) was used to characterize riparian integrity and ecological status for study reaches in streams across Europe (Sweden, Norway, Belgium and Romania) with varying levels of human impact. Assessment of the overall performance of the RCI showed that it was able to distinguish buffered and forested reference sites (higher scores) from the more degraded unbuffered and downstream "matrix" site types (lower scores) across all four case-study catchments. Differences between buffered and forested sites, and between unbuffered and matrix site types were not significant at $\alpha=0.05$, but general patterns were in most cases conserved across the case-study catchments (Figure 5). Contrasting patterns in the RCI that were recorded between the countries were reflected by the real differences in site characteristics. Scandinavian countries in general had higher RCI scores indicating better riparian conditions. Swedish sites situated in Lake Mälaren catchments had on average a higher level of riparian integrity than all the other countries, especially for the forested reference sites (Figure 5). The reason for the higher scores is due to the location of reference sites in Sweden which typically had a mix of mature deciduous and coniferous trees. Also, one of the sites was located within an important nature reserve ("Naturreservatet Fiby urskog" or Fiby primeval forest nature reserve) that has been mostly undisturbed by human intervention since the end of the 18th century. In comparison to the other case-study catchments, there was a greater difference between forested reference sites and buffered sites in Sweden. This was influenced by differences in vegetation composition at the forested riparian buffers, where a mix of deciduous tree species dominated and coniferous trees were mostly absent.

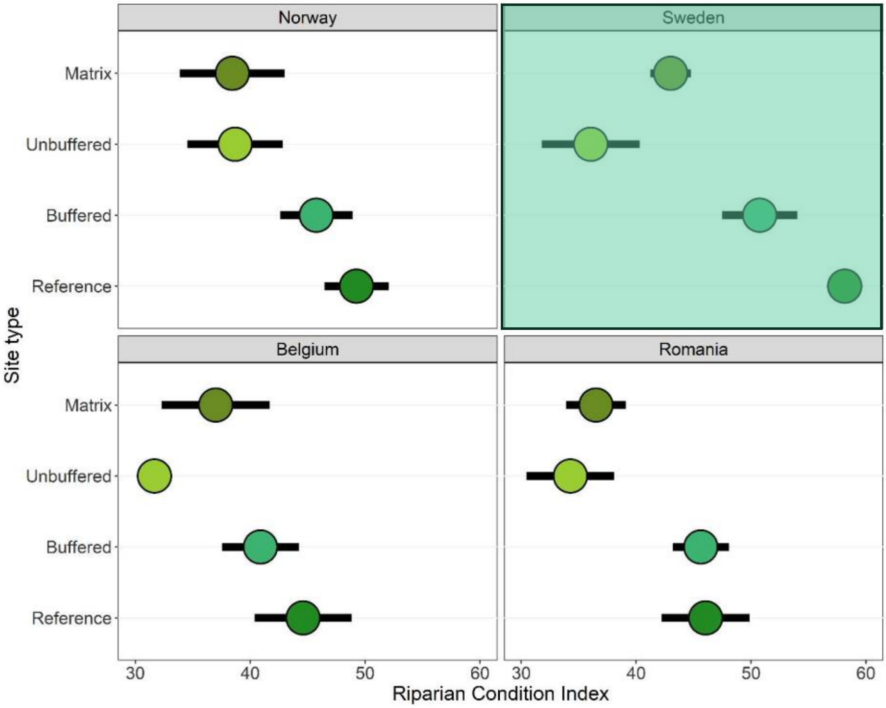


Figure 5. Mean values (± 95% CI) for each attribute used in the Riparian Condition Index for the stream reaches in four case-study catchments (Norway, **Sweden [green shadowing]**, Belgium, and Romania). Site type: 'Reference' (pristine or least-impacted sites), 'Unbuffered' (upstream sites of sites pairs lacking trees), 'Buffered' (downstream site of site pairs with a forested riparian buffer), 'Matrix' (sites located further downstream to capture cumulative land use impacts). (Figure adapted from Paper I).

One of the important outcomes from the RCI analysis was that forested riparian buffers had a strong positive effect on channel shading in all of the case-study catchments. Interestingly, in Sweden the adjacent vegetation and buffer width showed stronger effects between unbuffered and buffered sites, than the effect of shading (Figure 6). This was revealed using effect sizes (log response ratios) to measure which attributes have the strongest contribution to differences in the environmental conditions between unbuffered and buffered sites. Shading can contribute to improvement of ecological status by reducing proliferations of filamentous green algae and macrophytes, and indirectly helps in sustaining suitable instream conditions for more sensitive EPT species (Jacobsen et al., 2003). Moreover, shading potentially moderates stream temperatures (Broadmeadow et al., 2011, Battin et al., 2007, Johnson et al., 2016). These results are valuable for management, as shading is often seen as a key element in the restoration and rehabilitation of degraded streams (Rutherford et al., 1997, Clews et al, 2010).

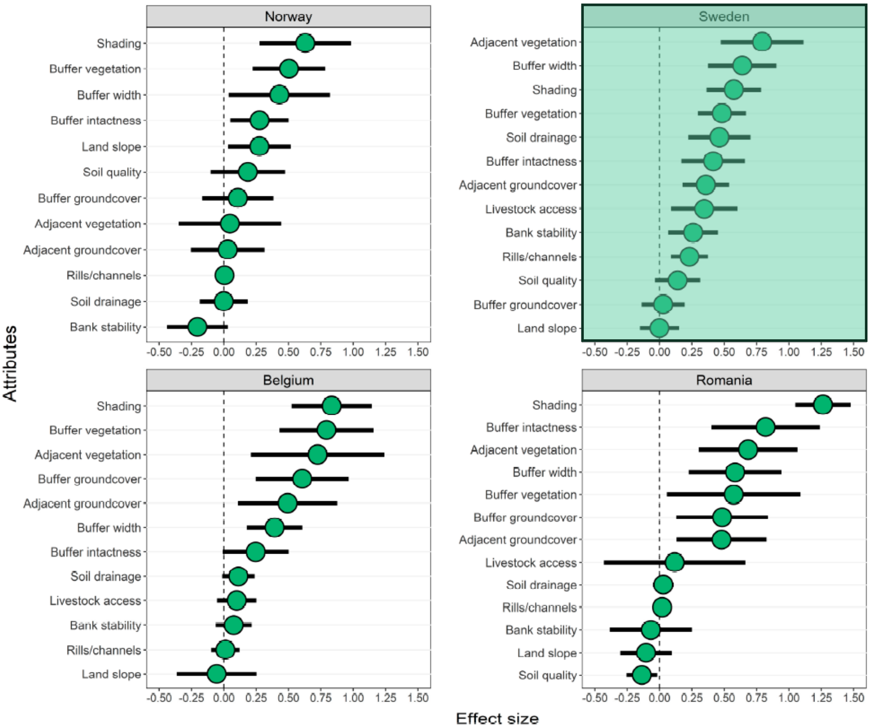


Figure 6. Mean log response ratios (±95% CI) for each case-study catchment (**Sweden [green shadowing]).** Effect sizes show the change between downstream buffered sites with forested riparian vegetation and upstream unbuffered sites for each attribute used in the Riparian Condition Index. (Figure adapted from Paper I).

The average Score Per Taxon (ASPT) macroinvertebrate index (an indicator of stream ecological integrity) was used to assess the utility of RCI as a predictor of stream ecological status, after accounting for upstream catchment-wide human impact (described using the Principal Component Analysis). Results showed that there was a positive, although weak, relationship between the RCI and the stream ecological status (ASPT). At the same time, there was a significant negative relationship between the level of human impacts in the upstream catchment (PC1) and the ASPT index. There was also evidence that the overall effect size of improved stream ecological status due to presence of buffers (improvement in riparian condition) declined overall when the existing upstream state was more degraded, which showed that the biotic response might be more contingent on the environmental context.

The results from this study demonstrate the efficiency of RCI as a useful measure of riparian ecological integrity. Study suggests that enhanced riparian management might have potential for a larger relative improvement in the degraded sites. However, both upstream anthropogenic influences and the selection of most appropriate indicators must be considered for the implementation of remediation strategies aiming to improve ecological status of the stream by rehabilitating degraded reaches.

4.2 Buffer properties and macroinvertebrate community responses (Paper II)

As seen in the previous chapter, the properties of riparian zones and the extent of human impacts can determine ecological response in streams, with buffers showing strong positive effects on ecological quality. Therefore, the focus of this chapter was to further investigate differences in the properties of unbuffered and buffered sites and their effects on the structural and functional composition of macroinvertebrates at paired sites from streams situated in Lake Mälaren catchment in Sweden. Additionally, the aim was to determine if local factors (i.e. properties of a buffer and instream habitats) have a stronger effect on community response than large-scale factors (i.e. catchment properties).

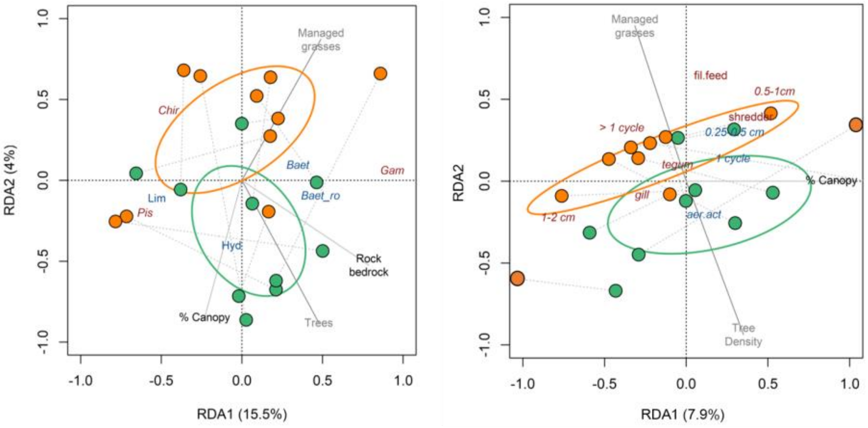
There was a clear difference between buffered and unbuffered sites, with the properties of instream habitats directly related to differences in riparian properties (e.g. vegetation cover). Buffered sites were characterized by increased shading (greater canopy cover), substantial amounts of large woody debris and CPOM. Unbuffered sites contained more fine sediments and greater macrophytes cover. These results confirmed findings from similar studies which showed that reaches of forested buffers can improve hydromorphological habitat quality when compared with open reaches in agricultural landscapes (Quinn et al., 1997, Sponseller et al., 2001). Based on the nutrient concentrations, there was no evident improvement in water quality in the presence of buffers, which suggests that local scale mitigation measures might not have the capacity to override the effects of land use at the catchment scale. Still, changes in the water quality in the presence of buffers might be subtle and therefore not easy to detect based on only few sampling events.

Inspection of (dis)similarities and visualization using NMDS and RDA analyses revealed differences in taxonomic and trait composition between buffered and unbuffered sites, with traits showing stronger discrimination than taxonomic composition (Figure 7). This finding was in line with previous studies which showed that traits reflect functional patterns and processes better than taxonomic composition (Townsend et al., 2008, Truchy et al., 2019). Taxa and traits that contributed the most to dissimilarity between buffered and unbuffered sites are shown in Table 3 (based on the analysis of similarity SIMPER).

Table 3. Analysis of similarity (SIMPER) of taxonomic and trait community differences between unbuffered (n = 10) and buffered (n = 10) sites. Species contribution to average between group dissimilarity is shown in column "Cont. %", and cumulative contribution in column "Cum. %". Community composition is shown as average of Hellinger transformed taxa abundance, while traits are shown as average community weighted means. (Table adapted from Paper II).

	Cont. %	**Cum. %**	**Unbuffered**	**Buffered**
Community composition				
Gammarus pulex	3.9	7.1	0.41 ± 0.2	0.39 ± 0.3
Pisidium sp.	3.1	12.7	0.35 ± 0.2	0.29 ± 0.2
Limnius volckmari	2.5	17.3	0.11 ± 0.1	0.23 ± 0.2
Simuliidae	2.4	21.6	0.16 ± 0.2	0.15 ± 0.1
Elmis aenea	2.3	25.9	0.14 ± 0.1	0.22 ± 0.2
Asellus aquaticus	2.1	29.7	0.21 ± 0.2	0.15 ± 0.1
Chironomini	1.9	33.1	0.19 ± 0.1	0.09 ± 0.1
Baetis sp.	1.8	36.3	0.09 ± 0.1	0.10 ± 0.2
Orthocladiinae	1.7	39.5	0.12 ± 0.1	0.15 ± 0.1
Oligochaeta	1.6	42.5	0.30 ± 0.1	0.31 ± 0.1
Trait composition				
# of cycles per year > 1	1.0	5.8	0.56 ± 0.1	0.48 ± 0.2
# of cycles per year = 1	1.0	11.6	0.41 ± 0.1	0.48 ± 0.2
shredder	1.0	17.4	0.39 ± 0.2	0.34 ± 0.1

	Cont. %	Cum. %	Unbuffered	Buffered
filter-feeder	1.0	23.0	0.28 ± 0.2	0.20 ± 0.1
gill respiration	0.9	28.3	0.63 ± 0.1	0.59 ± 0.1
scraper	0.9	33.5	0.21 ± 0.1	0.33 ± 0.1
size 0.25–0.5 cm	0.8	38.4	0.24 ± 0.1	0.30 ± 0.1
life cycle > 1 year	0.7	42.7	0.45 ± 0.1	0.45 ± 0.1
life cycle < 1 year	0.7	47.0	0.55 ± 0.1	0.55 ± 0.1
size 1–2 cm	0.7	50.8	0.34 ± 0.1	0.27 ± 0.1



(a) taxa **(b) traits**

Figure 7. Results of pRDA for taxonomic (a) and trait (b) composition. Riparian attributes used in the pRDA are shown in black (% canopy, % rock/bedrock) and two riparian variables that are significantly different between the study sites (% managed grass and % trees, based on t-tests) were shown in grey. The species and traits with the highest scores along the first two pRDA axes are shown in dark brown color (associated to unbuffered sites with orange ellipse and orange dots) and blue color (associated to buffered sites with green ellipse and green dots) color. RDA2 (b) is equivalent to PC1 axis, due to a one variable model (% Canopy). Taxa abbreviations: Baet = *Baetis* sp., Baet_ro = *Baetis rhodani*, Chir = Chironomidae, Gam = *Gammarus pulex*, Hyd = *Hydropsyche siltalai*, Lim = *Limnius volckmari*, Pis = *Pisidium*. Trait abbreviations: breathing: gills, tegum = tegument, dispersal: aer.act = aerial active; feeding: shredders, fil.feed = filter feeders; number of cycles per year: <1 cycle, 1 cycle; size: 0.25–0.5 cm, 0.5–1 cm, 1–2 cm. (Figure adapted from Paper II).

Metrics of α-diversity (e.g., taxa richness) did not differ between buffered and unbuffered sites, a result that might have reflected spatial proximities of our paired sites within the streams and the influence of dispersal rates and local diversity patterns, i.e. mass effects (Heino, 2013, Burdon et al. 2016). However, there was evidence of changes in β-diversity patterns, with abundances of key taxa changing between unbuffered and buffered sites due to environmental filtering. For example, taxa with preferences for gravel substrates and active aerial dispersal were more abundant at buffered sites, i.e. caddisflies *Agapetus ochripes* and *Rhyacophila nubile* and mayfly *Baetis rhodani*. EPT taxa in general, like these three species, prefer oxygen-rich, flowing waters and hard-bottom substrata, and are known to be pollution-sensitive (Johnson, 1993, Rosenberg & Resh, 1993). Thus, the changes in benthic habitat conditions in buffered sites likely contributed to their increased abundances. On the other hand, traits that were more prevalent in unbuffered sites were associated with depositional habitats (e.g. preference/tolerance of slow flow and CPOM substrate preferences), i.e. filter-feeding *Pisidium* molluscs commonly found in agricultural streams (Lenat & Crawford, 1989, Stone et al., 2005). Some traits showed the opposite trend from that predicted. Namely, despite the higher cover of CPOM and woody debris in the presence of buffers, shredders were more abundant at the degraded unbuffered sites: a trend that is most likely driven by high abundances of *Gammarus pulex* at unbuffered sites, which is a dominant shredder in southern Sweden (Nilsson, 1997). Similarly, scrapers were expected to benefit from higher primary production and algal biofilms at the open unbuffered sites, but instead were found in higher abundances at the buffered sites. This might be explained by differences in substrate, as buffered sites were characterised more by gravel substrates which are a suitable feeding habitat for scrapers. Instream habitats at the unbuffered sites had higher filamentous algae coverage, which is not considered as a good food resource and which might smother the substrates and algae that scrapers prefer to feed on.

RDA and variation partitioning (pRDA) revealed that the strongest predictors of macroinvertebrate communities between the paired sites were instream variables (e.g. related to nutrient enrichment, instream productivity and benthic habitat quality). Riparian characteristics alone explained only a minor proportion of the variability (4% of taxonomic composition) or were a negligible predictor for trait composition. By contrast, the shared variation component explained by riparian and instream factors was 7% for functional (trait) community composition. This finding indicates the strong linkage between aquatic and terrestrial habitats at the local (reach) scale. Likewise, the higher abundances of aquatic insects with a relatively large body size and aerial dispersal found in buffered sites suggests stronger cross-habitat connectivity and might reflect an increased reciprocal transfer of nutrients and energy back to terrestrial food webs by emerged insects.

4.3 Is there a link between aquatic and terrestrial habitats? (Paper III)

Findings from previous two chapters demonstrated the effectiveness of buffers to moderate land-use impacts on instream habitats and biodiversity. Furthermore, both studies highlighted the potential importance of aquatic-terrestrial linkages for biodiversity and function. However, there remains insufficient understanding of the key factors regulating ecological connectivity within stream–riparian meta-ecosystems. Knowledge gaps that are largely limited by the lack of studies that simultaneously quantify the drivers of biodiversity and ecosystem functioning in both aquatic and terrestrial systems and at both local and larger spatial scales (Turunen et al., 2017, Forio et al., 2020, Johnson et al., 2021).

This chapter investigated how the presence of buffers affects the composition and seasonal variability of aquatic and terrestrial communities with implications for cross-ecosystem connectivity. Organisms at the stream–riparian interface, putatively linked via trophic interactions, were analysed: larval aquatic insects, emerged adult aquatic insects and riparian spiders. Study sites comprised unbuffered and buffered paired sites in six streams situated in an agricultural catchment in Sweden. These sites are a subset of the streams included in the previous study (**Paper II**).

Instream aquatic insects were more abundant at buffered sites (Figure 8) and a similar pattern was observed for emerged adult insects. These findings of the positive effects of forested buffers on aquatic communities in agricultural streams agree with many earlier studies (e.g. Jerves-Cobo et al., 2017, Tolkkinen et al., 2021, Forio et al., 2021), and reinforces the use of buffers for sustaining more diverse instream biodiversity, especially disturbance sensitive EPT taxa. Unbuffered sites were characterised by higher inputs of sediments and cover of submerged macrophytes sites and increased abundances of sedentary non-insect macroinvertebrates (e.g. molluscs, snails and oligochaetes) (Figure 8). In addition, both instream and emerged aquatic insects showed seasonal differences in abundances (Figure 8), in line with the seasonal and synchronized emergence of aquatic insects in temperate zones (Sweeney and Vannote, 1982). The composition of emerged insects in degraded agricultural streams is often characterised by small body sizes and weak flying strengths (Raitif et al., 2018, McKie et al., 2018), traits indicative of limited dispersal capacity. In this study there was no support for these trait shifts at the unbuffered sites, most likely due to influence of Chironomidae (Diptera), since they dominated in abundance and seasonally at both site types (unbuffered and buffered). In the presence of buffers, improved environmental conditions should favour emergent insects with better dispersion abilities, such as larger body size and stronger fliers (Goss et al., 2020), shown here by the higher abundance of EPT taxa (e.g. Baetidae and Rhyacophilidae) at the buffered sites.

Buffers also affected riparian spider communities through changes in habitat availability (Figure 8), a finding that agrees with earlier studies that have shown the importance of vegetation types for spider communities (e.g. Greenstone, 1984, Chan et al., 2009, Galle and Schweger, 2014, Forio et al. 2021, Johnson et al., 2021). Trees and shrubs at buffered sites are an important habitat for web-building spiders (Laeser et al., 2005, Ramberg et al., 2020, Popescu et al., 2021), exemplified here by the abundance of Linyphiidae spiders. Free-living spiders such as Lycosidae, Pisauridae and Clubionidae were more abundant at the more degraded unbuffered sites. As active hunters, they seem to be less restricted by vegetation structure and therefore might benefit more from open areas (Ramberg et al., 2020). Contrary to predictions, the prevalence of the web-building Tetragnathidae at unbuffered sites might be explained by the abundance of overhanging and emergent vegetation close to the streams (Chan et al., 2009).

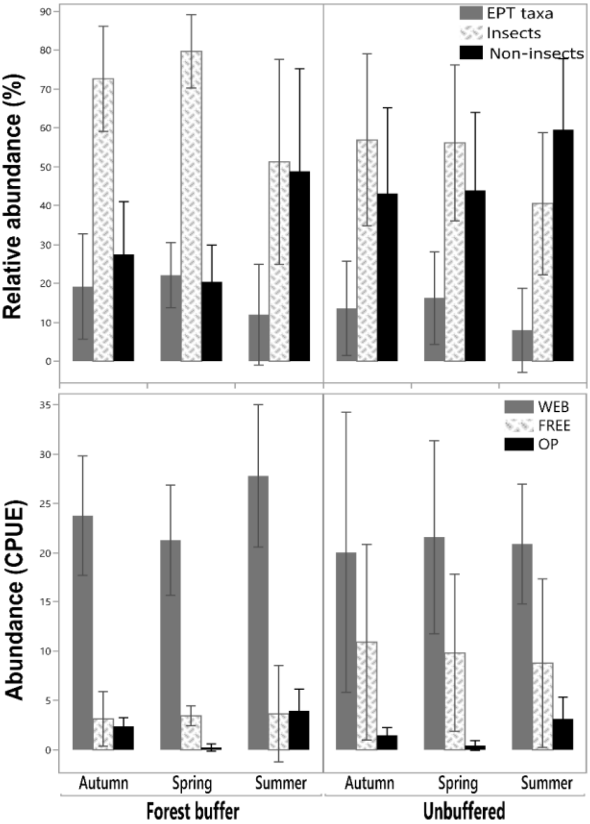


Figure 8. Relative abundance of insects, EPT taxa and non-insects, and catch per unit effort (CPUE) abundance of web-building (WEB) and free-living (FREE) spiders, and Opliliones (OP) between reach types and seasons. (Figure adapted from Paper III).

Variation partitioning revealed that significant variability in spider communities (21% families, 18% guilds) was explained by riparian variables (e.g. buffer length and canopy cover). Previous studies have also shown that riparian spiders can be strongly correlated with the abundance and taxonomic composition of emerged aquatic insects (e.g. Paetzold et al., 2006, Iwata, 2006, Krell et al., 2015). In this study seasonal differences were found for richness and abundances of a few web-building spider families (Araneidae, Theridiidae, Tetragnathidae) and Opiliones, but not for spider guilds (web-building and free-living) that show no difference between the seasons, despite seasonal differences in potential aquatic prey. RDA and variation partitioning did however reveal significant associations between spiders and the abundance/composition of aquatic and emerged insects (Figure 9).

Instream aquatic insects explained significant amounts of the variation in spider community composition (5.6% for spider families and 2.7% for guilds), while emerged insects were not significant, although they contributed to the shared variance component with environmental variables (34.4% for families and 19.6% for guilds).

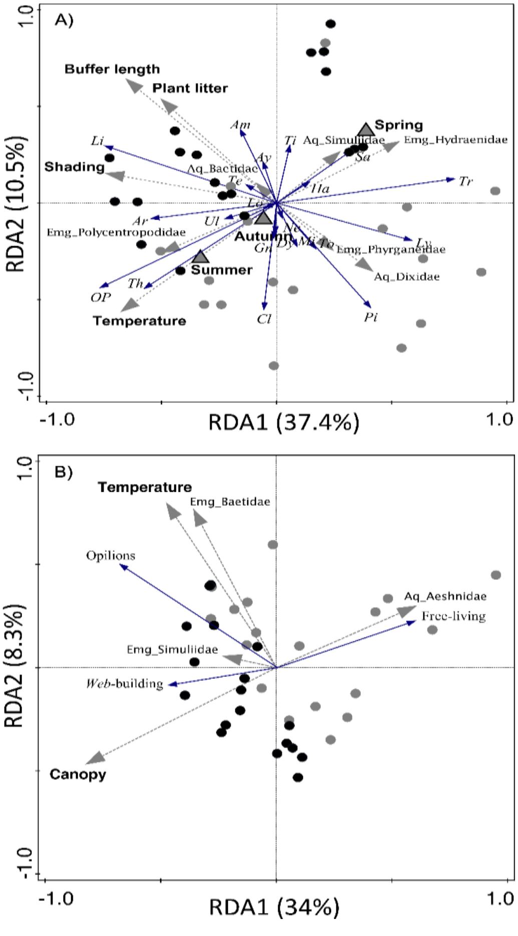


Figure 9. Redundancy analysis for A) spider families and B) spider guilds. Unbuffered (grey dots) and forested buffer (black dots) reaches. Abbreviations: **OP** (Opiliones); **WEB**-building: Am (Amaurobiidae), Ar (Araneidae), Ha (Hahnidae), Li (Linyphiidae), Ne (Nesticidae), Te (Theridiosomatidae), Th (Theridiidae), Ti (Titanoecidae), Tr (Tetragnathidae), Ul (Uloboridae); **FREE**-living: Ay (Anyphaenidae), Cl (Clubioniade), Dy (Dysderidae), Gn (Gnaphosidae), Lo (Liocranidae), Ly (Lycosidae), Mi (Mimetidae), Pi (Pisauridae), Sa (Salticidae), To (Thomisidae); **Aq** (instream aquatic insects), **Emg** (emerged aquatic insects). (Figure adapted from Paper III).

Collectively, results showed that the presence of buffers in agricultural streams affected the structure and function of both aquatic and terrestrial communities. Changes in the composition of aquatic insects due to habitat alterations and seasonal variation can have repercussions for cross-ecosystem connectivity in stream–riparian meta-ecosystems. Specifically, riparian food webs receive energy and essential nutrients transported via emerged adult insects. Significant correlations between spiders and aquatic insects found here highlight the importance for aquatic-terrestrial trophic linkages. Findings provide supporting evidence for the mitigating effects of forested buffers in maintaining functionally important linkages between stream–riparian meta-ecosystems in agricultural landscapes.

4.4 Trophic linkages in stream–riparian meta-ecosystems (Papers IV and V)

The previous chapter demonstrated relationships between aquatic and terrestrial communities in stream–riparian meta-ecosystems mediated via emerged insects. Although a few studies have quantified land-use effects on stream insect emergence (e.g. Carlson et al., 2016, Raitif et al., 2018, Goss et al., 2020), relatively little is known of effects on cross-habitat linkages in general and specifically the role of forested buffers on the transfer of energy and materials through stream–riparian meta-ecosystems (Larsen et al., 2016). This final chapter explored trophic connectivity between stream–riparian communities and addressed how alterations in riparian habitats and temporal changes (seasons) can affect these linkages. Cross-ecosystem connectivity was assessed through changes in the fatty acid (FA) composition of organisms comprising different trophic levels in the aquatic-terrestrial food web, i.e. aquatic insects and riparian spiders. Focus was on nutritionally important polyunsaturated FAs (PUFA), and especially eicosapentaenoic acid (EPA, 20:5ω3), that are almost exclusively produced in aquatic environments and known for their bioaccumulation and transfer to higher trophic levels, which makes them effective biomarkers for tracking aquatic subsidies into terrestrial food webs (Brett, 1997, Iverson, 2009, Twinning et al., 2016). The FA biomarker approach was complemented by molecular gut analysis (**Paper IV**) in order to determine the complete prey spectrum for Linyphiidae spiders and specifically the contribution of aquatic subsidies compared to terrestrial food resources. The strength of cross-ecosystem connections was additionally assessed (**Paper V**) using algal productivity, community weighted means (CMW) of macroinvertebrate dispersal traits and spiders' traits (body size) and environmental factors (i.e. network position and level of anthropogenic impact).

Results of the study that included only a subset of paired sites from streams in Sweden (**Paper IV**) indicated the importance of buffers in providing more aquatic-derived and highly nutritious food for riparian spiders. Specifically, buffered and unbuffered sites contributed to dissimilarities in PUFA content as shown by interactions between taxa and seasons in insects, despite the lack of between-site type differences. For example, larval Baetidae, which feed on benthic algae, had more essential PUFA (i.e. EPA, ALA and ARA) at reaches with forest buffer in spring, suggesting that buffers support algal communities with higher PUFA production (Figure 10). The results from the study that included headwater (reference) and paired sites on the European level (**Paper V**) confirmed the role of primary production in contributing to trophic connection through the shared variation component explained by aquatic invertebrate dispersal traits and algal productivity. However, the largest proportion of the variability in the FA composition of riparian arachnids was actually independently explained by insects' dispersal traits, especially dispersal traits associated with EPT insects. In contrast, structural equation modelling showed that increasing forested cover might negatively affect algal productivity, with shading leading to negative indirect effects on trophic connectivity. However, these effects might be balanced out by the increased abundances of EPT aquatic insects with dispersal traits that increase the transfer of aquatic subsidies to terrestrial food webs.

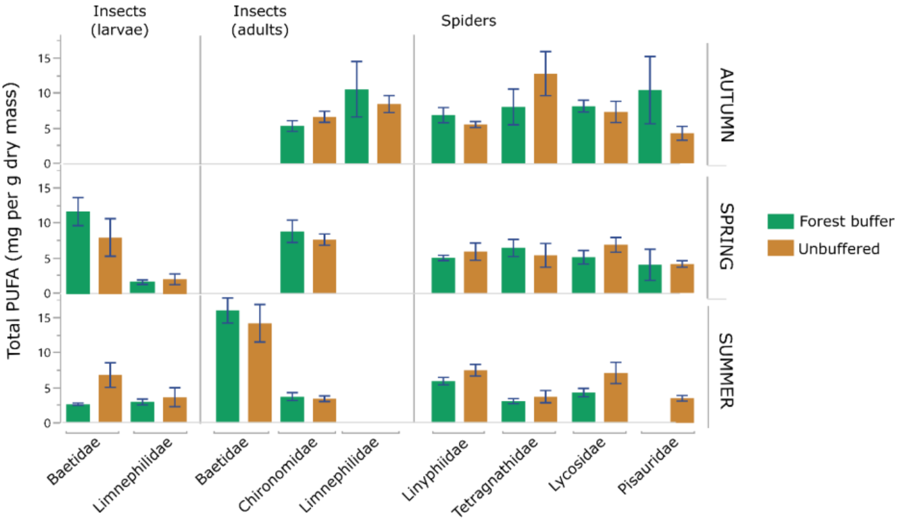


Figure 10. Mean ± 1SE of polyunsaturated fatty acids (PUFA) concentrations in mg per g for insect (larvae, adults) and spider taxa between reach types and seasons. (Figure adapted from Paper IV).

Previous studies showed that riparian land use can affect the longevity and dispersal ability of emerged adult aquatic insects, which in turn might influence the spatial extent and type of terrestrial consumers supported by aquatic prey (Greenwood and Booker, 2016, McKie et al., 2018, Kopp and Allen, 2020). In this study ‘aerial active’ aquatic insects were positively associated with the proportion of EPA in riparian spiders (Figure 11), while ‘univoltine life-cycles’ trait suggested that synchronised seasonal insect emergence could contribute to cross-ecosystem connectivity. Results that were in line with the study from the Swedish paired sites (**Paper IV**), where spiders in general had the highest PUFA concentrations in autumn, which coincided with the emergence of limnephilid caddisflies in September (**Paper III**). Emergence of baetid mayflies (that had the highest EPA in spring) was associated with the PUFA composition of spiders, e.g. web-building Linyphiidae had the highest ω-3/ω-6 ratio in spring, while ground-hunting Lycosidae had overall the highest EPA concentrations and were associated with ω-3 PUFA in spring (NMDS).

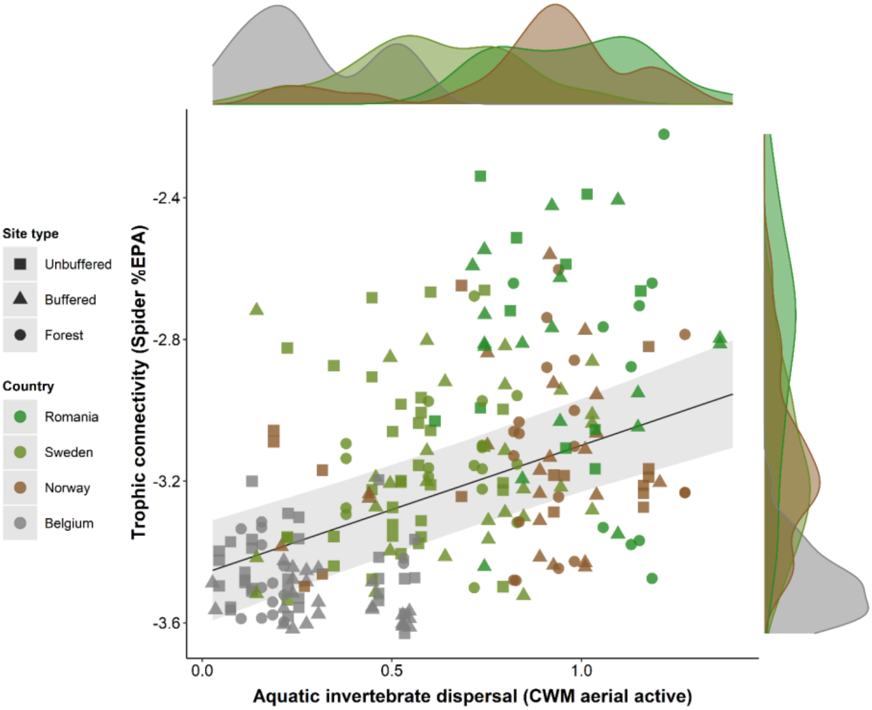


Figure 11**.** Trophic connectivity (logit % concentrations of EPA in riparian spiders) was correlated with log[*x* + 1] community-weighted mean (CWM) abundances of 'aerial active' dispersing aquatic insects. The regression line represents the parameter estimate (± 95% confidence interval) from a linear mixed-effects model. Density plots along margins show the distribution of values for each country. (Figure adapted from Paper V).

Finally, in combination with FA analysis, molecular methods (prey DNA) provided unique and valuable information about the composition of prey consumed by Linyphiidae spiders during autumn, spring and summer. Results showed the effects of reach types (i.e. presence of buffers) on the prey that spiders have consumed and the effects of seasonal changes for aquatic-derived food resources. Although aquatic prey in spiders' gut constituted less than 20% of all prey taxa, the greatest proportion of aquatic prey was found in spring (Figure 12). This finding confirms the effects of seasonal changes and emergence patterns on trophic connectivity, by indicating that spiders might rely on (and benefit from) more nutritious and abundant early-emerged aquatic prey when terrestrial prey is still limited. Further evidence of stronger aquatic - terrestrial connectivity at the buffered sites was also seen through spiders consuming more aquatic Diptera in autumn and Trichoptera in summer on those sites (Figure 12).

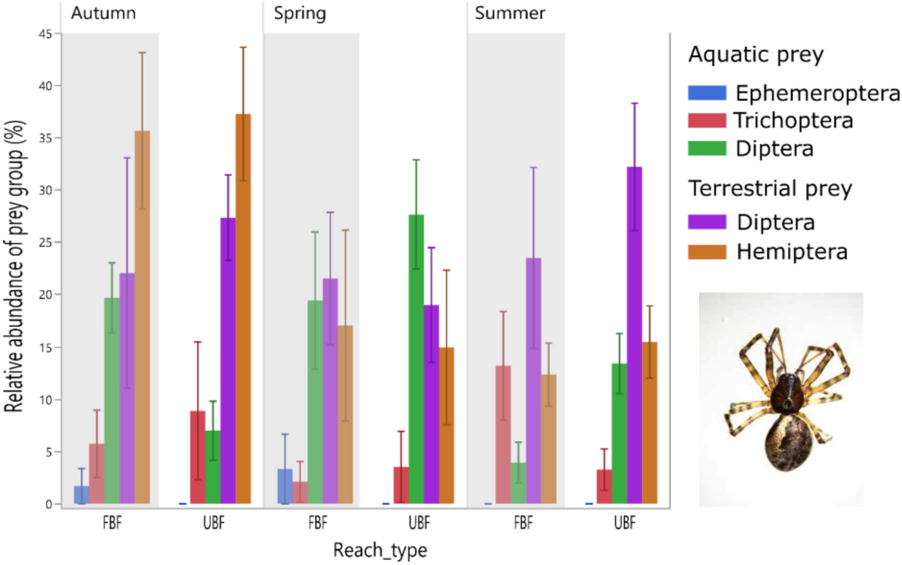


Figure 12. Mean abundance ± SE for the selected aquatic and terrestrial prey in Linyphiidae spiders detected using molecular methods, by reach types (FBF=forest buffer, UBF=unbuffered) and seasons (autumn, spring and summer). (Figure adapted from Paper IV).

These findings are in line with results from the European streams (**Paper V**) where contributions to trophic connectivity were associated with numerically abundant taxa characterised as having passive aerial dispersal and smaller body size (e.g. Diptera). However, in comparison with larger, more actively dispersing aquatic insects, their importance was relatively minor. Additionally, **Paper V** showed that spiders' body size explained most of the variation in the FA composition when combined with environmental predictors that included riparian vegetation structure. Therefore, even when the proportion of aquatic-derived food is modest, the presence of forest buffers can provide suitable habitats for small web-building Linyphiidae and significantly enhance their abundances (Ramberg et al., 2020, **Paper III**), resulting in overall positive effects and strengthening the connection between aquatic and terrestrial food webs and the transfer of high-quality aquatic PUFAs to higher trophic levels.

Combined, results presented in this chapter demonstrated that spiders rely on the availability of different prey, and that seasonal patterns in the emergence of aquatic insects can have significant effects on the receiving terrestrial food webs. Moreover, there was strong indication of the importance of environmental factors in moderating connectivity, which showed how riparian and catchment land uses mediate indirect effects on trophic connectivity.

5. Conclusion and outlook

Concerns about the impairment of freshwater ecosystems are increasing, especially with growing threats from pollution, land use and climate change. Land use activities such as clearance of riparian vegetation, urbanisation and hydrological modification contribute to the fragmentation of streams worldwide as well as alter the lateral connectivity between aquatic and terrestrial ecosystems. Human impacts, although particularly pervasive on streams and their riparian habitats, are often overlooked and neglected in comparison to larger rivers. However, the importance of these fragile systems in the river network is undeniable, as their abundance (stream km) greatly exceeds that of larger rivers, they provide subsidies for downstream ecosystems and they are intricately linked with surrounding terrestrial systems through cross-habitat flows of resources and energy.

Although there is no doubt that mitigation efforts are urgently needed in agricultural catchments, empirical evidence of the efficacy of forested buffers in reducing adverse effects on riverine ecosystems is scarce. Attempts to mitigate land-use impacts on streams (e.g. by creating buffers) also frequently results in conflicts between biodiversity conservation and human activities. By addressing key knowledge gaps, such as which environmental variables are important for explaining biological responses, how do buffers affect the composition of instream and terrestrial communities, and, most importantly for this study, how does the presence of forested buffers affect trophic linkages between stream–riparian meta-ecosystems, my thesis contributes to a better understanding of the importance of key processes and functions at the land-water interface that underpin biodiversity and function.

Throughout the thesis chapters, results showed the importance of riparian forest buffers for the structural and functional integrity of stream–

riparian meta-ecosystems and for mitigating adverse effects of agricultural land use. Main conclusions are summarised as:

- results highlighted important attributes of buffers, which included instream shading, provision of habitats for instream and riparian communities and improvement of stream ecological status (**Paper I-II**);
- local mitigation and rehabilitation interventions are appropriate for improving the environmental conditions that underpin instream biodiversity and terrestrial consumers in the riparian zone, as well as for strengthening food webs and resilience in stream–riparian meta-ecosystems (**Paper III**);
- results show the importance of buffers in providing highly nutritious, aquatic-derived food for riparian spiders, which strengthens cross-ecosystem connectivity and has the potential to affect a wide range of consumers in modified landscapes (**Papers IV – V**);
- the Riparian Condition Index is a useful measure of riparian ecological integrity and could be used more widely (e.g. in citizen science and by practitioners) to help monitor riparian ecological status (**Paper I**).

The knowledge gained in this thesis adds to the portfolio of ecosystem attributes to help improve rehabilitation of existing and implementation of new forested riparian buffers in agricultural landscapes. As confirmed here, management practices affecting riparian ecosystems can impact stream–riparian networks as they are intricately linked by spatiotemporal flows of resources. These findings will hopefully help policy makers, land managers and stakeholders to implement effective nature-based solutions and to prioritise and allocate greater resources towards the protection and management of these unique ecosystems.

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Popular science summary

I remember a river in the village where I grew up, surrounded by lush green trees. Taking a walk on a hot summer day, seeking shelter in the shadows, getting tangled in the spiders' webs, watching birds feeding their chicks with the same spiders. The water was flowing fast and clear, bugs were thriving, fish and otters graced us with their presence. I even dared to take a quick swim…but just that one time. And then the highway came, claimed parts of the stream which were replaced with a concrete channel. Next followed the conflicts, with landowners ploughing their fields all the way to the stream edge, complaining that leaves from the trees damage their crops, and that trees are responsible for the flooding…and finally, somebody out there in the shiny office in the middle of the concrete jungle decided that trees have to go. A whole ecosystem disappeared before my eyes in only few days. Trees were forever lost, replaced by steep slopes of managed grass. So I wondered, if this is the case with streams and rivers all over the world, how bad is the ecological status of streams and their associated stream-side habitats, and what can be done about it?

We live in an era which is commonly called the 'Anthropocene', where human activities are the main agent of ecological change on Earth in general, and in freshwater systems in particular. Despite the management efforts and legislations in place working towards water protection, ecological status in European freshwaters has declined by 50% in the last few decades. Widespread anthropogenic pressures like agricultural and urban land use intensification, in combination with climate change and hydromorphological alterations, are resulting in habitat fragmentation and degradation not only in stream and river ecosystems, but also in their associated stream-side or "riparian" habitats. Removal of trees and shrubs from riparian zones in agricultural areas can have negative effects on nutrient cycling, erosion, temperature regimes, and habitat quality, and subsequently on biodiversity and key organism groups in both stream and riparian ecosystems. Most importantly, human impacts can reduce the flows of nutrients and materials that connect stream–riparian habitats into a so-called “meta-ecosystem”. Returning to my earlier story, this means that the animals that I saw sheltering in the shadows of the riparian forest (spiders feeding on a delicious stream insect, birds eating the spiders) are hindered in the resource “subsidies” they are able to draw from the adjacent stream, with connectivity in the stream-riparian food web damaged or destroyed.

My thesis looked into the role of forested buffers for improving ecological status in linked stream–riparian habitats in human-modified landscapes across Europe, with focus on streams in Lake Mälaren catchment around Uppsala (Sweden). I specifically wanted to know how buffers affect composition of stream-living macroinvertebrates and riparian spiders, and on the trophic linkages between them. Further, I investigated whether riparian buffers as a local-scale management measure are sufficient for improving ecological status and connectivity in both stream and riparian habitats, or whether we need to look at the degradation further upstream in the catchment, and which local- and catchment scale environmental variables are important for the effects we observed. The results from both Swedish and European streams demonstrated positive effects on instream and riparian communities in the presence of buffers. Moreover, I have seen the evidence of stronger and more connected stream-riparian food webs mediated by emerged adult aquatic insects (e.g. mayflies and caddisflies) that carry important omega-3 fatty acids and serve as a source of food for terrestrial predators (spiders). My findings indicate that local actions could be sufficient for mitigating some of the impact from surrounding agricultural area, more so for riparian- than instream organisms.

Ultimately, my thesis provides scientific based evidence for the rehabilitation and implementation of buffers and preservation of functional integrity of our streams and riparian zones for future generations.

Populärvetenskaplig sammanfattning

Jag minns en flod i byn där jag växte upp, omgiven av grönskande träd. Att ta en promenad en varm sommardag, söka skydd i skuggorna, trassla in sig i spindelnäten, titta på fåglar som matar sina ungar med samma spindlar. Vattnet rann snabbt och klart, insekter frodades, fiskar och uttrar hedrade oss med sin närvaro. Jag vågade till och med ta ett snabbt dopp...men bara den enda gången. Och så kom motorvägen, gjorde anspråk på delar av floden som ersattes med en betongränna. Därefter följde konflikterna, med markägare som plöjde sina åkrar hela vägen till strandkanten och klagade på att löv från träden skadade deras skördar och att träden orsakade översvämningar... och slutligen någon där ute i ett blankt kontor i mitten av betongdjungeln som beslutade att träden måste bort. Ett helt ekosystem försvann framför mina ögon på bara några dagar. Träd som för alltid var förlorade, ersattes av branta sluttningar av skött gräs. Så jag undrade, om det här händer med bäckar och floder över hela världen, hur dålig är den ekologiska statusen i vattendrag och deras tillhörande livsmiljöer, och vad kan man göra åt det?

Vi lever i en era som vanligtvis kallas "antropocen", där mänskliga aktiviteter är den främsta orsaken till ekologisk förändring på jorden i allmänhet, och i sötvattensystem i synnerhet. Trots förvaltningsinsatser och lagstiftning som arbetar för att skydda vatten har den ekologiska statusen i europeiska sötvatten försämrats med 50 % under de senaste decennierna. Utbredda antropogena påfrestningar som intensifierad markanvändning av jordbruk och städer, i kombination med klimatförändringar och hydromorfologiska förändringar, resulterar i habitatfragmentering och försämring, inte bara i ström- och flodekosystem, utan också i deras associerade livsmiljöer längs strandkanten. Borttagning av träd och buskar från strandzoner i jordbruksområden kan ha negativa effekter på näringsämnens cirkulation, erosion, temperaturregimer och livsmiljökvalitet, och därefter på biologisk mångfald och nyckelorganismgrupper i både bäckars och floders ekosystem. Det allvarligaste är att mänsklig påverkan kan minska flödena av näringsämnen och material som sammankopplar livsmiljöer i vattendrag och på stränder till så kallade "meta-ekosystem". För att återgå till min tidigare berättelse betyder det här att djuren som jag såg söka skydd i skuggorna av strandskogen (spindlar som livnär sig på en läcker bäckinsekt, fåglar som äter spindlarna) hindras i vilka resurser de kan använda från den intilliggande floden, när sammankopplingarna i födoväven är skadade eller förstörda.

Min avhandling undersökte vilken roll skogklädda buffertar spelar för att förbättra den ekologiska statusen i sammanlänkade ström-strandlivsmiljöer i mänskligt modifierade landskap över hela Europa, med fokus på vattendrag i Mälarens avrinningsområde runt Uppsala (Sverige). Jag ville specifikt veta hur buffertar påverkar sammansättningen av strömlevande makroevertebrater och strandspindlar, och om de trofiska kopplingarna mellan dem. Vidare undersökte jag om strandbuffertar, som en lokal förvaltningsåtgärd, är tillräckliga för att förbättra ekologisk status och förbindelser i både vattendrags- och strandlivsmiljöer, eller om vi behöver titta på degradering längre uppströms i avrinningsområdet, och vilka miljövariabler på lokal- och avrinningsområdesskala som är viktiga för de effekter vi observerade. Resultaten från både svenska och europeiska vattendrag visade positiva effekter på organismsamhällen i vattendragen och längs stränderna i närvaro av buffertar. Dessutom har jag sett bevis på starkare och mer sammankopplade födovävar i strandkanten som möjliggörs när vattenlevande insekter kläcker från vattenlevande larver till terrestra adulter (t.ex. dagsländor och nattsländor) vilka innehåller viktiga omega-3-fettsyror och tjänar som födokälla för landlevande rovdjur (spindlar) . Mina resultat tyder på att lokala åtgärder kan vara tillräckliga för att mildra en del av påverkan från det omgivande jordbruksområdet, men mer för organismer längs strandkanten än de i vattendragen.

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To my favourite : no need for words, you know how much you mean to me…get ready, our new adventures are just around the corner ;)

To my old friends: look how far we've all come since that first day at PMF ☺ All these years, together through thick and thin…what more can I wish for?

To my new friends: you are the sunshine during the grey Swedish days. Hope we will continue our long conversations and laughs, wine & dine evenings, coffee/lunch breaks, game nights and dinners, road trips...and share many more journeys together, wherever we are.

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I

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MDPI

Article

Assessing the Benefits of Forested Riparian Zones: A Qualitative Index of Riparian Integrity Is Positively Associated with Ecological Status in European Streams

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Abstract: Developing a general, predictive understanding of ecological systems requires knowing how much structural and functional relationships can cross scales and contexts. Here, we introduce the CROSSLINK project that investigates the role of forested riparian buffers in modified European landscapes by measuring a wide range of ecosystem attributes in stream-riparian networks. CROSSLINK involves replicated field measurements in four case-study basins with varying levels of human development: Norway (Oslo Fjord), Sweden (Lake Mälaren), Belgium (Zwalm River), and Romania (Argeş River). Nested within these case-study basins include multiple, independent stream-site pairs with a forested riparian buffer and unbuffered section located upstream, as well as headwater and downstream sites to show cumulative land-use impacts. CROSSLINK applies existing and bespoke methods to describe habitat conditions, biodiversity, and ecosystem functioning in aquatic and terrestrial habitats. Here, we summarize the approaches used, detail protocols in supplementary materials, and explain how data is applied in an optimization framework to better manage tradeoffs in multifunctional landscapes. We then present results demonstrating the range of riparian conditions present in our case-study basins and how these environmental states influence stream ecological integrity with the commonly used macroinvertebrate Average Score Per Taxon

(ASPT) index. We demonstrate that a qualitative index of riparian integrity can be positively associated with stream ecological status. This introduction to the CROSSLINK project shows the potential for our replicated study with its panoply of ecosystem attributes to help guide management decisions regarding the use of forested riparian buffers in human-impacted landscapes. This knowledge is highly relevant in a time of rapid environmental change where freshwater biodiversity is increasingly under pressure from a range of human impacts that include habitat loss, pollution, and climate change.

Keywords: benthic invertebrates; land use; agriculture; urbanization; riparian management; riparian buffer; nature-based solutions; blue-green infrastructure; climate-change adaptation; protocols

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1. Introduction

Riparian zones are the interface between aquatic and terrestrial ecosystems that connect and help regulate ecological functions in both habitats [1,2]. They are three-dimensional zones encompassing hydrogeomorphic, vegetational, and food-web attributes which vary in space and time [1,3,4]. The importance of riparian zones far exceed their proportion of land cover because of their prominent location at the boundary between aquatic and terrestrial ecosystems [1,5]. For example, riparian zones are important habitats for maintaining biodiversity and provide multiple ecosystem services that include water purification, carbon storage, and recreational opportunities [6,7]. In particular, stream and terrestrial ecosystems can be highly connected by exchanges of organic matter and prey [3,8]. These ecosystem linkages include inputs of terrestrial detritus and prey that help sustain aquatic food webs [9,10], and the emergence of adult aquatic insects form an important source of prey for a wide range of riparian consumers that include spiders, birds, lizards, and bats [11,12]. However, human pressures from activities such as deforestation, agriculture, and urbanization frequently degrade stream-riparian networks [13], with potential consequences for cross-habitat linkages and ecosystem services through impacts on aquatic and terrestrial assemblages [7,11,14].

The impacts of human land uses on stream-riparian networks typify the “Anthropocene”—the current epoch of immense environmental upheaval caused by human activities [15,16]. These impacts disproportionately threaten freshwater biodiversity globally [17,18], and with land-use intensification set to continue there is a strong need for improved riparian management [19,20]. Thus, protecting and enhancing riparian zones are often seen as the first steps towards rehabilitating degraded waterbodies by buffering them from the impacts of adjacent human land uses. The conservation, rehabilitation, and restoration of riparian zones fits within the concept of nature-based solutions: “living solutions inspired and supported by nature that simultaneously provide environmental, social and economic benefits and help build resilience” ([21]; see also Table 1) and potentially mitigate adverse effects in catchments where human land uses have strong impacts [22]. In highly fragmented landscapes, riparian buffers (Table 1) can preserve natural habitat features, thus helping to ensure genetic and ecological connectivity amongst populations and communities [7,23–25]. Further, riparian buffers are often used to filter nutrients and fine inorganic sediment from adjacent land uses, and depending on the canopy-cover proffered, help shade stream reaches to reduce water temperatures and proliferations of aquatic vegetation [26–28].

However, the effectiveness of riparian buffers can depend on a variety of factors. For instance, buffers may be placed randomly on a stream network without integrated catchment management, meaning upstream human impacts can override any benefits of riparian management at the reach scale [29,30]. The uncertainties generated by this problem may contribute to the current situation where few countries have extensive national regulations for buffer properties, although some countries do require uniform riparian buffer strip widths (e.g., 5 m) [26]. Moreover, gaps in our current scientific knowledge and legal frameworks could mean such regulations are insufficient for meeting management goals (e.g., Water Framework Directive) or are impractical for land managers seeking to implement riparian buffers [26,31,32]. These challenges reflect the increased demand for knowledge on how freshwater ecosystems respond to various levels of perturbations (e.g., human land uses) and what level of mitigation is required for recovery to occur [33].

Here, we introduce the BiodivErSA-funded CROSSLINK project (see Table 1 for a glossary of terms) by highlighting the key questions it addresses and the methods underpinning the extensive data collection helping to better understand riparian zones in human-influenced landscapes (Table 2 and Supplementary Materials). CROSSLINK involves replicated field studies across four case-study basins (Figure 1) in Norway (forested and urban stream reaches in the Oslo Fjord basin), Sweden (forested and agricultural stream reaches in the Lake Mälaren basin), Belgium (forested, agricultural and urban reaches in the Zwalm river basin), and Romania (forested and agricultural stream reaches in the Argeş river basin). CROSSLINK conceptualizes stream-riparian networks as key components of blue-green infrastructure (BGI) that are subject to multiple human pressures including water extraction, hydropower generation, forestry, agriculture, and urbanization leading to ecological harm and stakeholder conflicts [18,34].

In the broadest terms, CROSSLINK aims to (1) evaluate how the extent, spatial arrangement and connectivity of riparian-stream BGI affects biodiversity, ecosystem functioning, ecosystem services, and resilience indicators in forested, rural, and urban settings; and (2) produce an optimization framework capable of balancing multiple values, uses and needs with longer-term adaptive capacity and resilience in riparian-stream BGI. Underpinning the latter objective is the multifunctionality of landscapes as a key concept for solving resource-use conflicts with an emphasis on trade-offs between agricultural production and other values [35].

In this introduction to the CROSSLINK project, we analyze data on riparian habitats described using the qualitative index of riparian integrity (the Riparian Condition Index—RCI) developed by Harding et al. [36] for New Zealand conditions and adapted here for Europe. The RCI is comprised of 13 attributes (Table 3) that are scored 1–5 (poor to good) for both banks and then averaged. Their summed total provides an overall index that can be associated with stream ecological responses (e.g., reference [37]). We first assessed the overall performance of the RCI for characterizing riparian integrity in study reaches with varying levels of human impact (from reference or least impacted to strongly impacted by adjacent and upstream agricultural and/or urban land uses). Our a priori expectation was that buffered sites would have higher RCI scores more similar to the reference site scores than unbuffered sites. Following Burdon et al. [37], we hypothesized that our estimates of riparian condition would be positively associated with stream ecological status after accounting for upstream human impacts. To test this hypothesis and thus assess the utility of the RCI for predicting stream ecological status we used the macroinvertebrate Average Score Per Taxon (ASPT) index [38], which is used in environmental reporting for the European Union's Water Framework Directive (WFD) [39–41].

Finally, we adapted the conceptual framework introduced by Burdon et al. [42] for understanding the role of forested riparian buffers in heterogenous landscapes. This framework considers how the extent of change in a biotic response may be determined by the magnitude of a local "transition" (here the change from an "unbuffered" riparian state to a woody vegetation patch providing a forested riparian buffer) or contingent on the environmental context (e.g., the level of catchment degradation). More specifically, biotic changes in response to riparian "buffering" can be predicted to be the product of a community's sensitivity (or tolerance; *sensu* "negative resilience" [43]) and the magnitude of the transition from an unbuffered to buffered state. In this example, the framework introduces a pivotal question: does the quality and quantity of the riparian buffer determine the ecological response, or is it environmentally contingent on other factors?

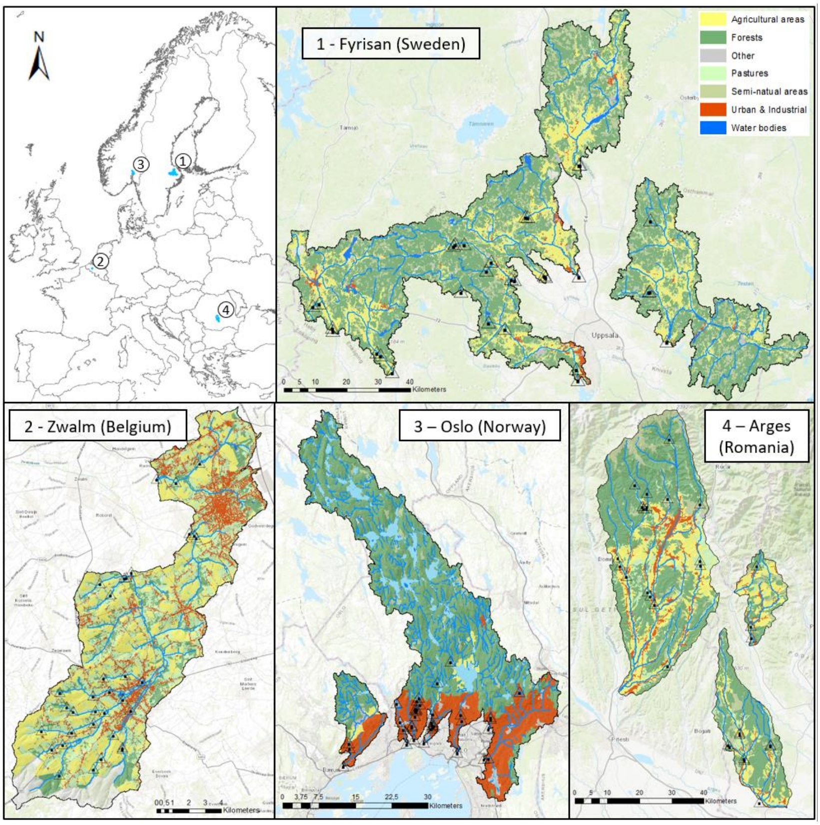

Figure 1. Map of Europe showing the locations of the four case-study basins used in the CROSSLINK project: (**1**) Sweden (forested and agricultural stream reaches in the Lake Mälaren basin), (**2**) Belgium (forested, agricultural and urban reaches in the Zwalm river basin), (**3**) Norway (forested and urban stream reaches in the Oslo Fjord basin), and (**4**) Romania (forested and agricultural reaches in the Argeş river basin).

Table 1. Glossary of key terms related to the CROSSLINK project.

Term	Definition
BiodivERsA	BiodivERsA is a network of national and regional funding organizations promoting pan-European research on biodiversity and ecosystem services, funded under the Horizon 2020 European Research Area (ERA-NET) COFUND scheme.
CROSSLINK	The full title of the CROSSLINK project is "Understanding cross-habitat linkages between blue and green infrastructure to optimize management of biodiversity, ecosystem services and multiple human uses." The CROSSLINK project is funded under the 2015 pan-European BiodivErSA call for international research projects on "Promoting synergies and reducing trade-offs between food supply, biodiversity and ecosystem services." Specifically, CROSSLINK addresses the theme "Understanding and managing biodiversity dynamics in land-, river- and seascapes (habitat connectivity, green and blue infrastructures, and naturing cities) to improve ecosystem functioning and delivery of ecosystem services."

Table 1. *Cont.*

Term	Definition
Blue-green infrastructure (BGI)	The concept of blue-green infrastructure emphasizes the importance of both "blue" (water) and "green" (vegetation) and the interaction between them [44]. The word infrastructure underscores the need for these different elements to be interlinked to work as a connected web of measures [45]. Elements of BGI are nature-based solutions that deliver multiple co-benefits to impacted environments such as urban ("grey") cityscapes; benefits include water supply, flood mitigation, terrestrial biodiversity, cooling and climate change resilience, and human well-being [46–48].
Nature-based solution (NBS)	Nature-based solutions are "living solutions inspired and supported by nature that simultaneously provide environmental, social and economic benefits and help build resilience" [21]. BGI can be NBS by providing natural ways to manage water resources and the environment [21]. The economic benefits of NBS have been promoted by the European Commission, as well as advocated by researchers [49].
Riparian buffer	A riparian buffer is a vegetated area (a "buffer strip") that helps to protect the stream from the impact of adjacent land uses [26]. A forested riparian buffer is a buffer strip dominated by woody vegetation, which in addition to helping protect the stream from human land-use impacts can also provide stream shading and crucial habitat diversity in fragmented landscapes.
Water Framework Directive (WFD)	The European Water Framework Directive (2000/60/EC; WFD) is a policy statement that establishes a framework for water protection so that all waterbodies in Europe reach "good ecological status" by 2021 or 2027.

2. Materials and Methods

2.1. Study Design

CROSSLINK has a tiered study design (Figure S1, Supplementary Materials). First, the "paired approach" tested aspects of lateral and longitudinal connectivity. This approach required 10–12 streams in each case-study basins flowing through an impacted (agricultural, urban or mixed agricultural and urban) landscape, each with two paired sites: an upstream site with no riparian buffer (i.e., "unbuffered"), and a downstream "buffered" site with a riparian buffer (i.e., leading to 20–24 sites in total). Second, the "network approach" testing aspects of longitudinal connectivity involved 10–12 additional sites distributed throughout the river network (e.g., upstream and downstream of the site pairs). Within these sites we sought pristine or least impacted headwater sites and more degraded, downstream longitudinal sites to help characterize the range of responses in ecosystem attributes to cumulative impacts of catchment land uses. Hereafter, the headwater sites are described as "reference" sites, and the downstream longitudinal sites are described as "matrix" sites because of their location further downstream in our landscape matrices (i.e., the portion of the heterogeneous landscape in which stream-riparian segments are "embedded").

To ensure consistency and feasibility, streams used were wadeable, 1st–3rd order (i.e., approximately 2–5 m wide), and with a stable streambed (i.e., not frequently hydrodynamically disturbed) dominated by gravels and cobbles. In the stream reaches categorized as reference, buffered, and unbuffered, we focused on the presence and extent of woody vegetation in the riparian zone. Reference sites typically had intact forest extending to the upstream catchment boundaries. Both buffered and unbuffered sites were in human-impacted landscapes (i.e., impacted by urban or agricultural land uses). Key criteria applied during site selection of buffered sites included requirements for minimum buffer length (i.e., >50 m moving upstream from the downstream end of the sampling reach), width (>2–3 × wetted stream width), extent (buffer on both banks of the stream segment), and composition (dominated by small and large trees). Unbuffered sites typically only had a few isolated trees within the riparian zone.

The main criteria for the matrix sites were their network position, being located lower down in the catchment and subjected to higher levels of human impacts. The matrix sites also lacked an extensive riparian buffer as defined by the criteria outlined above. At each site, the different components of sampling for CROSSLINK were conducted over two reaches differing in length, with a shorter effective sampling reach nested in a longer habitat assessment reach (Figure S2, Supplementary Materials). Key components of terrestrial and aquatic habitat sampling were conducted within the longer habitat assessment reach (50 m long). The biological sampling (i.e., biodiversity and ecosystem functioning measures) were conducted within the shorter effective sampling reach (30 m long), which had flowing water (i.e., run-riffle sequence) with hard-bottomed sections (i.e., with cobble, pebble, gravel, and/or bedrock substrates). Both reaches begun at the same point at the downstream end, which in the case of buffered sites was located as far downstream as possible within the woody riparian buffer. See Protocol S1, Supplementary Materials for more details.

2.2. Sampling Overview

We sampled multiple environmental, biodiversity, and ecosystem functioning attributes at sites in our CROSSLINK stream-riparian networks (Table 2). Detailed protocols for all our measured variables are provided in the Supplementary Materials.

Table 2. Overview of ecosystem attributes and approaches used in the CROSSLINK project to describe the multiple ecological benefits of forested riparian buffers in human-impacted landscapes.

Group	Response	Description
Environmental (Protocol S2)	Water quality	Grab water samples and spot measurements for a wide range of water chemistry parameters
	Thermal dynamics	Spot measurements and continuous logging of stream and riparian temperatures
	Instream habitat	Transect measurements of channel profiles and benthic habitat assessment
	Hydromorphological impacts	Assessment of human activities affecting hydrogeomorphic integrity
	Riparian habitat	Assessment of riparian condition and measurement of key habitat properties in six 50 m^2 plots (Figure S3, Supplementary Materials)
	Land use	Use of CORINE land cover inventory to describe catchment land uses
Biodiversity (Protocol S3)	Microbial	Environmental samples for microbial (e.g., bacterial) diversity from stream and riparian zone in effective sampling reach (ESR)
	Diatoms	Semi-quantitative sampling of benthic diatoms in ESR
	Macroinvertebrates	Quantitative sampling of aquatic macroinvertebrates in ESR
	Riparian invertebrates	Semi-quantitative sampling of terrestrial arachnids and predatory ground beetles in riparian plots
	Trees	Recording trees species and size (DBH) in riparian plots
Ecosystem functions (Protocol S4)	Algal accrual	Measurement of periphyton biomass on standardized substrates in ESR
	Sediment dynamics	Measurement of near-bed organic and inorganic particulate accrual on standardized substrates in ESR
	Organic-matter processing	Measuring stream and riparian organic-matter decomposition rates using litter bags and the cotton-strip assay
	Carbon sequestration	Using allometric scaling relationships to estimate tree biomass and carbon sequestration potential in riparian plots
Food webs (Protocol S5)	Trophic diversity	Use of stable isotopes (C and N) to describe community trophic niche breadths
	Energy flow	Using Bayesian mixing models to estimate consumer diets based on stable isotope measurements of basal resources and prey
	Trophic connectivity	Use of fatty acid biomarkers (e.g., poly-unsaturated FAs) to describe trophic connectivity between stream and riparian food-web compartments
Societal needs (Protocol S6)	Optimization framework	Applying collected data as objective functions in an optimization framework to balance land-user needs with biodiversity and ecosystem benefits of forested riparian buffers

2.3. Riparian Habitat Assessment

Riparian habitat characteristics were surveyed in the riparian zones adjacent to the habitat assessment reach (50 m) at each study site. The surveys were carried out in summer 2018, when leaf-out was complete for all tree/shrub species, and targeted both banks. We surveyed riparian condition using an assessment of 13 qualitative variables that could indicate poor riparian status. This assessment follows the protocol described by Harding et al. [36] but adapted here for European conditions (Table 3). The protocol requires observers to rank aspects of the riparian zone that might be indicative of poor quality and integrity. Attributes were graded from poor (1) to excellent (5) on each bank over the habitat assessment reach (50 m), and scores were summed to provide an index of riparian habitat quality (the Riparian Condition Index—RCI). For the analysis of total riparian condition and individual attributes, bank scores were averaged to provide a single value for riparian condition at each stream. To ensure consistency amongst observers we ran a technical workshop for the CROSSLINK project on field protocols where we discussed riparian attributes at representative sites in Sweden as a group to ensure attributes were characterized in a consistent manner.

2.4. Water Quality

Grab water samples were collected in plastic containers for water quality analyses during three different seasons (autumn 2017, spring and summer 2018). We collected water samples from just below the water surface (i.e., 10 cm) in the channel thalweg at the downstream end of each site. Site pairs were sampled on the same day. Water samples were stored cold and refrigerated upon return to the laboratory whereby they were analyzed within 24 h of collection following standard methods [50]. Water samples were analyzed for total organic carbon, total nitrogen, ammonium (NH_4–N), nitrite- and nitrate-nitrogen (i.e., oxidized nitrogen, NO_2–N + NO_3–N), total phosphorus, dissolved reactive phosphorus (PO_4–P), specific conductivity, pH, and alkalinity. Spot water measurements for turbidity (NTU), specific conductivity, dissolved oxygen (%), and temperature were collected at the time of water sampling using a handheld instrument (e.g., Manta +30 probe, Eureka Water Probes, Austin, TX, USA). Total organic carbon, alkalinity, turbidity, dissolved oxygen, and temperature were not measured at all sites at the same time so are excluded from our statistical analyses (see below). For further details on water quality sampling, see Protocol S2.

2.5. Macroinvertebrates

We sampled macroinvertebrates within the effective sampling reach (i.e., 30 m). The reach used had flowing water (i.e., run-riffle sequence) with hard-bottomed sections (i.e., with cobble, pebble, gravel, and/or bedrock substrates). The sampling area comprised the entire stream width along the predefined reach, but we avoided sampling areas affected by flow intermittency. Quantitative sampling requires that stream invertebrates are collected from a given area with a standard sampling effort. We standardized methods to ensure comparable data using one of two potential sampling methods: Surber sampling and quantitative kick-net sampling [51]. All samplers used 500 μm mesh netting, and Surber samplers were ≈ 0.0625 m^2 (e.g., 25 × 25 cm) in dimensions. Kick-nets used were equivalent to the dimensions of the Surber sampler by using an area defined by a quadrat equaling the width of the net. Sampling effort was standardized for 60 s where coarse substrate was disturbed to a maximum depth of 10 cm from the surface of the streambed. A total of six replicate subsamples were collected (three from erosional/riffle-run habitats, and three from depositional/run-pool habitats) using identical protocols within the effective sampling reach. All subsamples were pooled together. Woody material and leaves were retained separately in a plastic bag to contribute to estimates of standing coarse particulate organic matter (CPOM). The final, pooled macroinvertebrate sample was sieved (500 μm mesh) to remove excess water and then preserved in a 500–1000 mL container with 96% ethanol to reach a final concentration of 70% for later sorting.

Macroinvertebrate samples were identified to the lowest practicable taxonomic level (e.g., species or genus) using standard identification guides. From this data, we calculated the Average Score Per Taxon (ASPT) index [38]. The ASPT index is calculated as the ratio of the score obtained in the Biological Monitoring Working Party (BMWP) index to the number of taxa scored in the sample (Equation (1)):

$$\text{ASPT} = \frac{\text{BMWP Index}}{\sum \text{Taxa}} \tag{1}$$

The BMWP index assigns scores from one to 10 to each macroinvertebrate taxa based on their sensitivity to organic pollution, ranging from zero (tolerant) to 10 (sensitive) [38]. The BMWP index is calculated as the sum of scores for all taxa present in a sample. BMWP index values greater than 100 are associated with unpolluted ("clean") streams, whilst scores less than 10 typify heavily polluted streams. Similarly, a high ASPT score is considered indicative of a "clean" (i.e., unpolluted) site containing large numbers of high scoring taxa. The ASPT index is suitable for assessing the impact of organic pollution [38]. We calculated ASPT scores with Family-level macroinvertebrate data using the function "calcBMWP" in the R package "biotic" [52].

2.6. Data Analysis

Here, we analyzed data from the CROSSLINK project on riparian conditions, stream macroinvertebrates, and catchment-wide human impacts (land use and water quality) to demonstrate the potential value of the Riparian Condition Index (RCI) for management. We used linear mixed models (LMM) to test overall differences in the summed totals of the RCI, with site type and country and their interaction as the fixed effects and site "Block" (for site pairs) as the random effect. To determine which individual attributes were contributing to impairment, we used two approaches. First, we tested each attribute individually in an LMM with site type and country and their interaction as the fixed effects and site "Block" (for site pairs) as the random effect. To visualize how attributes differed across site types, we performed a Non-Metric Multidimensional Scaling (NMDS) ordination for sites using RCI attribute scores. The function "metaMDS" in the R package "vegan" with Euclidean distances were used for the NMDS analysis of the untransformed data matrix [53]. Each attribute was treated as a "species" in the data matrix with a mean score between 1–5 at each site surveyed. We used the "adonis" and "pairwiseAdonis" R functions in the "vegan" package [53] to test for differences across all attributes between site types.

Second, we calculated log response ratios (LRR) between site pairs with the "batch_calc_ES" function in the R package "SingleCaseES" [54] to determine which attributes were most improved by the presence of a forested riparian buffer (i.e., compared with the upstream unbuffered reach). The log response ratio (LRR) is a common effect size metric (i.e., the log proportional change in the means of a treatment and control group) [55]. The LRR is particularly used in meta-analyses of ecological research [56], and for quantifying simple two-group experimental designs (i.e., buffered (B) vs unbuffered (U)) the calculation of LRR is straightforward (Equation (2)):

$$\text{LRR} = \ln\left(\frac{\overline{X}_B}{\overline{X}_U}\right) \tag{2}$$

Effect sizes of attributes were ranked and presented graphically at the overall European level and for each case-study basin. The livestock access attribute was excluded in the analysis of the Norwegian sites because it was given a constant value (5) reflecting the urban nature of the catchment. Similarly, the soil quality attribute was excluded in the analysis of the Belgian sites because it consistently had an intermediate value (3) at both sites. The linear mixed models were fitted with the "lmer" function in the "lmer4" R package, and post-hoc tests conducted using the "lmertest" R function [57].

We used an indicator of stream ecological integrity (i.e., the Average Score Per Taxon index (ASPT)) to assess the utility of the Riparian Condition Index (RCI) whilst controlling for catchment-wide human impacts. We used Principal Components Analysis (PCA) to describe catchment-wide anthropogenic impacts (i.e., upstream influences). The PCA decomposed log-transformed water quality variables (i.e., total inorganic nitrogen, ammonium, nitrite- and nitrate-nitrogen, total phosphorus, dissolved reactive phosphorus, specific conductivity, and pH) and logit-transformed upstream land-use cover variables (i.e., % of the catchment area covered by urban, arable cropping, orcharding and vineyards, pasture, forest, natural features, water, wetlands, and other) into site scores (Axis 1, henceforth PC1) explaining 37% of total variation. Upstream land-cover estimates were obtained from the CORINE Land Cover inventory [58]. First, we tested the association of the RCI with the ASPT index using a mixed model where we included PC1 as a fixed control variable and specified "country" and "site pairs" as random effects. We excluded forested reference sites from Sweden (n = 5) in the mixed model because these streams went into extreme low flows (or dried completely) in the summer prior to macroinvertebrate sampling, potentially explaining the lower than expected values for the ASPT index. This omission did not alter the conclusions inferred from the statistical test, although it did improve the model fit. The linear mixed model was fitted with the "lmer" R function.

Table 3. Scores for riparian attributes used to calculate the Riparian Condition Index (RCI) (adapted for European conditions from Harding et al. [36]).

Attributes		Score 1	Score 2	Score 3	Score 4	Score 5
Shading of water		Little or no shading	10%–25% shading	25%–50%	50%–80%	>80%
Buffer width		<1 m	1–5 m	5–15 m	15–30 m	>30 m
Buffer intactness		Buffer absent	50%–99% gaps	20%–50% gaps	1%–20% gaps	Completely intact
Vegetation comp. of buffer and/or adjacent land to 30 m from streambank	Buffer Adj. land	Short grazed pasture grasses to stream edge, or impervious surfaces	Weedy shrubs or mainly long grasses 0.3–2 m or herbs/forbs	Deciduous tree dominated; small tree dom. (2–5 m); or forest plantation with < 25% cover of > 5 m trees; or natural grassy veg.	Regenerating forest or woodlot evergreens with > 25% cover sub-canopy (>5 m) trees but < 10% canopy trees (>12 m); or natural grassy veg.	Maturing forest including >10% cover canopy trees (>12 m); or natural wetland or natural grassy vegetation
Bank stability		Very low: uncohesive sediments and few roots and > 40% recently eroded	Low: uncohesive sediments and few roots/low veg. cover and > 15%–40% recently eroded	Moderate: stabilized by geology (e.g., cobbles), veg. cover and/or roots and > 5%–15% recently eroded	High: stabilized by geology (e.g., bedrock), veg. cover and/or roots; and 1%–5% recently eroded	Very high: stabilized by geology (e.g., bedrock), veg. cover and/or roots; < 1% recently eroded
Livestock access		High: unfenced and unmanaged with active livestock use	Moderate: some livestock access	Limited: unfenced but low stocking, bridges, troughs, natural deterrents	Very limited: temporary fencing of all livestock or naturally very limited access	None: permanent fencing or no livestock
Riparian soil denitrification potential		Soils dry/firm underfoot or moist–wet but frequent tile drains bypass riparian soils (≥3 per 100 m)	1%–30% streambank soils moist but firm or moist-wet with infrequent bypass drains (1–2 per 100 m)	≥30% streambank soils moist but firm underfoot. No drains.	1%–30% streambank soils water-logged, soft underfoot with black soil. No drains.	≥30% of streambanks water-logged, surface moist/fluid underfoot. No drains.
Land slope 0–30 m from stream bank		>35°	>20–35°	>10–20°	>5–10°	0–5°
Groundcover of buffer and/or adjacent land to 30 m from streambank	Buffer Adj. land	Bare	Short/regularly grazed pasture (<3 cm)	Pasture grasses or crops with bare flow paths or 2–3 cm tree litter layer	Moderate density grass or crops dense (>3 cm) tree litter layer	High density long grasses or crops
Soil drainage		Impervious (e.g., sealed) or extensively pugged and/or compacted soil	Low permeability (e.g., high clay content) or moderately pugged/compacted soil	Low-moderate permeability (e.g., silt/loam) and not pugged/compacted	Mod-high permeability (e.g., sandy loam) and not pugged/compacted	Very high permeability (e.g., pumice/sand) and not pugged/compacted
Rills/channels		Frequent rills (>9 per 100 m) or larger channels carry most runoff	Common rills (4–9 per 100 m) or 1–2 larger channels carry some runoff	Infrequent rills (2–3 per 100 m) and no larger channels	Rare rills (1 per 100 m) and no larger channels	None

The framework introduced in Burdon et al. [42] describes how the magnitude of change in a biotic response can be determined by the size of a local "transition" (here the change from an "unbuffered" riparian state to a woody vegetation patch providing a forested riparian buffer) or be context-dependent, reflecting the prevailing upstream environmental conditions. In our study, changes in response to riparian "buffering" can be predicted to be the product of the community's sensitivity (or tolerance; *sensu* "negative resilience" [43]) and the magnitude of the transition from an unbuffered to buffered state (Equation (3)). This approach corresponds to a (local) sensitivity analysis [42]:

$$\Delta \mathrm{Y_i} = \frac{\partial \mathrm{Y_i}}{\partial \mathrm{D}} \times \Delta \mathrm{R} \tag{3}$$

where Y is an ecological metric characterizing the status of an ecosystem. The state of the system at i (i.e., a point in time or space) can be defined as the ecological status Y_i relative to an existing level of perturbation D (e.g., $\partial \mathrm{Y_i}/\partial \mathrm{D}$). Here, $\Delta \mathrm{Y_i}$ describes the response of the ecosystem to the transition between "impacted" and "buffered" states as defined by ΔR. By quantifying $\Delta \mathrm{Y_i}$, ΔR, and the existing level of impairment (e.g., $\partial \mathrm{Y_i}/\partial \mathrm{D}$) it is possible to test the extent to which the sensitivity (or tolerance) varies with ecological status (i.e., environmental context). We apply the general approach described in Equation 3 to assess stream macroinvertebrate responses using change in the ASPT index between unbuffered and buffered sites. We hypothesized that using the RCI to measure the "magnitude of transition" between buffered and unbuffered states would reveal the benefit conferred to the stream invertebrate community whilst controlling for "environmental context" (i.e., the existing level of environmental degradation at the upstream site).

To test our hypothesis regarding the magnitude of transition and environmental context, we calculated log response ratios for the ASPT and the RCI, with the latter being the response variable (ΔASPT) and the former a predictor (i.e., the "magnitude of transition" hypothesis, ΔRCI). We used upstream site scores of catchment-wide human impacts (PC1) to represent the "environmental context" hypothesis. We tested the contribution of each hypothesized driver [i.e., the magnitude of transition (ΔRCI) vs environmental context (PC1)] and their interaction to the change in stream ecological status (ΔASPT) between site pairs using a mixed model with "country" as the random effect. To fit the mixed models, we used the R function "blmer" and tested for significance using Wald tests. The variance explained by the fixed and random effects was determined following Nakagawa and Schielzeth [59]. We visualized the results using the "scatter3D" function in the "plot3D" R package. All analyses were conducted in R [60].

3. Results

3.1. Riparian Integrity across Case-Study Basins

The Riparian Condition Index (RCI) was able to distinguish buffered and forested reference sites from the more degraded unbuffered and downstream "matrix" site types across our four case-study basins (Figure 2). However, the differences between buffered and forested sites ("lsmeans," $t = -2.593$, $P = 0.052$) and unbuffered and matrix site types ($t = 2.450$, $P = 0.074$) were not significant at $\alpha = 0.05$. These differences were typically conserved across the case-study basins, with a few exceptions. In Belgium and Romania, RCI scores for the downstream matrix sites did not differ significantly from the buffered and forested sites (Figure S4, Supplementary Materials).

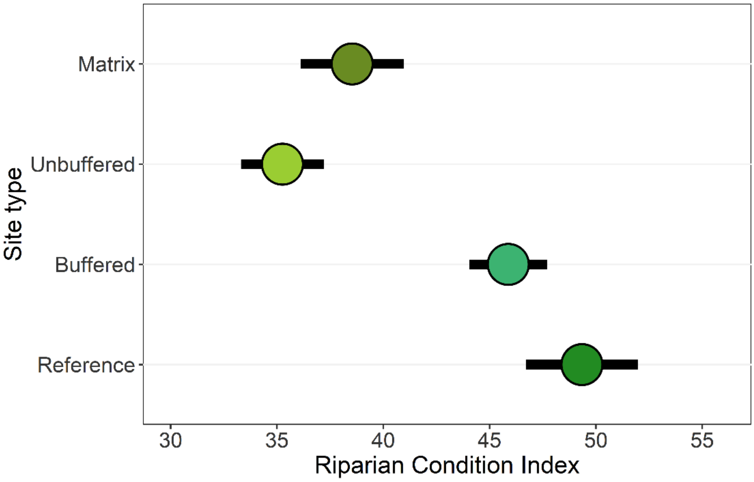

Figure 2. Mean values (± 95% CI) of the Riparian Condition Index for site types in the CROSSLINK project (including sites in Norway, Sweden, Belgium, and Romania). "Matrix" refers to sites that were typically located further downstream in our catchment landscape matrices (i.e., the portion of the heterogeneous landscape in which stream-riparian segments are "embedded").

Overall, the Scandinavian countries generally had higher RCI scores indicating better riparian conditions, with Sweden achieving on average a higher level of riparian integrity than the other three countries, and Norway having overall better riparian status than Belgium (Figure S4, Supplementary Materials). However, accounting for interactions between country and site type revealed that Norway only had significantly better riparian status in their unbuffered sites when compared with Belgium ($t = -2.831$, $P < 0.05$). In contrast, Sweden had significantly better riparian status in their buffered sites when compared with Belgium ($t = -3.962$, $P < 0.001$), and the Swedish forested reference sites had consistently higher index scores when compared to the other case-study basins (e.g., Norway–Sweden, $t = -2.713$, $P < 0.05$).

There were differences in the 13 attributes used to calculate the RCI across site types (Figure S5, Supplementary Materials). An NMDS ordination highlighted the key differences between forested reference, buffered, unbuffered and downstream matrix site types (Figure 3). This analysis showed that forested and buffered sites differed from the more degraded sites (PERMANOVA, $F_{3125} = 13.6$, $R^2 = 0.26$, $P < 0.001$) and were generally associated with high scores for shading, buffer properties such as vegetation composition, intactness, width, groundcover, and properties of adjacent land to the riparian zone (>30 m from the stream) including vegetation composition and groundcover. In contrast, the more degraded unbuffered and matrix sites typically had lower scores for these attributes and other undesirable features, such as low scores associated with increased access for livestock. The land slope attribute showed slightly higher scores in these degraded sites, indicating riparian banks that were less steep than the reference and buffered sites (Figure 3).

3.2. Effects of Forested Riparian Buffers

In statistics, an effect size is a quantitative measure of the size of the difference between two groups. We used effect sizes (log response ratios) to explicitly measure which attributes most strongly contributed to improved environmental conditions between the site pairs (i.e., unbuffered and buffered sites). This analysis strongly reflected the differences in attributes elucidated in Figure 3, with forested riparian buffers having a strong positive effect on channel shading (Figure 4). Attributes that responded with a moderate effect size to the presence of a forested riparian buffer included buffer properties such as vegetation composition (including adjacent land >30 m from the stream), width, and intactness. There were only weak positive effects sizes on the following attributes: buffer groundcover, soil drainage and livestock access. Effects sizes were negligible for rills and channels, soil quality, bank stability, and land slope.

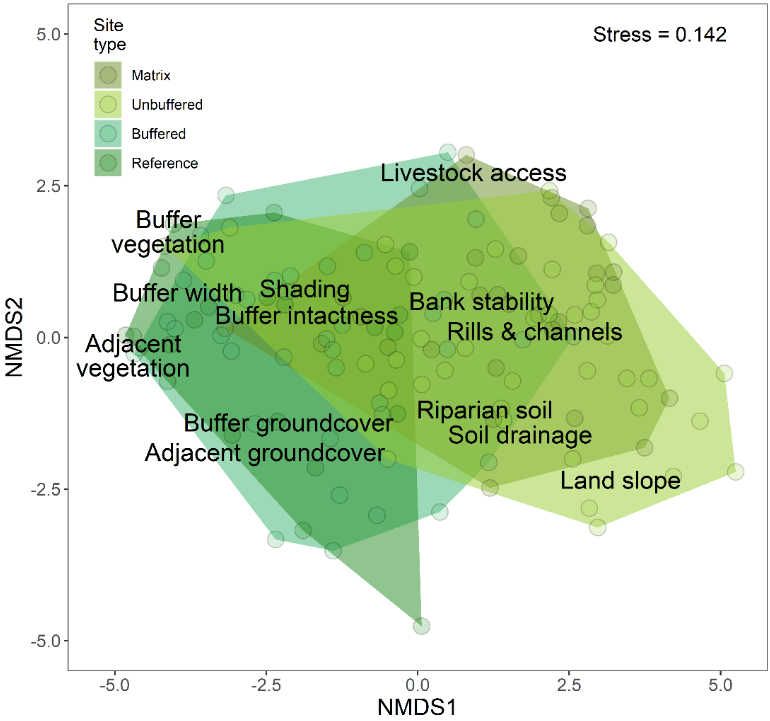

Figure 3. Unconstrained ordination (non-metric multidimensional scaling) of values for each attribute used in the Riparian Condition Index for the four site types used in the CROSSLINK project. This plot include data from sites in Norway, Sweden, Belgium, and Romania.

We also used this approach (i.e., comparing effect sizes between unbuffered and buffered sites) in each case-study basin (Figure 5). Again, the dominant trends were conserved across countries, but with some notable exceptions. The presence of a forested riparian buffer had the strongest effect on shading in three of the case-study basins (Norway, Belgium, and Romania), with the exception of Sweden where adjacent vegetation and buffer width showed stronger effects between unbuffered and buffered sites (Figure 5). In the mostly urbanized catchments of the Oslo Fjord basin in Norway, effects of buffer presence on vegetation composition and groundcover of adjacent land to the riparian zone (>30 m from the stream) was negligible. Another interesting feature in the Oslo basin was the negative effect for bank stability in the presence of a forested riparian buffer, yet the land slope attribute showed a weak to moderate positive effect size. In contrast, land slope did not change with the presence of a forested riparian buffer in the three other case-study basins.

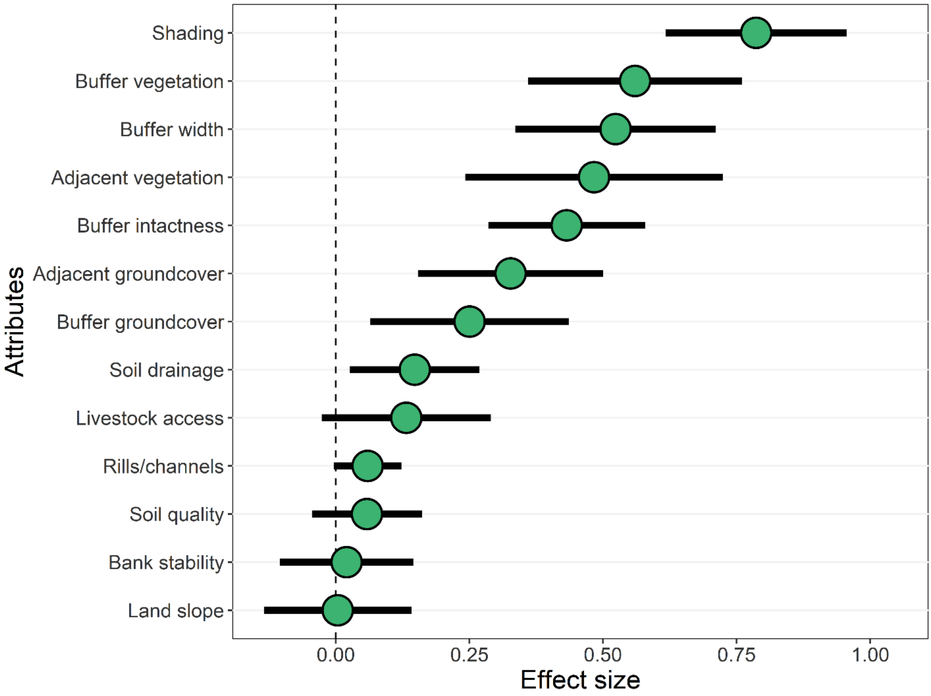

Figure 4. Mean log response ratios (±95% CI) showing the change between downstream sites buffered with woody riparian vegetation (i.e., forested) and upstream sites without this type of buffer for each attribute used in the Riparian Condition Index. This plot include data from sites in Norway, Sweden, Belgium, and Romania.

In the more agricultural catchments of Sweden's Lake Mälaren basin, the sites also showed weak to moderate positive effect sizes in the presence of a forested riparian buffer for attributes typically associated with poor land management practices such as livestock access, soil drainage, and bank stability (Figure 5). Notably, in the agriculture-dominated Argeş basin of Romania, the presence of a forested riparian buffer only brought a negligible improvement in livestock access with a large uncertainty (Figure 5).

3.3. *Riparian Condition and Stream Ecological Status*

We found a positive, albeit weak, relationship between the Riparian Condition Index (RCI) and stream ecological status as indicated by the Average Score Per Taxon (ASPT) macroinvertebrate index (Figure 6A, Table 4). There was also a significant negative relationship between the level of human impacts in the upstream catchment (PC1) and the ASPT index (Table 4). There was no significant association between the change in stream ecological status (ΔASPT) between site pairs and the size of the improvement in riparian condition (ΔRCI) after accounting for the influence of catchment-wide human impacts (PC1) and their interaction (Figure 6B, Table 4). The effect size for an improvement in stream ecological status (ΔASPT) was negatively associated with the influence of catchment-wide human impacts (PC1) after accounting for the size of riparian improvement (ΔRCI) and their interaction (Figure 6C, Table 4).

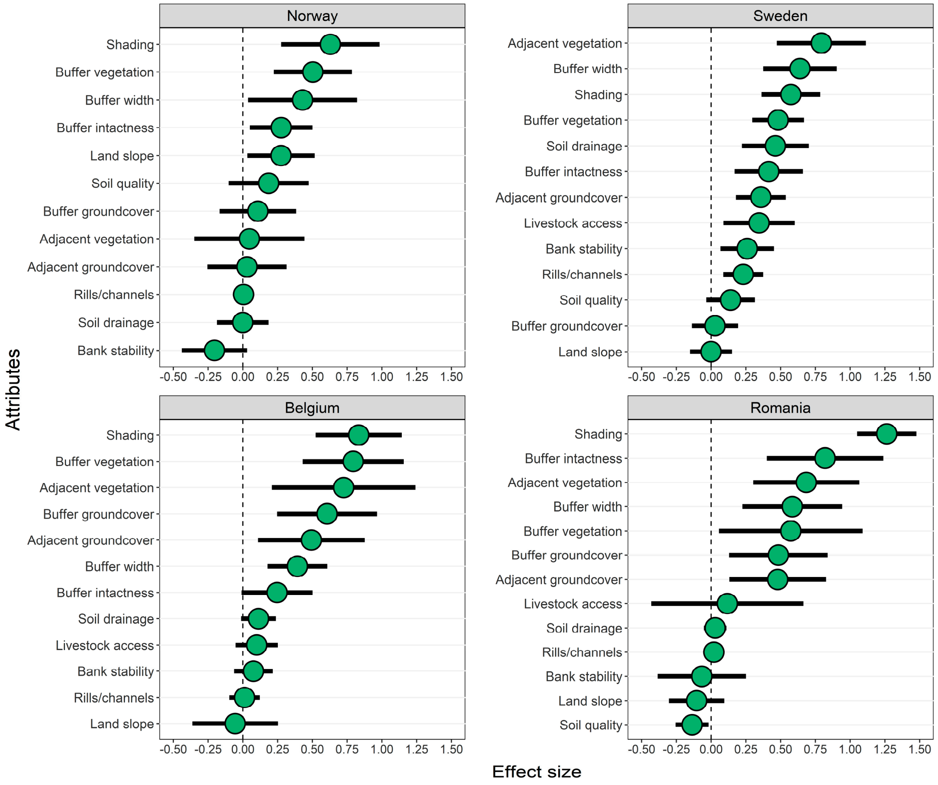

Figure 5. Mean log response ratios (±95% CI) for each CROSSLINK case-study basin. These effect sizes show the change between downstream sites buffered with woody riparian vegetation (i.e., forested) and upstream sites without this type of buffer for each attribute used in the Riparian Condition Index.

Table 4. Results from mixed models testing the influence of the Riparian Condition Index (RCI) on the macroinvertebrate Average Score Per Taxon (ASPT) index whilst controlling for catchment human impacts (PC1). The second model use log response ratios to describe the magnitude of change in the response (ΔASPT) and predictor (ΔRCI) variables between site-pairs (i.e., unbuffered upstream sites and downstream, buffered sites) whilst controlling for the existing level of ecological impairment (i.e., upstream PC1). PC1 is the Axis 1 sites scores from a Principal Components Analysis (PCA) explaining 37% variation in catchment-wide human impacts. CI, 95% confidence interval.

Response	Predictors	Estimates	CI	*P*	Marginal R^2	Conditional R^2
ASPT	(Intercept)	0.997	0.497–1.498	<0.001	0.278	0.887
	log (RCI)	0.174	0.048–0.300	0.007		
	PC1	−0.267	−0.365–−0.169	<0.001		
ΔASPT	(Intercept)	0.265	−0.043–0.430	0.050	0.140	0.467
	ΔRCI	−0.675	−1.387–0.206	0.093		
	PC1	−0.177	−0.268–0.025	0.046		
	ΔRCI × PC1	0.531	−0.035–0.922	0.031		

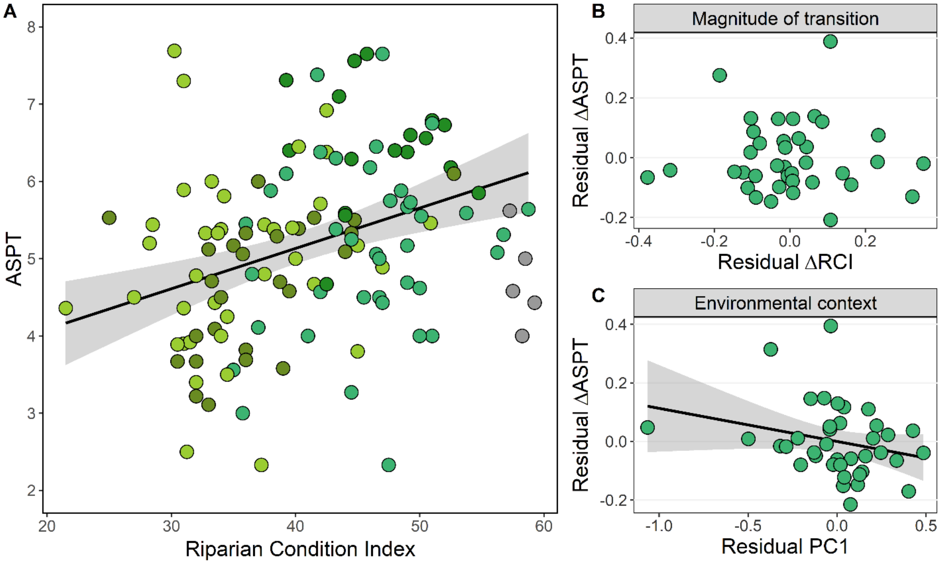

Figure 6. Plot A shows the association of the Riparian Condition Index with a commonly used stream macroinvertebrate indicator, the Average Score Per Taxon (ASPT) index. Outlier sites in grey indicate "reference" forested sites in Sweden which went dry in the summer prior to macroinvertebrate sampling, potentially explaining lower than expected values for the ASPT. Plots B and C show the individual strength of the riparian "magnitude of transition" and the "environmental context" hypotheses after accounting for other influences across our four case-study basins. These analyses use the log response ratio for the Average Score Per Taxon index score at paired sites as the response variable (ΔASPT). The riparian "magnitude of transition" predictor uses the log response ratio for the Riparian Condition Index at paired sites (ΔRCI). The "environment context" predictor (PC1) is the Axis 1 sites scores from a PCA explaining 37% variation in catchment-wide human impacts (i.e., indicating the level of upstream degradation). See Table 4 for results from mixed models testing these responses.

However, the interaction between the improvement in riparian condition (ΔRCI) and catchment-wide human impacts (PC1) was significant (Table 4), and Figure 7 shows that the "magnitude of transition" hypothesis (i.e., ΔRCI) was contingent on the level of upstream degradation. In less impacted sites, the improvement in riparian condition (ΔRCI) had a negative relationship with the improvement in stream ecological status (ΔASPT; Figure 7). Contrasting with this result, sites that were more affected by upstream human activities showed that the size of the improvement in riparian condition (ΔRCI) was positively associated with the improvement in stream ecological status (ΔASPT; Figure 7).

4. Discussion

Riparian zones hugely influence fluxes that connect aquatic-terrestrial habitats, making them disproportionately important in terms of land area for these coupled meta-ecosystems [1,5]. For the CROSSLINK project we adapted and developed multiple approaches for measuring environmental, biodiversity, and ecosystem functioning attributes in stream-riparian networks (Table 2 and Supplementary Materials). Here, we demonstrate the value of our project by analyzing data collected for the Riparian Condition Index (RCI), a qualitative index of riparian integrity developed by Harding et al. [36] in New Zealand and adapted for European conditions. We used the RCI to describe the riparian ecological status of sites in four European countries (i.e., Norway, Sweden, Belgium, and Romania). We were able to demonstrate how our site types differed and what attributes used in the index were contributing to those changes. Our forested reference sites and sites with a forested riparian buffer typically had high scores for shading; buffer properties including vegetation composition, width, and intactness; and the vegetation composition of land adjacent to the riparian zone (i.e., >30m from the stream edge). In contrast, unbuffered sites and downstream "matrix" sites typically had lower overall scores, with key attributes indicating poor land management practices such as increased livestock access. Finally, we detected a weak positive association between riparian condition and stream ecological status, based on the macroinvertebrate ASPT index. Notably, in the presence of a forested riparian buffer, the effect size of improved stream ecological status did not scale with the effect size of the improvement in riparian condition ("magnitude of transition"). Instead, we saw evidence for the "environmental context" hypothesis, where improved stream ecological status in the presence of a forested riparian buffer declined overall when the existing upstream state was more degraded.

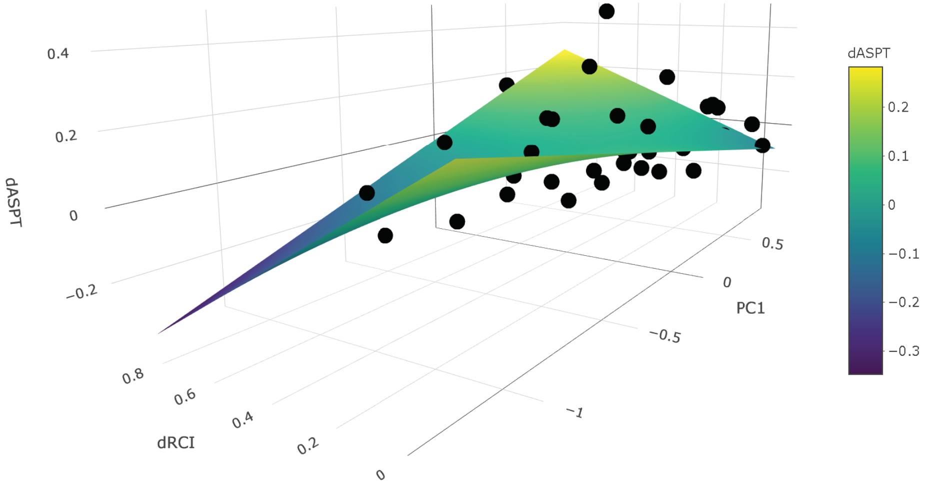

Figure 7. At paired sites across our four case-study basins, the size of the improvement in stream ecological status (ΔASPT) was positively associated with an interaction between the magnitude of the improvement in riparian condition (ΔRCI) and level of upstream human impacts (PC1). The result in this figure (see also Table 4) suggests that the riparian "magnitude of transition" effect on ΔASPT is dependent on the "environment context" (PC1). PC1 explains 37% variation in catchment-wide human impacts (i.e., indicating the level of upstream degradation).

4.1. The Riparian Condition Index (RCI) in the European Context

Overall, the RCI was an effective means to describe characteristics among site types and was shown to be ecologically relevant with a positive influence on stream ecological status measured by a commonly used macroinvertebrate index (ASPT). We saw variation in index scores because of real differences in site properties between case-study basins. In the heavily urbanized streams of the Oslo Fjord catchment, we found that bank stability decreased in the presence of a forested riparian buffer (Figure 5), owing to the box culverting and impervious surfaces of upstream reaches resulting in higher scores indicating more stable bank habitat. Likewise, the presence of these features (i.e., box culverts) helped explain the shallower bank slopes recorded in the Norwegian buffered sites. Another real feature of the sites in Norway was the negligible effects of buffer presence on adjacent vegetation and groundcover, indicating that riparian buffers were typically constrained in the cityscape and did not "spillover" into the land >30m from the streams edge. Similarly, there were contrasting patterns in Sweden that reflected real differences in site characteristics. The forested reference sites in Sweden typically contained a mixture of mature coniferous and deciduous trees, with one of the sites located within an important nature reserve "Naturreservatet Fiby urskog" (Fiby primeval forest nature reserve) that has had relatively little human intervention since the end of the 18th century. For these reasons, the Swedish forested reference sites on average recorded better ecological status than the reference sites in the other European countries. Within the Lake Mälaren basin, the greater difference between the Swedish forested reference sites and buffered sites was influenced by differences in vegetation composition, with the forested riparian buffers strongly dominated by a mixture of deciduous tree species and a conspicuous absence of conifers. In contrast, the negligible difference between forested reference sites and buffered sites of the Argeş River basin in Romania was likely due to logging activity and the presence of livestock in both site types.

Indices that rely on observer-based judgements have obvious weaknesses but can be very useful for efficiently capturing the "gestalt" characteristics of an environment (i.e., an overall summation better perceived than the individual parts). Perhaps the most well-known observer-based index in stream ecology is the Stream Reach Inventory and Channel Stability Evaluation [61], also referred to as the Pfankuch Stability Index (PSI). The PSI is used extensively for catchment assessment and studies investigating relationships between channel stability and biota in North America and internationally [62,63]. The PSI is calculated by summing the scores assigned to 15 attributes (weighted in relation to their perceived importance) in three regions of the stream channel (i.e., upper banks, lower banks, and stream bottom), according to the observer's evaluation of predetermined criteria [61]. The PSI has been shown to be a highly efficient means of describing bed-stability characteristics, but is prone to observer bias [64]. Despite using a technical workshop on field protocols to help ensure consistency in the determination of the RCI across case-study basins, we still detected evidence for differences in scoring arising from observer-specific judgements. For instance, field workers in Belgium were unable to perceive differences between buffered and unbuffered sites in the soil quality attribute, meaning we excluded this variable from our analyses of effect sizes (Figure 5). Other problems included anthropogenic features in urban landscapes indicating that some attributes of the RCI (e.g., "bank stability") could be further modified to account for these properties. Overall, the RCI is an efficient and useful measure of riparian ecological integrity as demonstrated here, but not without some limitations (e.g., urban features, observer-specific biases). Despite these problems, the benefits for rapid habitat assessment are evident, and the RCI could be used widely by practitioners and citizen scientists to help monitor riparian ecological status.

4.2. Effects of Shading by Forested Riparian Buffers

A clear outcome from our analysis was the importance of forested riparian buffers for channel shading (Figure 4). Whilst unsurprising, this is a non-trivial result because management of shade is often seen as a key element in rehabilitating and restoring degraded streams [65,66]. Shading can reduce proliferations of filamentous green algae and macrophytes that contribute to impaired ecological status [67,68]. For example, excessive autotrophic biomass can increase ecosystem respiration in the water-column and interstitial spaces of the streambed [69,70], potentially leading to adverse impacts on pollution-sensitive EPT (Ephemeroptera, Plecoptera, Trichoptera) species through reductions in dissolved oxygen concentrations [71]. Excessive algal growth can also smother benthic substrate, thus reducing habitat availability for sensitive grazing mayflies and increasing abundances of tolerant taxa that include oligochaetes and chironomids [72]. Consequently, negative effects of shading on aquatic autotrophs may help explain why we saw a positive association between the RCI and ASPT indices after accounting for upstream impacts that included nutrient concentrations (i.e., PC1).

Further, a central tenet of the CROSSLINK project is that riparian buffers help rehabilitate stream habitats and enhance resilience for the impending problems posed by climate change [73]. Streams and river ecosystems are sensitive to climate change because they are intimately linked with the global hydrological cycle, are strongly influenced by atmospheric thermal regimes, and are frequently at risk from interactions between warming and existing anthropogenic stressors [42,74,75]. The strong influence of our forested riparian "buffers" in providing shade potentially also helps moderate stream temperatures; a pattern well supported by evidence [76–78]. For instance, planting deciduous riparian trees along temperate streams as an adaptation to climate change can reduce temperatures by 2–3 °C through channel shading [79]. Thus, our future analyses will be geared towards better understanding the magnitude of temperature regulation in the presence of forested riparian buffers and the environmental contingencies (e.g., water residence times) that influence this moderating influence.

4.3. Magnitude of Transition and Environmental Context

Cost effective ecosystem management requires consideration of additional stressors, both locally and at whole catchment scales, that might limit or enhance the success of any given mitigation or restoration measure, including the rehabilitation of riparian buffers [80]. In our example (Figure 6B,C), the effect size of improved stream ecological status did not scale with the effect size of the improvement in riparian condition (i.e., the magnitude of transition hypothesis). Instead, we saw evidence for the overall effect size of improved stream ecological status by "buffering" becoming smaller when the existing upstream state was more degraded, supporting the environmental context hypothesis. However, adding another layer of complexity, we also detected a significant interaction between the drivers representing the magnitude of transition and environmental context hypotheses. This result indicated that the relationship between improved stream ecological status and the magnitude of transition (i.e., the improvement in riparian condition) was dependent on the level of upstream human impacts. Thus, although the maximum possible improvement in stream ecological status declined overall with increasing upstream degradation, the potential for a larger relative improvement with enhanced riparian management was more likely in degraded sites. Intriguingly, the negative influence of improving riparian condition on the ASPT index at low levels of upstream degradation (Figure 7) may have reflected a "subsidy-stress"-type response, where increased light availability in the more open unbuffered sites conferred a benefit (*sensu* "subsidy") to normally sensitive invertebrate taxa [81]. The subsidy-stress relationship describes how at low levels, anthropogenic perturbations may enhance ecosystem functioning and species responses, whereas higher levels depress these responses [82]. Nonetheless, the pattern in our study (low upstream stress, negative response to riparian afforestation) may also have reflected the distribution of data points, and a study in small Danish streams showed there was no difference in invertebrate community composition between forested streams and sites in open landscapes [83].

As a caveat, we only used one metric (i.e., the ASPT) as a response in our example. In Burdon et al. [42], we found that ecological responses to the same environmental driver (i.e., here forested riparian buffers) was not only dependent on the environmental context but also the community metrics used. Thus, remediation strategies aiming to improve stream ecological status by rehabilitating degraded reaches not only need to consider upstream anthropogenic influences but also the most appropriate indicators [42]. Future research will consider other ecological responses and better describe riparian buffer properties and the key environmental contingencies that may alter responses (e.g., catchment size, network position, etc.). However, our findings here as a proof of concept should interest managers, because it suggests that the potential for improvement in stream ecological status using forested riparian buffers may be greater in more degraded streams for certain ecological metrics, provided sufficient effort goes into improving riparian conditions.

5. Conclusions

Acquiring a general, predictive understanding of ecological systems requires knowing how much structural and functional relationships can cross scales and contexts to form broader patterns. Here we introduced the BiodivERsA-funded project CROSSLINK that investigates questions about the role of forested riparian buffers in human-impacted landscapes by measuring a wide range of ecosystem attributes in stream and riparian habitats at a continental scale. Riparian zones are important because they provide habitat for biodiversity and act as the interface between land and water, thus influencing cross-habitat food-web interactions, system functioning, and the provision of ecosystem services in heterogenous landscapes. Our results have highlighted important attributes of forested riparian buffers, which include the provision of habitat and shading of the stream channel. We also saw evidence for improving stream ecological status through the presence of these landscape features, and the potential for improvement in certain metrics (i.e., ASPT) may be greater in more degraded streams, provided sufficient effort goes in to improving riparian conditions. Enhancing existing and planting new forested riparian buffers as "nature-based solutions" is increasingly required in modified catchments, where multiple pressures are causing ecological degradation and decreased resilience to climate change. However, evidence for the multifunctionality of riparian buffers is needed to inform and persuade regulators and land managers to implement effective nature-based solutions and devote greater resources towards this goal [84]. Our introduction to CROSSLINK highlights the potential for this project with its broad portfolio of ecosystem attributes to help improve management of forested riparian buffers in human-impacted landscapes.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/4/1178/s1, Figure S1. Four CROSSLINK site types; Figure S2. The effective sampling reach and the habitat assessment reach; Figure S3. Riparian plots used to sample biodiversity indicators; Figure S4. Riparian Condition Index (RCI) values for site types in each CROSSLINK case-study basin; Figure S5. Mean RCI attributes for the four CROSSLINK site types; Protocol S1. Study sites and design; Protocol S2. Environmental data (Descriptions of field methods for environmental data); Protocol S3. Biodiversity data (Descriptions of field methods for biodiversity indicators); Protocol S4. Ecosystem functioning data (Descriptions of field methods for functional indicators); Protocol S5. Food web data (Descriptions of methods for biomarker analyses); Protocol S6. Societal Needs (Description of optimization framework).

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Supplementary Materials for:

Assessing the Benefits of Forested Riparian Zones: a Qualitative Index of Riparian Integrity is Positively Associated with Ecological Status in European Streams

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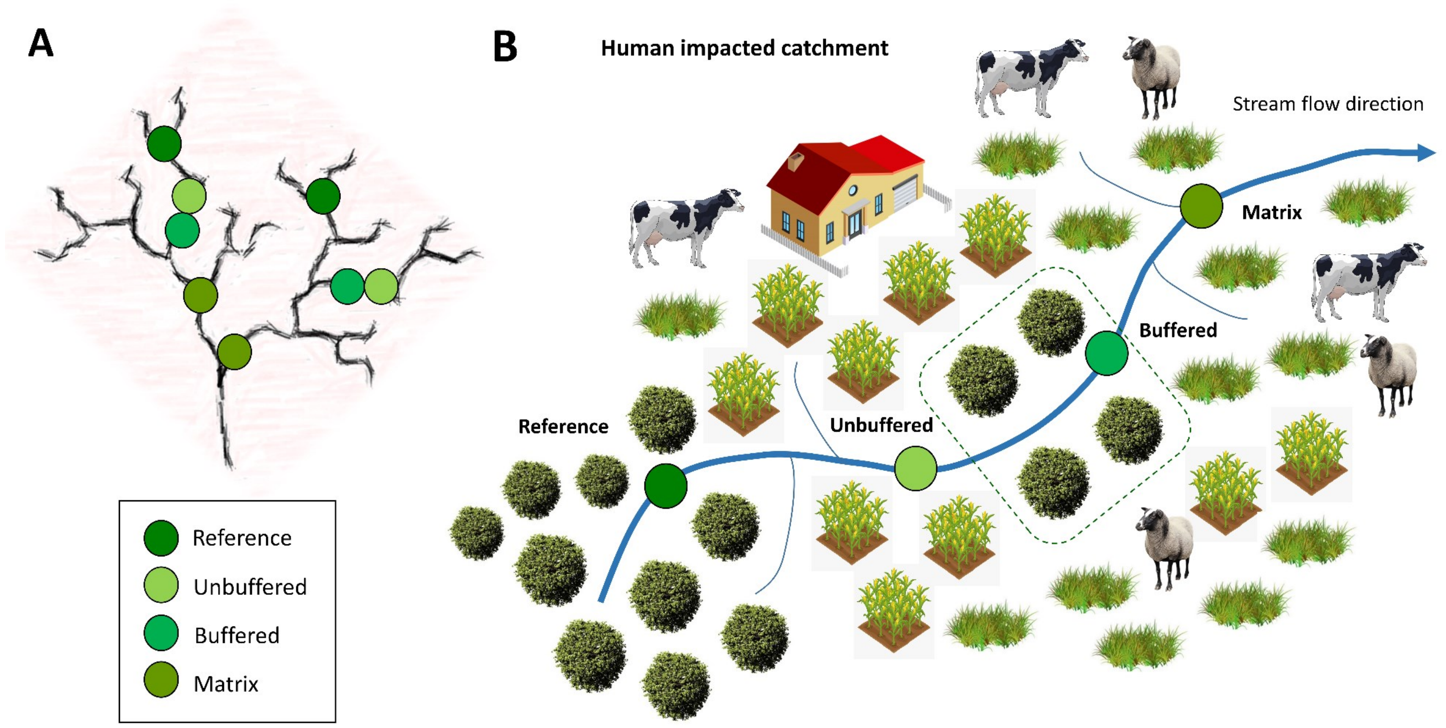

Figure S1. Four types of site were used in the CROSSLINK project: Pristine or least-impacted ‘Reference’ sites, site pairs with an ‘Unbuffered’ upstream site and a downstream ‘Buffered’ site with a woody riparian buffer on both banks, and ‘Matrix’ sites that were typically located further downstream from the other site types to capture cumulative land use impacts. Panel **A)** shows how the sites were typically distributed in a stream-riparian network, whereas panel **B)** highlights the potential pressure from a human land use (agriculture) on these networks and how our study design aimed to characterize different site types.

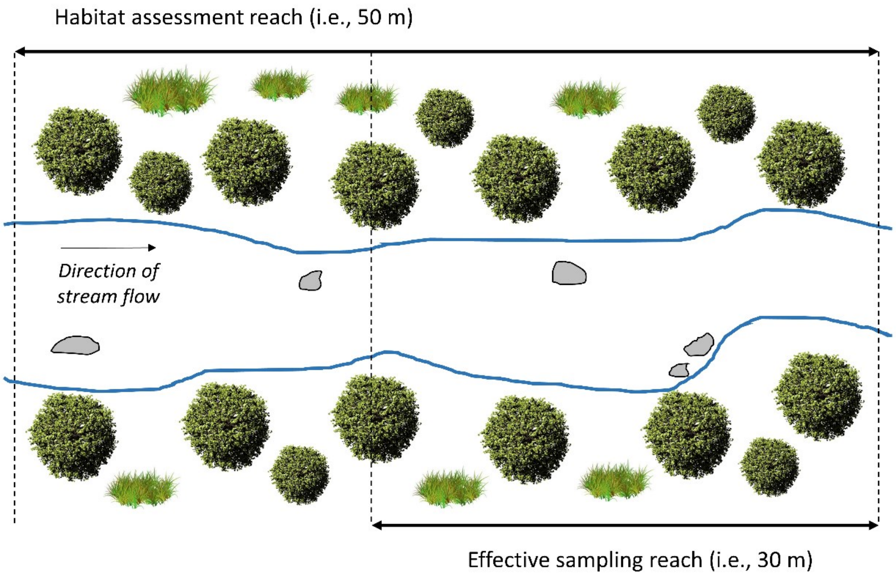

Figure S2. The effective sampling reach and the habitat assessment reach used in CROSSLINK. At each site, the different components of sampling for CROSSLINK were conducted over two reaches differing in length, with a shorter effective sampling reach nested in a longer habitat assessment reach.

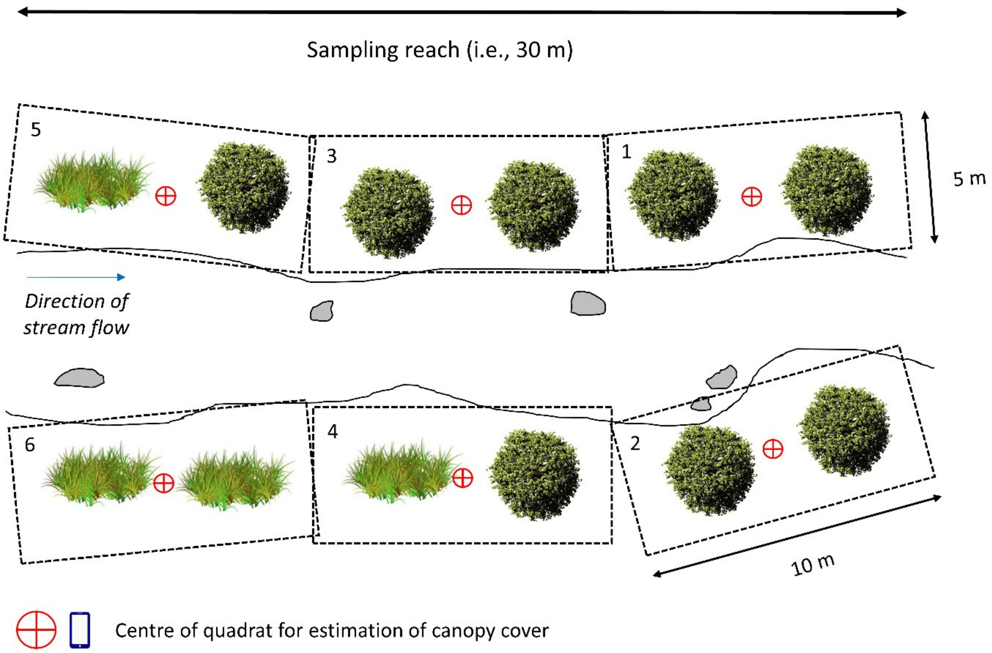

Figure S3. Riparian plots used to sample biodiversity and functional indicators (Protocols S3 and S4). We also measured aspects of riparian habitat within these plots (Protocol S2).

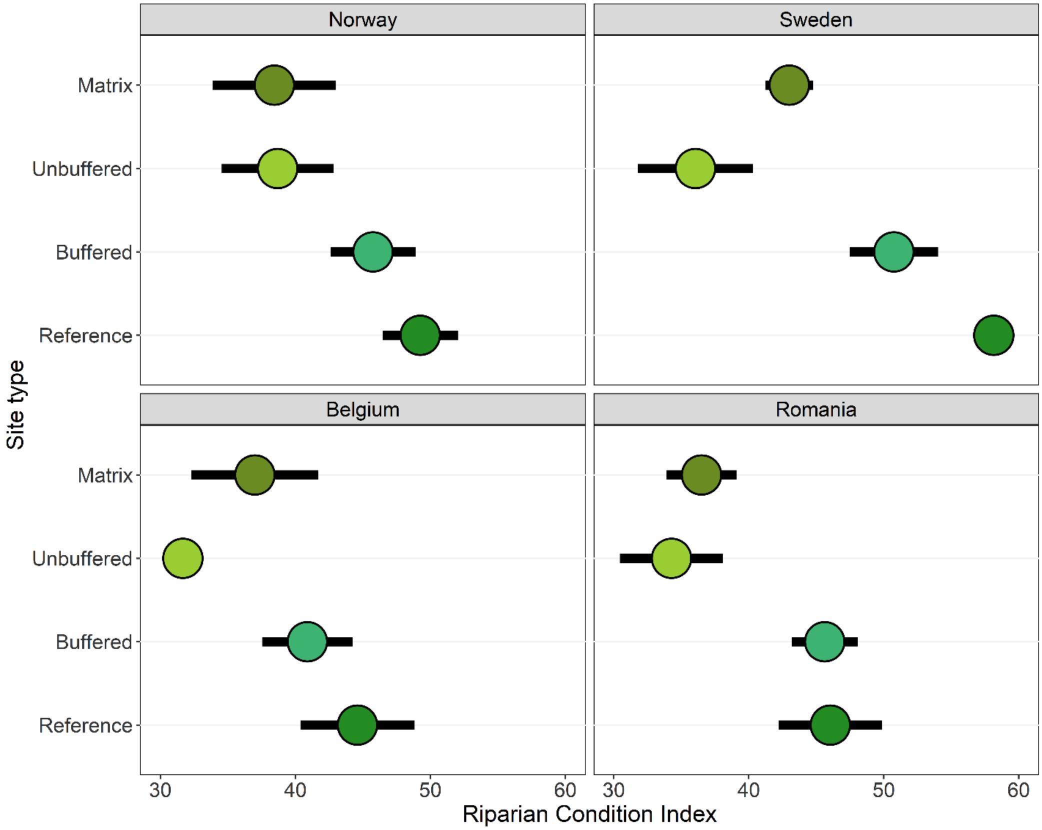

Figure S4. Mean values (± 95% CI) of the Riparian Condition Index for site types in each CROSSLINK case-study basin. The "Site type" refer to the sites used: pristine or least-impacted 'Reference' sites, site pairs with an 'Unbuffered' upstream site and a 'Buffered' downstream site with a woody riparian buffer on both banks, and 'Matrix' sites that were typically located further downstream to capture cumulative land use impacts.

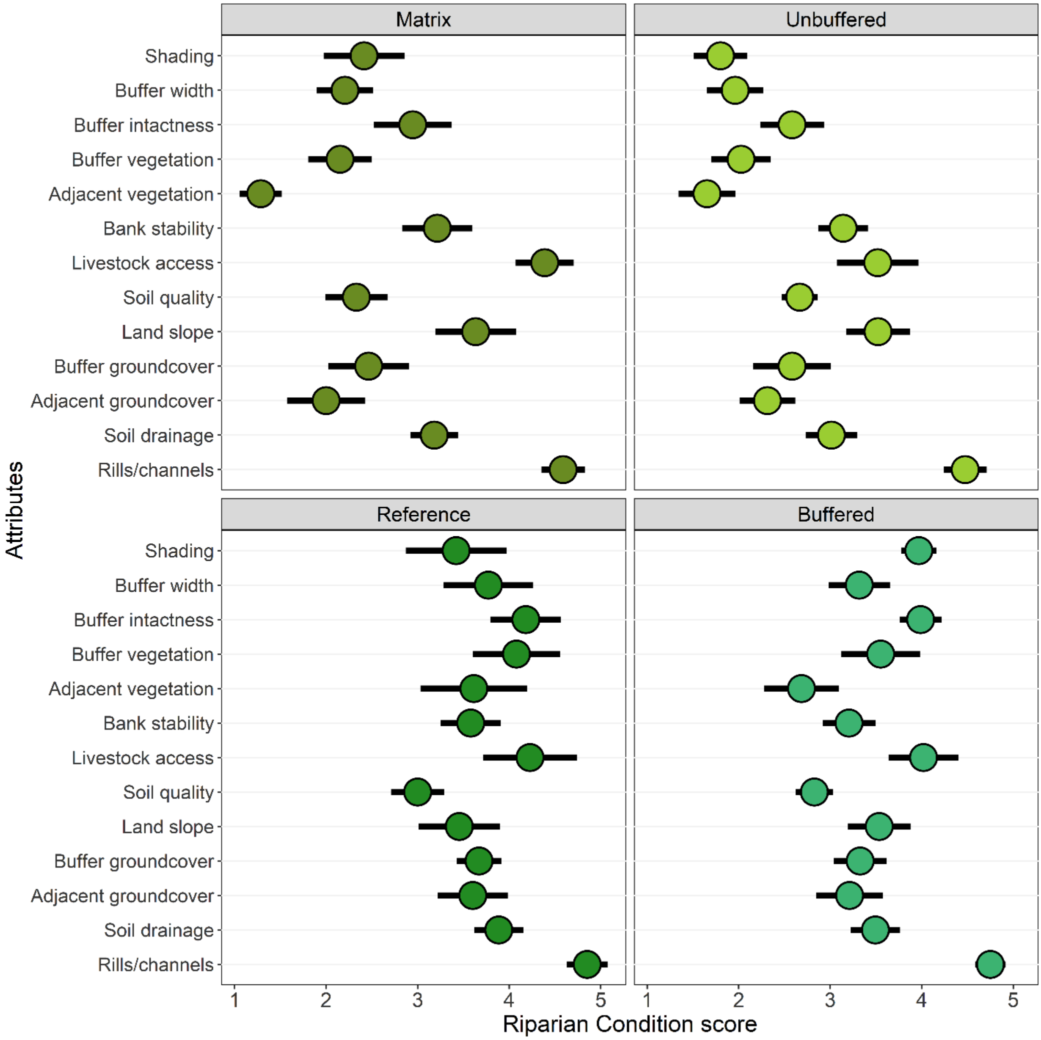

Figure S5. Mean values (± 95% CI) for each attribute used in the Riparian Condition Index for the four site types used in the CROSSLINK project. These plots include data from sites in Norway, Sweden, Belgium, and Romania. The “Site type” refer to the sites used: pristine or least-impacted ‘Reference’ sites, site pairs with an ‘Unbuffered’ upstream site and a ‘Buffered’ downstream site with a woody riparian buffer on both banks, and ‘Matrix’ sites that were typically located further downstream to capture cumulative land use impacts.

Protocol S1. Study sites and design

CROSSLINK has a tiered study design. Firstly, the *paired approach* tested both lateral and aspects of longitudinal connectivity. This approach required 10-12 streams in each case-study basins flowing through an impacted (agricultural, urban or mixed agricultural and urban) landscape, each with two paired sites: an upstream sites with no riparian buffer, and a downstream site with a riparian buffer (i.e., leading to 20-24 sites in total). Secondly, the *network approach* testing aspects of longitudinal connectivity involved 10-12 additional sites distributed throughout the river network (e.g., upstream and downstream of the site pairs). Within these sites we sought pristine or least impacted headwater *reference* sites and more degraded, downstream *matrix* sites to help characterize the range of ecosystem attributes possible and to show the potential for cumulative impacts of catchment land uses. Fig.S1 further describes the four site types.

There were exceptions to this design in the Belgian case-study basin. The majority of woody riparian buffers in the Zwalm River network were located at the headwaters of streams. This situation meant that either the stream source was located within the woody riparian buffer or the upstream, unbuffered reach only had intermittent flows and thus violated the site selection criteria below. To deal with this problem, additional downstream *matrix* sites were used as surrogates for the unbuffered reach in 4 site pairs.

Streams were wadeable, 1st-3rd order (i.e., approximately 2-5 m wide), and with a stable streambed (i.e., not frequently hydrodynamically disturbed) dominated by gravels and cobbles, with the exception of the Belgian sites which were dominated by fine sediments. Key buffer properties considered during site selection included buffer length (i.e., >50 m moving upstream from the downstream end of the sampling reach), width (>2-3 × wetted stream width), extent (buffer on both banks of the stream segment), and composition (dominated by woody vegetation). At each site, the different components of sampling for CROSSLINK were conducted over two reaches differing in length, with a shorter reach nested in a longer reach. Key components of the terrestrial and aquatic habitat sampling were conducted within the longer *habitat assessment reach* (50 m long). Most of the biological sampling (i.e., biodiversity and ecosystem functioning measures) were conducted within the shorter *effective sampling reach* (30 m long). The most downstream end for each reach was located at the same point in the stream.

Protocol S2. Environmental data

Water quality

Grab water samples were collected in plastic containers for water quality analyses during a maximum of three different seasons (autumn 2017, spring and summer 2018) following standard methods (i.e., samples were collected from below the water surface in the channel thalweg) at the downstream end of each site. Site pairs were sampled on the same day. Samples were stored cold and refrigerated upon return to the laboratory whereby they were analyzed within 24 hours of collection. Water samples were analysed for total organic carbon, total nitrogen, ammonium, nitrite- and nitrate-nitrogen (i.e., oxidized nitrogen, NO2-N + NO3-N), total phosphorus, dissolved reactive phosphorus, specific conductivity, pH, and alkalinity. Spot water measurements were also made at the case-study basin level. In Sweden, we measured for turbidity (NTU), specific conductivity, % dissolved oxygen, and temperature using a Manta +30 probe (Eureka Water Probes, Austin, TX, USA) at five different times of the year (e.g., November and December 2017; May, June, and July 2018). In Belgian the same parameters were measured using two YSI probes (YSI 6600 V2 & YSI 6600 V1, Yellow Springs, OH, USA) and a WTW probe (Three-Multi 3430 IDS, WTW GmbH, Weilheim, Germany). Although some methods and protocols differed among basins we expect these to have minor influence on data evaluation given the analytical approach with comparisons of pairs and exploring relationships along pronounced gradients of environmental stress.

Instream habitat assessment

We recorded aspects of hydrogeomorphology, including cross-sectional measurements of width, depth, and flow at 5-6 transects distributed in a stratified random approach throughout the *habitat characterisation reach*. At each transect we estimated bankfull width and depth (based on evidence of the highest waterline) following Rosgen [85]. We also recorded wetted channel widths, water depths, and flow at the time of sampling in two seasons: summer and autumn. Flow measurements were made using a flow meter located at 2/3 depth of the channel thalweg of each transect. Channel slopes were measured over the entire reach using a clinometer or the ‘smart’ phone application “Clinometer” (plaincode™, Munich, Bayern, Germany). We measured channel shading at zenith during summer (i.e., peak leaf cover) using the “CanopyApp” (University of New Hampshire, Durham, NH, USA) for smart phones. Channel shading was quantified in the middle of the channel at each of the 5-6 transects mentioned above.

We recorded counts of naturally occurring large wood and debris dams (i.e., detrital structures) in the *habitat characterisation reach*. We defined debris dams as accumulations of particulate organic matter (POM) > 0.3 m^3 (estimated by width, length, and height) or POM-accumulations occupying more than half of the wetted stream width. The number of large wood elements (i.e., logs > 10 cm diameter) were recorded if they were at least partly located in the wetted channel.

We estimated the % cover of different substrate types (inorganic and organic) subjectively over the effective sampling reach (30 m reach) and habitat assessment reach (50 m reach). This assessment of instream habitat involved estimating the % cover of streambed for organic and inorganic substrate types (e.g., macrophytes, large woody debris, coarse particulate organic matter, fine particulate organic matter, fine sediment, gravel/pebbles/cobbles, boulders, and bedrock). The inorganic substrate classes followed the Wentworth scale [86]. The estimates were recorded after stream walking the effective sampling reach, and then again after stream walking the habitat assessment reach. We separately estimated the % cover of bryophytes and filamentous algae as their extent on the underlying substrate.

We also recorded a quantitative estimate of inorganic substrate composition from 100 random substrate measurements following the “Wolman walk” methodology [87]. We adapted the method so that the first 60 stones were recorded within the effective sampling reach, with the remaining 40 stones recorded from the habitat assessment reach upstream of 30 m from the downstream end of both reaches. The walk was conducted moving upstream from the downstream end of the site, with particles randomly selected at the front of the recorder foot. We selected 100 particles within the wetted width of the sampling reach, and measured the B-axis (i.e., the intermediate axis perpendicular the longest axis) of each particle. We used Wentworth [86] categories for fine sediment (i.e., <0.06 mm = silt, <2 mm = sand).

We qualitatively assessed hydromorphological impacts (HMI) over the habitat assessment reach. We described HMI based on the Standardisation of River Classifications (STAR) classification.

The STAR framework is a method for calibrating different biological survey results against ecological quality classifications developed for the Water Framework Directive (http://www.eu-star.at/). Firstly, we recorded the % extent that banks and the stream bed were fixed by artificial or living materials (separately for the stream bed, right and left banks). We used the following categories: concrete without seams, a solid concrete structure without interstices; concrete with seams, concrete plates with interstices; stones, gabion baskets or riprap; stone plastering with interstices; stone plastering without interstices; wood, dead wood in fixed structures (including bridges); other materials; and no bank fixation. We also recorded the presence of other HMI - water extraction (i.e., visible evidence or water abstraction for irrigation, hydropower or other purposes); channel straightening or channelization; and culverting (i.e., if the channel was partly culverted in the habitat assessment reach).

Additional hydromorphological attributes were recorded from existing data, including the number of dams, transverse structures (e.g., step weirs), and total barriers upstream and downstream of the site. Where possible we obtained flow alteration data cumulative from upstream, which included at least one of the following: the volume of water regulation, the deviation from the natural hydrograph, or another equivalent measure. The volume of water regulation is relevant to catchments with impounded water, and could be actual data on volume of water regulated (or held in reservoirs), or alternatively a Water Framework Directive (WFD) status classification reflecting impacts from regulation (i.e., from bad to high, 1-5). The deviation from the natural hydrograph used modelled values when available, otherwise a WFD status classification (i.e., 1-5).

Riparian habitat assessment

Riparian habitat characteristics were surveyed in the riparian zones adjacent to the habitat assessment reach (50 m) at each study site. The surveys were carried out in summer 2018 when leaf-out was complete for all tree/shrub species, and targeted both banks. Six 50 m^2 rectangular plots (10 × 5 m) were used to describe vegetation characteristics. These plots were located close to the stream edge approximately running parallel on their longest edge as indicated in Fig.S3. Plots did not overlap, and spread across the habitat assessment reach to capture the full heterogeneity present at the study sites. Canopy cover was measured at zenith from the center of each plot (see Fig.S3) using the smartphone app “CanopyApp” (University of New Hampshire, Durham, NH, USA). Multiple measurements were recorded from each plot if required to capture the full heterogeneity present. We estimated the pooled cover (% area within the sample plot) of different vegetation/habitat categories within each plot. The vegetation/habitat categories used were: Managed, short grasses (e.g., grazed or mown); Unmanaged grasses, long grasses including rushes and sedges; Herbs, herbaceous vegetation including forbs; Mosses and lichens growing on the ground; Small trees and shrubs (DBH < 5 cm); Rocks and bedrock; Bare ground; Plant litter including leaves; and other (e.g., roads, fences, embankments). The cover of each category was estimated as a vertical projection on to a horizontal plane (i.e., the ground), meaning that if plants in one category occurred in multiple layers then it was still only the vertical projection on the ground of that category that was considered. We identified and measured the girth (circumference) of all trees with a diameter at breast height (DBH: 130 cm) ≥ 5 cm in each of the six riparian plots described above. These measurements included dead trees that were still standing. We used local identification guides and the smart phone app “PlantSnap” (PlantSnap Inc., Telluride, CO, USA) to identify trees to species level. We also recorded dead wood attributes (i.e., dead wood on forest floor) in addition to the vegetation/habitat categories mentioned above. Firstly, we recorded the number of logs (> 10 cm diameter) at least partly located in each plot, consistent with the instream habitat assessment. We also recorded the approximate areal cover (i.e., % of the 50 m^2 plot) and length and diameter of the trunk for dead wood volume estimates.

We also surveyed riparian condition using an assessment of 13 qualitative variables that could indicate poor riparian status (see Main Text). This assessment follows the protocol described by Harding et al. [36] but adapted here for European conditions (Table 3). Attributes were graded from poor (1) to excellent (5) on each bank over the habitat assessment reach (50 m), and scores summed to provide an index of riparian habitat quality. For the analysis of total riparian condition and individual attributes, bank scores were averaged to provide a single value for riparian condition at each stream.

Protocol S3. Biodiversity data

Microbes – We collected environmental samples for molecular analyses describing stream and riparian microbial diversity from within the effective sampling reach (30 m). Aquatic sediment samples were collected from three aggregations of fine particulate organic matter (FPOM) randomly located within the effective sampling reach. We also collected riparian samples for molecular analyses describing terrestrial microbial diversity. Sediment samples were collected from three aggregations of fine particulate organic matter (FPOM) in the riparian zone close to water's edge but in areas that were only flooded occasionally. We targeted top soils while avoiding plant material and roots in the sample. Wearing nitrile gloves and using a disposable plastic spoon to collect samples, each subsample was placed in a sterile, disposable plastic trough and homogenised using a sterile plastic spatula before being transferred into a 10ml and 5mL cryovial. Care was taken to avoid larger gravel, stones and excess water. We used new gloves and plastic implements at each site to avoid cross-contamination. Samples were stored on ice and then frozen at -80 °C for later processing.

Diatoms – We sampled diatoms within the effective sampling reach (30 m). The area of flowing water used was representative of the site in terms of bottom substrate, vegetation, water depth and water velocity. The sampling area covered the entire stream width, with the areas closest to the stream edge avoided. Diatom samples were taken from rocks without filamentous algae or moss and attempts were made to ensure that the stones we submerged for >4 weeks prior to sampling. Areas with low current or high shading were avoided, except when they were characteristic of the sampling site. A minimum of five stones (10-25 cm in diameter) were collected. At nutrient-enriched sites and when only small stones were present, the number of stones was increased (≈10). The upper surface of the stones was brushed repeatedly three times with a new toothbrush and the material rinsed into a plastic tray with approximately 250 ml of stream water (or distilled water). The number of brushed stones and volume of water used was recorded. In addition, a digital photo was taken of the stones on a light background (i.e., a white plastic sorting tray) using a clearly marked ruler for a scale. Area estimates for the stones were made using the digital image with the software "ImageJ" [88]. After brushing, the water and organic material was mixed carefully and poured into two 250 ml containers to settle. Where required we then decanted about 2/3 of the liquid and filled the container with 96% alcohol. One container was sent for analysis and the other was saved as a contingency.

Macroinvertebrates – We sampled macroinvertebrates within the effective sampling reach (i.e., 30 m). The area used was a stretch of flowing water (i.e., run-riffle sequence) with hard-bottomed sections (i.e., with cobble, pebble, gravel and/or bedrock substrates). The sampling area comprised the entire stream width along the predefined reach, but efforts were made to ensure that sampling did not include areas that were dry in the recent past. Quantitative sampling requires that stream invertebrates are collected from a given area with a standard sampling effort. We standardized methods to ensure comparable data using one of two potential sampling methods: Surber sampling and quantitative kick-net sampling [51]. All samplers used 500 µm mesh netting, and Surber samplers were ≈ 0.0625 m^2 (e.g., 25 × 25 cm) in dimensions; kick-nets used were equivalent to the dimensions of the Surber sampler by using an area defined by a quadrat equaling the width of the net. Sampling effort was standardized for 60 seconds where coarse substrate was disturbed to a maximum depth of 10 cm from the surface of the streambed. A total of six replicate subsamples were collected (three from erosional/riffle-run habitats, and three from depositional/run-pool habitats) in the same way within the effective sampling reach. All subsamples were pooled together. Woody material and leaves were retained separately in a plastic bag to contribute to estimates of standing coarse particulate matter (CPOM). The final, pooled macroinvertebrate sample was sieved (500 µm mesh) to remove excess water, then preserved in a 500-1000 mL jar with 96% ethanol to reach a final concentration of 70% for later sorting.

Trees – We identified and measured the girth (circumference) of all trees with a diameter at breast height (DBH: 130 cm) ≥ 5 cm in each of the six riparian plots described above. We used local identification guides and the smart phone app "PlantSnap" (PlantSnap Inc., Telluride, CO, USA) to identify trees to species level. We use genus level where species were unable to be determined. DBH was calculated from circumference data using the following equation (Eq.1):

$$d = \frac{C}{\pi} \tag{1}$$

where d is the diameter and C is the circumference (girth) of the tree.

Arachnids and ground beetles - We surveyed and collected two groups of predatory invertebrates commonly found in riparian zones that are known to use aquatic prey subsidies: Arachnids, web-building and free-living spiders including Opiliones; and ground beetles: Carabid and Staphylinid beetles. The sampling method used a semi-quantitative approach involving timed visual searches to obtain a relative indication of abundances and provide material for analyses. Sampling only occurred in dry weather conditions during the summer of 2018. We surveyed both banks over the habitat assessment reach using the same plots (i.e., 5 x 10 m = 50 m^2) described above for riparian habitat assessment. The maximum total area searched was the plot area (i.e., 50 m^2), but typically the area searched was a fraction of 50 m^2 recorded from the plot boundaries. The exact time taken for the search was recorded with a target of 10 minutes per plot using two to three people. We systematically started searching from the shoreline (i.e., near the water's edge) with each collector following a transect parallel to stream edge moving further from the streams edge. Attempts were made to standardize the allocation of effort to reflect the proportion of different habitat types present. We sampled a minimum of four plots, but where necessary we sampled all six plots. This sampling effort was required because we were also collecting individual predatory invertebrates for biomarkers analysis, meaning we had targets regarding the requisite number of spiders and beetles needed for analyses (e.g., >20 individuals). We use a "catch per unit effort" (CPUE) approach to calculate a relative measure of abundance, making abundances between sites comparable. The number of people searching multiplied by the time taken was used to calculate search duration. Total sampling duration (h), area sampled (m^2), and the total number of invertebrates collected were used to calculate the CPUE (Eq.2):

$$\mathrm{CPUE} = \frac{\text{No. of invertebrates}}{(\text{Total area sampled}/\text{Duration of sampling})} \tag{2}$$

We recorded several additional parameters that could additionally explain variation in our sampling effectiveness and catch efficiency including sampling methods used (see below), the time of day that sampling occurred, air temperature, wind speed, weather conditions, and water levels.

Sampling techniques used for collection included visual searching and collection by hand (the preferred method for most habitat types) and sweep-netting in long grasses. Visual searching for spiders and beetles was conducted by investigating habitat types in each sampling plot. We attempted to find web-building spiders in their webs or retreats (curled leaves, silken cases) on vegetation or other structures. We also turned over loose bark, fallen wood, rocks etc. for free-living spiders and ground beetles. We searched the interstices of exposed gravel bars adjacent to the stream because ground-dwelling beetles often inhabit this habitat. Invertebrates were captured by guiding them into a larger sample container, before transferring them to a smaller sample container, or by using an aspirator. Sweep netting was used for sampling unmanaged grass habitat (e.g., long grasses, sedges, and reeds) and some herbs/forbs. The general "sweep-netting" method involves the use of a heavy insect net being vigorously swept through the surface of the vegetation. After repeated sweeps (e.g., a standardized level of effort involving five passes for an area of 1 m^2), the contents were put onto a flat white sheet and spiders and beetles removed.

Large individuals (e.g., *Carabus* spp., Pisauridae, Lycosidae) were kept in separate containers. Smaller individuals of the same guilds (e.g., web-building spiders) were pooled. We recorded information about the plot and habitat types where individuals were recovered, and the distance from the streams edge. The samples were kept on ice in the field, and frozen at -20°C prior to identification and preparation for biomarker analyses (see Protocol S5).

Protocol S4. Ecosystem functioning data

Algal biomass accrual

To quantify algal growth we placed eight unglazed tiles (16 x 16 cm in size) in each effective sampling reach, arranged in four pairs. Each pair was fixed to a plastic trellis frame using cable-ties over each tile corner. In turn, each frame edge parallel to the stream flow was fixed to a metal stake driven into the streambed. For each tile pair, one tile had a strip of Vaseline smeared around the outside edge to restrict access for algal grazers. The strip was approximately 1-1.5 cm wide, and applied evenly in a relatively thin layer. The four pairs were distributed over the reach as evenly spaced as possible, but with care to ensure that habitat conditions were comparable (i.e., moderate to fast flowing reaches with rocky substrate). The tiles were deployed for approximately 30 days during summer to allow sufficient time for algal colonization and growth. At the end of the study period, algal biomass accrual was assessed using one of two methods: in the field with the "Benthotorch" (BBE Moldaenke, Schwentinental, Schleswig-Holstein, Germany) which quantifies the fluorescence of chlorophyll *a* and converts this information to chlorophyll biomass [89], or using pigment extraction and spectrophotometry in the laboratory [e.g., 90]. During the period that tiles were deployed in the field, stream temperatures were logged (iButton, Maxim Integrated, San Jose, CA, USA) to provide an estimate of degree-days (i.e., cumulative mean daily temperatures).

Sediment dynamics

The same frames and metal stakes used for the algal accrual assays were used for the sediment deposition assays. To assess short-term fine particle deposition rates, we fixed four pairs of "Astroturf" (or similar type) mats (16 × 16 cm) to the frames described above, with the turf facing upwards [91]. The mats act in a similar way to macrophyte and byryophyte beds by trapping fine particles moving near the stream bottom. The mats were fixed *in situ* for approximately three days, after which they were retrieved, placed into labeled zip-lock plastic bags, and within 24 hours either processed or frozen.

Organic matter processing

We assessed organic matter using two complementary methods: the litter pack assay (LPA), and the cotton strip assay (CSA). The LPA was applied exclusively in streams, whereas we conducted the CSA in both stream and riparian habitats following Tiegs [92]. The LPA involved alder (*Alnus glutinosa*) leaf litter enclosed in bags of two mesh sizes (10 mm and 0.5 mm, respectively) following Woodward [93]. Coarse mesh bags (10 mm mesh) allowed access for the majority of detritivorous macroinvertebrates, whereas the fine mesh bags (0.5 mm mesh) prevented access for most macroinvertebrates. Alder leaves were collected at abscission from a single homogenous stand for each case-study basin. Leaves were sorted, well-mixed, and air-dried at room temperature until weight change was negligible. We weighed 5.00 ± 0.25 g of air-dried leaves and put the leaves in individual trays so that they could be wetted with distilled water. Once the damp leaves were malleable enough to be handled without fragmentation they were then placed into a litter bag. Each litter hag was closed so that it formed a tetrahedral shape; for coarse mesh bags they were closed with plastic cable ties, for the fine mesh bags they were sewn shut with nylon thread. A total of 160 fine mesh bags and 160 coarse mesh were prepared for each case-study basin.

Leaf packs were placed in the field during the period of peak organic-matter inputs (late October – early November 2017). A total of five coarse and five fine mesh litter bags were deployed at each field site. In each stream, the litter bags were distributed between five experimental blocks (preferable in riffles), with one fine and one coarse litter bags in each block. Leaf bags were fixed to chains that were then attached to a metal stake driven into the streambed. We used five consecutive riffle-type habitats for the replicate leaf bags, and placed them at the midpoint between the water edges and the channel thalweg. A subset of leaf packs (i.e., 10 coarse and 10 fine) were taken to field sites, immersed in stream water for 30 seconds, and then immediately placed in individual plastic zip-lock bags. These leaf packs were then returned to the laboratory and soaked in distilled water for 48 hours before laboratory processing to estimate our handling and leaching losses. The bags incubated in the field were deployed for approximately 30 days (± 7 days) to achieve approximately 50% leaf mass loss in coarse mesh bags at reference sites. At the time of collection, leaf packs were placed in individual zip-lock bags, placed on ice, and frozen upon return to the laboratory for later processing. During the period that leaf packs were deployed in the field, stream temperatures were logged (iButton, Maxim Integrated, San Jose, CA, USA) to provide an estimate of aquatic degree-days (i.e., cumulative mean daily temperatures).

The cotton strip assay (CSA) was conducted in parallel with the LPA. Cotton strips (25 mm × 80 mm) were cut from the same bolt of 12-ounce, heavy-weight cotton fabric (Style 548; Fredrix, Lawrenceville, GA, USA) equivalent to the "Artists canvas" fabric described by Slocum [94]; each strip was 28 threads in width following Tiegs [95]. The "Artists canvas" fabric has been demonstrated to be a highly effective cotton material for stream biomonitoring purposes [96]. Our field methods for the CSA followed Tiegs [97], but briefly here we fixed two pairs of cotton strips to chains holding leafpacks in the stream, and two pairs of cotton strips were fixed to nylon cords tied to metal stakes in the riparian zone. In riparian zones, cotton strips were placed on the soil surface to simulate organic-matter input by senescent leaves. The cotton strips were distributed evenly between two locations in each habitat (i.e., each site) that were separated by a distance of approximately five to seven bankfull channel widths. During the period that leaf packs and cotton strips were deployed in the field, riparian air temperatures were logged (iButton, Maxim Integrated, San Jose, CA, USA) to provide an estimate of terrestrial degree-days (i.e., cumulative mean daily temperatures).

Protocol S5. Food web data

We assessed aspects of stream and riparian food-webs using biomarkers (stable isotopes and fatty acids).

Stable isotope analysis

We analysed the stable isotope composition ($\delta^{13}C$, $\delta^{15}N$) of basal resources, macroinvertebrates, and fish (Norway only). We sampled each site within the effective sampling reach (identical to the area used for the diatom and macroinvertebrate community sampling). Similar to the methods described in Protocol S3, we sampled erosional/riffle and depositional/run habitats using a kicknet or Surber sampler. A total of six replicate samples (3 erosional/riffle habitat, 3 depositional/run habitat) were collected with same sampling effort. Samples were pooled together and pre-sorted in the field by placing the collected material in white sorting trays and removing invertebrates with forceps. Following Burdon et al. [9], we collected individuals from 1) the most abundant taxa groups and 2) larger-bodied macroinvertebrates that disproportionately contributed to total invertebrate biomass. We used taxonomic units at the Family or Genus-level (e.g., Hydropsychidae, *Baetis* spp.). We aggregated smaller individuals (e.g., Chironomidae) at a relatively coarse level to get enough material for a viable isotope sample (≈1.5−2 mg dry mass). We also aimed for approximately 10 taxonomic groups per site to enable meaningful comparisons of community metrics across sites. Macroinvertebrate samples (i.e., each taxonomic group) were stored in a separate plastic containers with enough moisture and keep invertebrates damp. Large predatory invertebrates were stored individually (e.g., Aeshnidae). At same time detritus (coarse particulate organic matter) and biofilm (scraped from cobbles) samples were collected as representative basal resources and placed in plastic bags. Samples were stored cold in the field and transported to the laboratory for further processing.

In the laboratory, we allowed predatory invertebrates to purge their gut contents prior to further processing. We only accounted for gut contents in predatory invertebrates because the isotopic content of herbivores typically shows close fidelity with their diets [97]. We placed individual predators in petri dishes with a small amount of filtered water to keep animals damp during gut clearance. We covered the petri dishes with parafilm and holes punched in the film to allow gas exchange and reduced evaporative losses. Animals were kept for 12 – 24 hours in refrigerated conditions (≈4 °C). Prior to being frozen for sample storage and final preparation, invertebrates were further sorted taxonomically and voucher specimens removed for more detailed identification.

We freeze-dried (LyoDry compact, Mechatech systems LTD, Bristol, UK) frozen samples of basal resources, invertebrates, and fish for a minimum of 48 hours at -45°C prior to homogenization (i.e., grinding) and encapsulation. We pooled multiple individuals for small-bodied invertebrates (e.g., a minimum of 50 chironomid individuals) to get sufficient biomass for a sample, and where possible, enough for technical replication (i.e., >1 pooled samples per taxa). For larger-bodied taxa we attempted to sample multiple specimens individually (i.e., ≥3 and a maximum of 10 individuals). Animal samples (~1.0-mg) and basal resources (~2.0-mg) were encapsulated into 8 x 5-mm tin capsules (OEA Laboratories Ltd., Cornwall, UK) and sent to the Stable Isotope Facility (University of California, Davis, CA, USA) where they were analysed on a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Fatty acids

Riparian invertebrates collected for fatty acid analyses following methods outlined in Protocol S3 were first identified prior to sample preparation. We identified frozen individuals using a stereo microscope, reassigned them to re-labelled containers, and placed the samples back in the freezer for storage until sample preparation. Spiders (Araneae) were identified to the Family-level using the Araneae key to families [98], and with the aid of Jocqué and Dippenaar-Schoeman [99] and Kronestedt [100]. Huntsmen (Opiliones) were left at the Order level. Ground beetles (Carabidae) were identified to genus level using Lindroth [101] and Hackston ([102], online key adapted from Lindroth [101]). Rove beetles (Staphylinidae) were determined to sub-family level using Hackston [103].

Following taxonomic identification, all invertebrates went through the initial preparation stages, including biomass quantification. The target invertebrates prepared for fatty acid analysis were: all the ground beetle genera, Staphylinidae, Opiliones, and the spider families, Linyphiidae, Tetragnathidae, Lycosidae and Pisauridae. For each site all invertebrates belonging to the same family or genus were pooled together to one sample. The pooling was done to average individual variations in fatty acid content, and to reach fatty acids analysis mass requirements (≈5 mg dry weight per sample). The number of individuals per sample was recorded. The samples were freeze-dried (LyoDry compact, Mechatech systems LTD, Bristol, UK) for a minimum of 48 hours at -45°C. The samples were weighed and the mass recorded. Non-target taxa were stored in the freezer for future analysis.

The target samples for fatty acid analysis were homogenized (i.e., grinding with a mortar and pestle), then re-weighed and the mass recorded. The samples were then stored in a freezer (-20°C) until processing for fatty acid analysis using methods similar to those reported in Grieve and Lau [104]. These methods involve three main steps: lipid extraction, methylation, and gas chromatography-mass spectrometry (GC-MS). We analyzed samples at the Swedish Metabolomics Centre in Umeå, Sweden.

Protocol S6. Optimization Framework

The results of CROSSLINK case studies have been used to develop an optimization framework for stream-riparian BGI capable of balancing different socio-economic and environmental objectives. The focus of this optimization framework is on the identification of spatial configurations that minimize trade-offs and support the multifunctionality of the case study areas. We first identified model parameters and relationships (e.g. between land-use, spatial connectivity and ecosystem services) required for optimization models in each case-study basin. Input from local stakeholders was used to help tailor socio-economic and environmental objectives according to needs in each case study basin. Indicators and services identified as model objectives (explanatory model variables) of the case study basins include biodiversity, functional indicators (species traits), supporting processes (e.g., litter decomposition, algal productivity) as well as socio-economic trade-offs (e.g. loss of arable land). As predictors (explanatory model variables) for the optimization models we tested a variety of spatial parameters gained by a comprehensive GIS analysis of the catchments. The parameters derived can be grouped into 3 categories: (1) local properties related to a specific river segment upstream of the sampling sites, (2) catchment properties related to the riparian catchment and total catchment upstream of a sampling site, (3) connectivity properties including a set of distance measures. Based on the parameters and relationships identified we then constructed linked biophysical-statistical models to quantify the influence of forested riparian buffers, land use and other human activities on the identified model objectives. These tools were then integrated into a multi-objective optimization framework to identify synergies and trade-offs between ecosystem services, biodiversity and functional indicators at multiple spatio-temporal scales. The optimization is carried out using the Python environment CoMOLA (Constrained Multi-objective Optimization of Land-use Allocation; [105]). CoMOLA utilizes the Non-dominated Sorting Genetic Algorithm-II (NSGA-II) to optimize (riparian) land-use maps for multiple objectives under consideration of basic land-use change constraints. Therefore numerous (tens of thousands) simulations of different potential spatial land use configurations are generated, to explore the ‘potential solution space’ at each study site and to identify optimal solutions along a Pareto front.

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Forested Riparian Buffers Change the Taxonomic and Functional Composition of Stream Invertebrate Communities in Agricultural Catchments

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Abstract: Riparian zones form the interface between stream and terrestrial ecosystems and play a key role through their vegetation structure in determining stream biodiversity, ecosystem functioning and regulating human impacts, such as warming, nutrient enrichment and sedimentation. We assessed how differing riparian vegetation types influence the structural and functional composition (based on species traits) of stream invertebrate communities in agricultural catchments. We characterized riparian and stream habitat conditions and sampled stream invertebrate communities in 10 independent site pairs, each comprising one "unbuffered" reach lacking woody riparian vegetation and a second downstream reach with a woody riparian buffer. Forested riparian buffers were associated with greater shading, increased gravel content in stream substrates and faster flow velocities. We detected changes in invertebrate taxonomic composition in response to buffer presence, with an increase in sensitive Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa and increases in key invertebrate species traits, including species with preference for gravel substrates and aerial active dispersal as adults. Riparian vegetation independently explained most variation in taxa composition, whereas riparian and instream habitat together explained most variation in functional composition. Our results highlight how changes in stream invertebrate trait distributions may indirectly reflect differences in riparian habitat, with implications for stream health and cross-ecosystem connectivity.

Keywords: riparian zone; riparian vegetation; riparian buffer strip; stream macroinvertebrates; agriculture; taxonomic composition; functional traits

1. Introduction

The habitats that form the interface between a stream and its adjacent terrestrial landscape are known as "riparian zones" [1]. Streams and their riparian habitats are strongly connected hydrologically and ecologically and together provide valuable ecosystem services including intrinsic aesthetic values, recreational activities such as fishing, birdwatching and hiking and the supply of clean drinking water to human populations [2]. Riparian habitats also support unique biodiversity and strongly influence the functioning of stream ecosystems [3,4]. The properties of riparian zones (e.g., vegetation structure) can determine stream ecosystem processes and the extent of human impacts by moderating flow regimes and stabilizing banks during floods and regulating the extent of warming (e.g., during summer heat waves) and inputs of nutrients, fine sediments and micropollutants (e.g., pesticides) [5]. Consequently, riparian vegetation may strongly influence the structural and functional composition of stream communities in modified catchments by helping to mitigate impacts of human land uses.

Riparian vegetation directly affects instream primary production by altering incident light and secondary production indirectly by affecting the basal resources that support instream food webs, including the quantity and quality of terrestrially derived (allochthonous) detritus (e.g., leaf litter, woody debris). The presence of riparian trees can further contribute to the physical structuring of instream habitats, by contributing structural elements such as instream wood, stabilizing banks and providing habitats for both invertebrate and vertebrate breeding [6]. The level of control that riparian vegetation has on a stream ecosystem largely depends on the density and type of riparian vegetation and its position in the river network [7]. Headwater streams are often strongly embedded in the terrestrial (forested) landscape, while the riparian corridors of large rivers are characterized by complex floodplains and diverse terrestrial vegetation [4]. Small to mid-sized stream food webs are often driven by the quality and quantity of allochthonous organic matter entering the stream [8–10]. The significance of allochthonous matter is also reflected in the diversity, taxonomic composition and feeding guilds (e.g., shredders and filter-feeders) comprising forested communities. Thus, human activities, such as deforestation and landscape disturbances, that alter riparian vegetation composition can impact both terrestrial and stream ecosystems.

Land use impacts on riparian vegetation can directly or indirectly affect instream biodiversity and function through changes in instream conditions, habitat and basal resources [11–13]. Erosion and increased inputs of fine sediments and pollutants result in loss of instream microhabitats and sensitive species [14,15], whilst at high levels of disturbance, macroinvertebrate communities are often composed predominantly of species preferring soft-bottom sediments (e.g., certain Diptera and Oligochaeta). These stress-tolerant invertebrates are often adapted to low dissolved oxygen levels (e.g., modes of respiration including tegument). Adverse conditions and availability of instream food resources are also reflected through species life-history traits including smaller body size, shorter development times and adult life spans [16,17]. Loss or altered riparian vegetation also reduces shading and increases water temperatures, which, when combined with elevated nutrient levels from land use, can result in an increase in primary production (algae and macrophytes) [18,19], ultimately shifting basal resources from allochthonous to autochthonous matter [8]. These changes can impact stream food webs by altering the composition of functional feeding guilds and the transfer of energy to higher trophic levels [5]. Such changes to biodiversity and ecosystem processes require management practices that mitigate the impacts of human activities and restore key functional processes.

Riparian buffer strips, defined as patches of habitat alongside streams planted either with a more open mixture of grasses and herbs or with shrubs and trees (or a mixture of both), are increasingly used as a management tool to reduce the effects of agriculture and forestry on streams [20,21]. Such buffers can help to control erosion, filter nutrients and play an important role in hydrological processes including runoff and groundwater recharge [22–24]. Forested riparian buffers have the potential to be a particularly useful management tool, because increasing riparian forest quality (i.e., forest cover, structure, quality and channel integrity) can support the greatest number of ecosystem services in stream-riparian networks [2]. Forested riparian buffers have multiple benefits, including increased shading thereby moderating temperature fluctuations, reducing proliferations of aquatic plants and adding inputs of allochthonous organic matter [1,25,26]. While the ecological importance of stream-riparian ecosystems for local and regional biodiversity is well-recognized [4], the lack of robust guidelines for riparian buffer attributes and the potential management conflicts (e.g., drainage values, invasive plants) underscore a pressing need to quantify and understand how forested riparian buffers mitigate human impacts on streams and rivers. Riparian buffer management practices may significantly improve biodiversity [27], but knowledge gaps remain on the responses and drivers of instream communities, hindering efficient and effective management by land owners and decision makers [20,21,28].

Using a replicated field study, we addressed these knowledge gaps by quantifying the effects of forested riparian buffers on changes in instream attributes and macroinvertebrate communities across 20 stream reaches located on 10 streams within an agricultural catchment. On each stream, we sampled two reaches: an upstream unbuffered reach lacking riparian woody vegetation and the paired forested buffer reach downstream. We chose benthic macroinvertebrates as our biological response because (1) these organisms are often used in biomonitoring of stream ecosystems, (2) combining taxonomic and trait-based approaches allows for making strong mechanistic inferences and (3) macroinvertebrate responses to anthropogenic disturbance are well known and predictable [29–31].

Building on previous studies, we expected significant differences in instream habitat, physicochemical conditions and biological responses between unbuffered and buffered reaches. Specifically, we hypothesized that:

(1) Buffered reaches would have high amounts of organic matter (e.g., coarse particulate organic matter (CPOM) and woody debris), while unbuffered reaches were expected to have higher temperatures, nutrients concentrations (e.g., nitrogen, phosphorous) and finer benthic sediments (e.g., sand, silt).

(2) Altered riparian vegetation and differences in instream habitat between unbuffered and buffered reaches were expected to result in lower diversity and shifts in community and trait composition at unbuffered reaches. For example, differences in instream habitat were expected to result in the loss of sensitive species such as EPT taxa [30,32] and changes in the relative abundances of sensitive and tolerant taxa. Moreover, if substratum differed between the study reaches (e.g., shifts from hard to soft-bottom substrata with more macrophytes), we expected these differences to be reflected in macroinvertebrate traits describing microhabitat and flow preferences and adaptions for breathing.

(3a) Altered conditions in agricultural streams are often associated with changes in life history traits such as polyvoltinism and adult dispersal (i.e., smaller adult size and weak fliers) [14,16,33]); accordingly, we expect species with relatively shorter development times, small body sizes and passive dispersal to be more abundant at the unbuffered reaches.

(3b) Differences in feeding guilds were also anticipated between unbuffered and buffered reaches. For example, in unbuffered reaches, increased incidence of photosynthetically active radiation (PAR) is often associated with increased algal production [18,19]. Therefore, we expected a higher proportion of scraper feeding traits (i.e., traits specialized for consuming algal biofilms). Alternatively, if substrata at unbuffered reaches were dominated by fine sediment (i.e., sandy or silty substrates) we expected to find a higher proportion of deposit-feeders [5]. In buffered reaches, higher inputs of allochthonous organic matter (e.g., coarse particulate matter and woody debris) were expected to result in a higher diversity and proportion of shredders [34].

(4) Finally, we predicted that macroinvertebrate communities would respond more to local (i.e., riparian and instream habitat) than the large-scale (i.e., catchment land use) drivers. This prediction was consistent with the strong differences in local habitat characteristics that we anticipated between our unbuffered-buffered site pairs and the key role that local habitat features play in helping to structure macroinvertebrate communities [35].

2. Materials and Methods

2.1. Study Sites

Ten 1st to 3rd order lowland (<200 m a.s.l.) streams in the Lake Ekoln basin (part of the larger Lake Mälaren catchment), located in central Sweden, were studied in spring 2018. The catchment land cover is dominated by forest (49%), agriculture (36%) and urban (4%) land uses and in the sub-catchments of studied streams agricultural land use averaged 38%. In order to investigate impacts of local and upstream catchment land uses and the potential of riparian buffers to mitigate environmental impacts, one unbuffered (upstream) reach and one buffered (downstream) reach was selected for sampling within each stream (hereafter described as paired reaches; Figure 1).

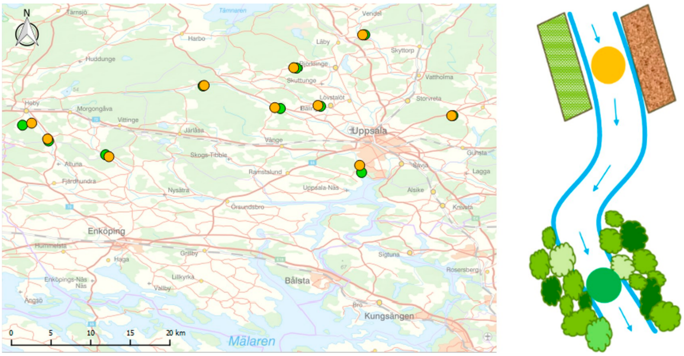


Figure 1. Position and schematic representation of streams and their paired reaches (orange dots—upstream unbuffered reaches; green dots—downstream forested buffered reaches).

Buffered reaches were selected based on the presence of a "forest riparian buffer" and following criteria described in Burdon et al. [5]: length (a woody buffer extending >50 m on both sides of the stream over the sampling reach), width (>2–3 x wetted stream width), extent (buffer on both banks of the stream segment) and composition (dominated by small and large trees). Unbuffered reaches typically only had a few isolated riparian trees or woody vegetation was completely absent, instead being dominated by grasses and herbaceous vegetation. Streams were chosen to be as similar as possible in key environmental characteristics including a stable streambed dominated by hard substrates (gravel/cobbles), similar stream widths, depths and flow characteristics. Riparian and aquatic habitat properties at each reach were surveyed within a 50 m habitat assessment reach (HAR), while macroinvertebrate sampling was conducted within 30 m effective sampling reach nested within the HAR [5].

2.2. *Sampling Methods*

2.2.1. Environmental Variables

We measured stream properties along 5–6 transects distributed in a stratified random approach over each study reach [5], including bankfull width and depth (based on evidence of the highest waterline), as well as wetted channel widths and water depths at the time of sampling. Flow measurements were made at 2/3 depth of the channel thalweg on the transect using a flow meter (MiniAir20 Flowmeter, Schiltknecht Messtechnik AG, Gossau, Switzerland).

Water samples were collected from below the water surface in the channel thalweg at the downstream end of each reach, stored cold and analyzed within 24 h for total organic carbon, total nitrogen, ammonium, nitrates, total phosphorus, conductivity, pH and alkalinity. Water temperature was measured using spot measurements and continuous logging (e.g., Manta + 30 probe, Eureka Water Probes, Austin, TX, USA).

We estimated the percentage cover of instream inorganic and organic substrate types (Table 1) over the effective sampling reach. Inorganic substrate classes followed the Wentworth scale [36], while the percentage cover of bryophytes and filamentous algae was estimated as an extent on the underlying substrate. Instream shading was recorded as a percentage of canopy cover using CanopyApp (for Android OS, Version 1.0.3, University of New Hampshire, Durham, NH, USA). Six measurements (digital images using a smartphone at the breast height) were taken in the middle of the stream channel to calculate the average stream shading (% canopy cover) for each study reach.

Table 1. List of environmental (explanatory) variables used to assess the difference in the structure of the macroinvertebrate communities between buffered and unbuffered reaches.

1. SPATIAL	**3. RIPARIAN**	**4. INSTREAM**	
Latitude	Riparian buffer size (m^2)	Bankfull width (m)	Algae (%)
Longitude	Length (m)	Bankfull depth (cm)	Bedrock, boulders (%)
Principal Coordinates of Neighbourhood Matrix ((PCNM)	Width (m)	Channel width (m)	Bryophytes (%)
2. CATCHMENT	Tree density (trees/m^2)	Channel depth (cm)	Coarse particulate organic matter (CPOM %)
Elevation	Unmanaged grass (%)	Flow (m/s)	Large woody debris (%)
Catchment area (m^2)	Herbs (%)	Temperature (°C)	Fine particulate organic matter (FPOM %)
Forest: broad-leafed, coniferous and mixed (%)	Moss/lichens (%)	Instream shading (%)	Gravel (%)
Arable land and crops (%)	Trees/shrubs (%)	pH	Macrophytes (%)
Pasture (%)	Rocks/bedrock (%)	Conductivity (mS/m^2)	Fine sediment (%)
Natural areas (%)	Plant litter (%)	Total organic carbon (mg/l)	
Urban and industrial (%)	Bare ground (%)	NH_4 (µg/l)	
Water (%)	Managed grass (%)	NO_3–N (µg/l)	
Wetlands (%)		Total N (µg/l)	
Other (%)		Total P (µg/l)	

Woody riparian vegetation was quantified by measuring, identifying and counting the number of trees (Diameter at Breast Height > 5 cm) as the mean density (m^2) of trees from six 50 m^2 (30 m × 5 m) rectangular plots in the riparian zones adjacent to the stream at each sampling reach. Similarly, riparian habitat characteristics (% area of different habitat types) were estimated as a vertical projection onto the ground from each 50 m^2 riparian plot. Canopy cover was estimated in the middle of each plot, using the same method as described above (for instream shading).

We used a national database from Sweden's meteorological and hydrological institute (SMHI, https://www.smhi.se/data/hydrologi/vattenwebb, accessed on 2 November 2020) and Swedish mapping, cadastral and land registration authority (Lantmateriet, https://www.lantmateriet.se/en/about-lantmateriet/Samverkan-med-andra/internationell-samverkan/corine-land-cover/, accessed on 2 November 2020) to extract the spatial data (e.g., river network, digital elevation) for catchments of the 10 stream reaches. We used land use data from the most current (2018) CORINE Land Cover (CLC) inventory. Riparian buffer size, width and length were estimated using aerial photographs from Google Earth and reach elevation, longitude and latitude were obtained from Geographical Information System (GIS) data.

2.2.2. Macroinvertebrate Communities

We sampled macroinvertebrates once in spring 2018, using a Surber sampler with a metal frame (dimensions 0.25 × 0.25 m = 0.0625 m^2) and 500 µm mesh netting. The sampling effort was standardized for 60 s where coarse substrate was disturbed to a maximum depth of 10 cm. A total of six replicate subsamples were collected (three samples from erosional run-riffle habitats and three from depositional run-pool habitats). The six samples were pooled, sieved through 500 µm mesh and preserved in 70% ethanol. Macroinvertebrate samples were identified to the lowest taxonomic level possible (e.g., species or genus) using standard identification guides. Taxa were counted and the abundance was expressed as the number of individuals per m^2. EPT taxa were expressed as a percentage of the sensitive orders (E = Ephemeroptera, P = Plecoptera, T = Tricoptera) to the total taxa found.

2.3. Functional Traits Selection

Benthic macroinvertebrate functional traits were used to quantify differences in functional structure of macroinvertebrate communities. We focused on biological and ecological traits that reflect changes in the aquatic environment attributable to differences in riparian vegetation (e.g., feeding and respiration), instream habitat (e.g., substratum preferences) and traits that can reflect changes in the transfer of nutrients and energy between aquatic and terrestrial systems, therefore affecting lateral connectivity of streams (e.g., life history and dispersal traits). Accordingly, we selected 39 trait modalities from the 8 traits categories to quantify changes between unbuffered and buffered reaches.

Biological traits:

(1) body size (in cm): $\leq$0.25, 0.25–0.5, 0.5–1, 1–2, 2–4, 4–8, >8;
(2) life cycle duration: $\leq$1 year, >1 year;
(3) potential number of cycles per year: <1 (semivoltine), 1 (monovoltine), >1 (polyvoltine);
(4) dispersal: aquatic passive, aquatic active, aerial passive, aerial active;
(5) respiration: tegument, gill, plastron, spiracle;
(6) feeding: deposit feeder, shredder, scraper, filter feeder, predator, parasite.

Ecological traits:

(7) substratum preferences: boulders/cobbles/pebbles, gravel, sand, silt, mud, macrophytes, microphytes, twigs/roots, organic detritus/litter (hereafter CPOM);
(8) flow: null (<5 cm/s), slow (5–25 cm/s), medium (25–50 cm/s), fast (>50 cm/s).

We extracted available trait information from the database of Tachet et al. [37] and an online database (https://www.freshwaterecology.info/TaxaDB_mzbSearch.php, accessed on 2 November 2020) for 89 taxa (out of 94). Traits scores were based on fuzzy coding, which uses positive scores to describe the affinity of a species for different modalities (i.e., categories) of a given variable [38]. Fuzzy coding allows membership of a given species in more than one trait state simultaneously to account for trait plasticity, with trait scores weighted individually for each species. Macroinvertebrate traits were expressed as community weighted means (CWM) calculated as: $\sum_{i=1}^{n} relative\ abundance_{\mathrm{i}}\ x\ trait_{\mathrm{i}}$ (for a species i, [39]).

2.4. Statistical Analysis

2.4.1. Hypothesis 1

Each of the 10 investigated streams comprised a pair of study reaches (unbuffered and buffered). To detect if there was a difference between these two categories, means of study reaches were tested using a paired t-test implemented in the JMP Pro 15.0.0 (SAS Institute, Cary, North Carolina). Prior to the analyses, environmental metrics data were *log* or *log (x + 1)* transformed, while the percentage data were *logit* transformed to approximate normal distribution. Principal Component Analysis (PCA) on standardized environmental variables (mean of 0 and a standard deviation of 1) and correlation (Pearson Product Moment) was used to visualize the difference between the study reaches and reduce the dimensionality of environmental variables. Data were normalized using R packages *car* and *vegan* and standardized by the *decostand* R function.

2.4.2. Hypotheses 2, 3a and 3b

Changes in macroinvertebrate biodiversity metrics between unbuffered and buffered reaches were tested using paired t-tests. We included taxa richness, EPT taxa richness, Simpson index, Shannon–Wiener index, evenness and dominance. To visualize (dis)similarities and test the differences in the taxonomic and trait compositions between the study reaches, we used Non-Metric Multidimensional Scaling (NMDS), followed by PERMANOVA (PERmutational Multivariate ANalysis Of VAriance), using the *adonis* function in R. Next, to investigate the contribution of individual taxa and traits to dissimilarity between study reaches [40], we used the *simper* function in R package *vegan*, tested with 999 permutations. The invertebrate abundance data were Hellinger transformed using the *decostand* R function to down weight rare species and avoid the influence of the "double zero" problem in our data [41].

2.4.3. Hypothesis 4

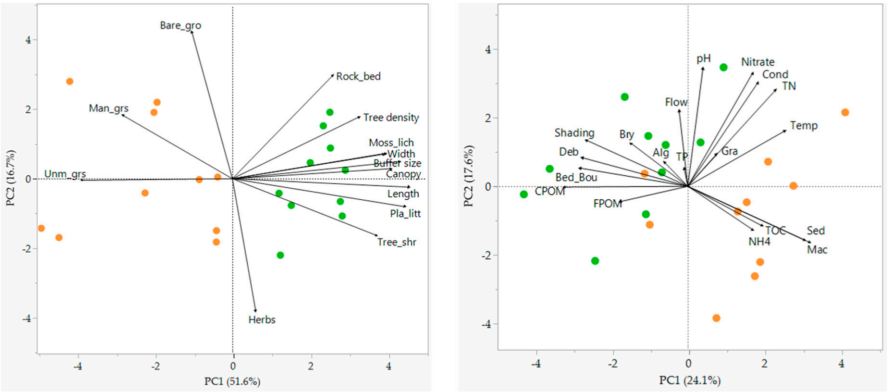
Redundancy analysis (RDA) was used to relate the variation in the composition of macroinvertebrate taxa and functional traits with environmental variables among the study reaches, using R package *vegan* and *adespatial* and related functions *pcnm*, *rda* and *varpart* [42]. We tested independent effects of various environmental variables describing spatial structure (PCNM, Principal Coordinates of Neighborhoods Matrix), catchment properties (land use), riparian characteristics and instream characteristics (Table 1). PCNM was used to decompose the spatial (geographical) relationship among study reaches into PCNM functions, which were obtained by principal coordinate analysis of a truncated matrix of Euclidean (geographic) distances [43]. PCNM is based on the diagonalization of a spatial weighting matrix and produces orthogonal maps that maximize spatial autocorrelation. It creates spatial components that can be directly linked to the spatial patterns of the environmental variables. Consequently, geographical distances in rectangular form are similar to normal explanatory variables used in RDA, for ecologically relevant spatial scales. To select model predictors and avoid over-parameterizing models, we first removed highly collinear predictors (e.g., $r > 0.75$). Then, we used *forward.sel* function in the *adespatial* R package (v 0.3–8) to select a subset of the best explanatory variables to describe the most variability in taxonomic and trait composition. The significance of the environmental variables was tested with 999 Monte Carlo permutations. Forward selection was carried out with the stopping criteria at the alpha significance level $\alpha = 0.1$ and the adjusted coefficient of multiple determination ($R^2{}_{adj}$) calculated using all potential explanatory variables [44]. A more relaxed significance level was necessary to retrieve explanatory variables within each of the four observed sets of variables. Finally, we checked the variance inflation factors (VIF) using the *vif.cca* function. Predictor variables were excluded if they had VIF score > 4 [45].

To further investigate the independent effects of the local habitat attributes and direct contribution of riparian buffers to differences between study reaches, we performed partial RDA (pRDA). Here, we included riparian habitat structure and vegetation attributes selected by forward selection and conditioned out the confounding influence of instream properties as well as spatial structure and catchment characteristics. Additionally, variation partitioning analysis (VP) was used (*varpart* function in R) to separate variation in taxonomic and trait differences of communities explained by each environmental fraction (spatial, catchment, riparian and instream) using the strongest predictors from RDA analysis (i.e., forward selection) (*varpart* function in R).

3. Results

3.1. Riparian and Instream Habitats (Hypothesis 1)

Principal Component Analysis (PCA) of selected environmental variables, describing riparian buffer properties (Figure 2a) and instream characteristics (Figure 2b), showed clear differences between unbuffered and buffered reaches. The first two axes combined explained more variations for riparian properties (68.3%) than instream variables (41.7%), with the first axis for riparian buffer properties explaining the majority of the variation between reaches (51.6%). Furthermore, the two axes in both plots clearly reflected gradients related to changes in the riparian characteristics and vegetation cover between study reaches, with buffered reaches correlated with riparian buffer size (length/width) and percentage of canopy cover and plant litter, as well as instream CPOM (loadings > 0.8 and −0.7 along the first PC axis). Unbuffered reaches, on the other hand, were correlated with the higher presence of unmanaged grass in the riparian zone, as well as instream macrophyte cover and fine sediment (loadings −0.8 and > 0.7 along first PC axis).



(a) riparian **(b) instream**

Figure 2. Principal Components Analysis (PCA) of the unbuffered and buffered reaches using selected environmental variables for: (**a**) riparian; and (**b**) instream habitats. Orange dots represent unbuffered and green dots represent buffered reaches. Abbreviations: riparian (**a**): Bare_gro = Bare ground (%), Man-grs= managed grass (%), Moss_lich = moss/lichens (%), Pla_litt = plant litter (%), Rock_bed = rocks/bedrock (%), Tree_shr = trees/shrubs (%), Unm_grs = unmanaged grass (%). Abbreviations: instream (**b**): CPOM = coarse particulate organic matter, FPOM = fine particulate organic matter, TOC = total dissolved carbon, Cond = conductivity, TP = total phosphorus, TN = total nitrogen, Alg = algae (%), Bed_Bou = Bedrock, boulders (%), Bry = bryophytes (%), Gra = gravel (%), Mac = macrophytes (%), Sed = fine sediment (%).

Paired *t*-tests showed significant differences for 17 out of the 34 environmental variables tested ($p < 0.05$, Table S1). The size, length and width of the riparian buffer zone, percentage of trees/shrubs, plant litter and moss/lichens were higher at buffered than unbuffered reaches, whilst for instream habitat, flow, shading (% canopy cover), pH, CPOM and woody debris differed significantly. Unbuffered reaches had a deeper channel depth, higher percentage of unmanaged and managed grass in the riparian zone, as well as instream macrophyte cover and fine sediment. Nutrient levels did not differ between the reaches. At both unbuffered and buffered reaches, forest was the dominant type of land cover, but catchment land use categories were excluded from the *t*-tests as the stream pairs (unbuffered, buffered streams reaches) were nested within the same sub-catchments.

3.2. *Taxonomic and Trait Differences Between Study Reaches (Hypotheses 2, 3a and 3b)*

A total of 94 taxa were recorded among the study reaches, belonging to 62 families and six taxa classified at a higher taxonomical level due to difficulties in identification (e.g., Oligochaeta) (Table S2). In total, 33,097 individuals were identified. Taxon richness and abundance did not differ between the study reaches: 26 ± 6 taxa and 4310 ± 2601 ind/m^2 at the buffered reaches and 24 ± 5 taxa and 6705 ± 4393 ind/m^2 at the unbuffered reaches. The most abundant families at both study reaches, based on overall abundance means, were Pisidiidae and Gammaridae, followed by Chironomidae, Oligochaeta, Asellidae and Elmidae (Table S2). The percentage of individuals of EPT taxa (Ephemeroptera, Plecoptera and Trichoptera) was higher at buffered (21.1 ± 16.5%) compared to the unbuffered (14.1 ± 7.2%) reaches, even though this trend was not significant at the 5% level (*t*-ratio = 1.98, $p = 0.078$).

Contrary to our expectations in the second hypothesis, alpha diversity, measured as Shannon–Wiener, Simpson, evenness and dominance (Table S3), did not differ between the study reaches (paired *t*-test, $p > 0.05$). Relative abundances of several individual taxa differed, however (here, we also included nearly significant taxa that might indicate changes between the reaches): Chironomini midges (*t*-ratio = 2.33, $p = 0.045$) and the nemourid stonefly *Nemoura* sp. (*t*-ratio = 2.31, $p = 0.046$) were more abundant at unbuffered reaches, whilst elmid riffle beetles *Limnius volckmari* (*t*-ratio = −2.57, *p* = 0.030), the glossamatid *Hydraena* sp. (*t*-ratio = −2.17, *p* = 0.058), the mayfly *Baetis rhodani* (*t*-ratio = −2.18, *p* = 0.057) and the caddisflies *Agapetus ochripes* (*t*-ratio = −2.43, *p* = 0.038) and *Ryacophila nubila* (*t*-ratio = −2.25, *p* = 0.051) were more abundant at buffered reaches, thus supporting our second hypothesis.

Similarly, a number of macroinvertebrate traits differed between unbuffered and buffered reaches, lending support to our third hypothesis. As predicted, community-weighted mean (CWM) traits showing a preference for gravel substrates (*t*-ratio = −3.33, *p* = 0.009) and active aerial dispersal (*t*-ratio = −2.66, *p* = 0.026) were more prevalent at the buffered reaches, whilst preferences for null flow (<5 cm/s, *t*-ratio = −2.84, *p* = 0.019), were more common at the unbuffered reaches. Contrary to our expectations, scraper abundances and plastron respiration (t-ratio = −3.16, *p* = 0.012) were higher at buffered than unbuffered reaches (t-ratio = −3.57, *p* = 0.006), while CPOM substrate preference (organic/detritus/litter) (*t*-ratio = 2.2, *p* = 0.055) was higher at the unbuffered reaches. Shredder abundances did not differ between reaches (paired *t*-test, *p* > 0.05).

Visual inspection of unconstrained ordination (NMDS) of taxonomic and trait community compositions also supported predictions in hypotheses 2, 3a and 3b as unbuffered and buffered reaches tend to group separately (Figure S1). However, PERMANOVA did not detect a systematic differentiation overall (taxa: F = 0.87, *p* = 0.199; traits: F = 1.28, *p* = 0.12). Nevertheless, SIMPER analysis showed that the top 10 ranking taxa accounted for 42.5% and top 10 traits accounted for 50.8%, of the macroinvertebrate community dissimilarities between buffered and unbuffered reaches (Table S4). The four top ranked taxa (*Gammarus pulex, Pisidium* sp., *Limnius volckmari* and Simuliidae) accounted for >20% of the dissimilarity between reaches. For functional traits, between-reach type dissimilarities were mainly due to differences in life-history traits (size and voltinism), feeding preferences and respiration. The overall dissimilarity between the unbuffered and buffered reaches revealed by SIMPER analysis was higher for taxa (54.6%) than traits (11.2%).

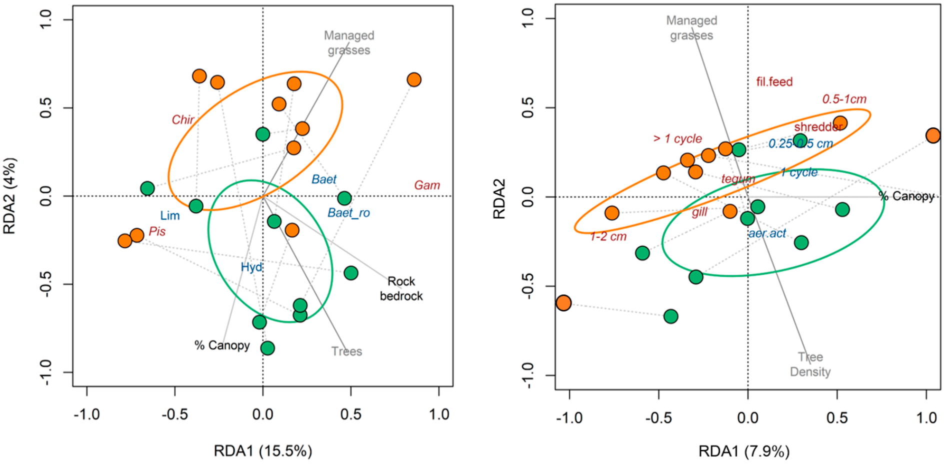
3.3. Taxonomic and Trait Responses to Environmental Variables (Hypothesis 4)

The forward selection procedure (i.e., for redundancy analysis (RDA)) resulted in parsimonious eight-variable model for both taxonomic and trait composition (Table 2). For taxonomic composition, the first two RDA axes explained 34% of total between reach variation. Instream characteristics explained between 4% (% gravel) to 16% (% algae) of the variability, followed by spatial variables (e.g., PCNM1 11%), catchment area (8%) and riparian characteristics (3–7%). By comparison, the first two RDA axes explained 51.3% of between-reach variation in trait composition. Instream variables explained the most variability (13–24%), but catchment area (19%) was a stronger predictor than spatial variability (6–13%), followed by riparian characteristics (8%).

In ordered to test the independent effects of the local habitat attributes and the direct contribution of riparian buffers to macroinvertebrate community composition, as considered in Hypothesis 4, we used partial redundancy analysis (pRDA). These results are presented in Tables S5 and S6. The pRDA model for taxonomic composition (Figure 3a) included two variables (% of canopy cover and % of rock/bedrock) which explained 19.5% variability between the study reaches. The pRDA model for trait composition included only one variable (% canopy cover), which independently explained 7.9% of variation (Figure 3b). The pRDA results are also consistent with the results from our SIMPER analysis.

Table 2. Results of RDA analysis (forward selection) showing environmental variables from each tested model with significant conditional effects on taxonomic (n = 94) and trait (n = 39) composition.

Environmental Variables	Taxa			Traits		
	Adj R^2	F	p	Adj R^2	F	p
Spatial						
PCNM1	0.11	1.75	0.044			
PCNM9	0.03	1.65	0.089	0.13	2.42	0.062
PCNM10				0.06	2.24	0.066
Catchment						
Catchment area (m^2)	0.08	1.61	0.081	0.19	2.03	0.089
Riparian						
Canopy (%)	0.03	1.55	0.094	0.08	2.65	0.034
Rock/bedrock (%)	0.07	1.86	0.041			
Instream						
Nitrate	0.09	2.96	0.002	0.24	3.39	0.018
TP				0.30	2.29	0.054
Algae (%)	0.16	2.49	0.004	0.13	3.9	0.009
Gravel (%)	0.04	1.72	0.047	0.15	4.35	0.004



(a) taxa **(b) traits**

Figure 3. Results of pRDA for taxonomic (**a**) and trait (**b**) composition. Riparian attributes used in the pRDA are shown in black (% canopy, % rock/bedrock) and two riparian variables that are significantly different between the study reaches (% managed grass and % trees, based on *t*-tests) were shown in grey. The species and traits with the highest scores along the first two pRDA axes are shown in dark brown color (associated to unbuffered reaches with orange ellipse and orange dots) and blue color (associated to buffered reaches with green ellipse and green dots) color. RDA2 (**b**) is equivalent to PC1 axis, due to a one variable model (% Canopy). Taxa abbreviations: Baet = *Baetis* sp., Baet_ro = *Baetis rhodani*, Chir = Chironomidae, Gam = *Gammarus pulex*, Hyd = *Hydropsyche siltalai*, Lim = *Limnius volckmari*, Pis = Pisidium. Trait abbreviations: breathing: gills, tegum = tegument, dispersal: aer.act = aerial active; feeding: shredders, fil.feed = filter feeders; number of cycles per year: <1 cycle, 1 cycle; size: 0.25–0.5 cm, 0.5–1 cm, 1–2 cm.

Variation partitioning showed that riparian characteristics (% of canopy cover and rock/bedrock), catchment area nor spatial location were significant ($p > 0.05$). However, the shared variation between instream habitat and riparian characteristics explained 2%

of the variability in taxonomic composition and 7% in trait composition. More results are available in Figure S2.

4. Discussion

Stream ecosystems are strongly connected to their adjacent terrestrial ecosystems, with riparian characteristics known to be strong predictors of instream physicochemical characteristics and biological communities [46]. Altered or loss of riparian vegetation from human land uses can result in biodiversity loss and shifts in species composition of aquatic communities [15,18]. However, studies of the effects of riparian land use on stream communities are inconsistent. For example, studies have shown both strong [12,46–49] or only weak effects [5,19] of riparian buffers on instream macroinvertebrate communities. We quantified the importance of forested buffers on instream habitats and macroinvertebrate biodiversity and trait composition using a paired-site approach, with upstream unbuffered and downstream buffered reaches in 10 streams. Our study showed significant differences in instream habitat and macroinvertebrate taxonomic and trait composition between unbuffered and buffered reaches, thus corroborating previous studies [11–13].

4.1. Changes in Riparian and Instream Habitats

Consistent with our predictions, ordination analyses and paired *t*-tests showed clear differences in instream habitat related to riparian characteristics. Indeed, 50% of the variables tested (nine riparian and eight instream) differed significantly between the study reaches. The strongest instream differences were related directly to differences in riparian habitat (e.g., vegetation cover). Buffered reaches had greater canopy cover, increased shading, higher amounts of large woody debris and CPOM, whilst unbuffered reaches were characterized by fine sediments and greater cover of macrophytes. That riparian buffers result in altered instream habitat is well established. For example, Burdon et al. [14] found that degraded riparian condition was associated with changes to instream habitat that included reduced flow velocities and increased sedimentation in agricultural streams. Other studies have similarly shown improved hydromorphological habitat quality in forested reaches when compared with open agricultural reaches [50,51]. While benefits of woody riparian buffers for shading, stream flow and benthic habitats were evident in our study, they were not matched with a significant improvement in water quality (i.e., nutrient concentrations). Our characterization of nutrient levels were based on only a few sampling dates and may not have been sufficient to detect subtle differences between our paired reaches. Nevertheless, these results suggest that the capacity for a local scale mitigation measure (i.e., forested buffers) to achieve dramatic improvements in nutrient pollution arising from land use at the catchment scale may be limited.

4.2. Taxonomic and Trait Changes in Buffered and Unbuffered Reaches

Changes in instream habitat due to the loss of riparian vegetation can have strong effects on invertebrate communities [14,46,50], manifesting in altered macroinvertebrate taxonomic and trait composition between buffered and unbuffered reaches in our study. NMDS and RDA analyses showed that species traits discriminated instream differences between the paired reaches with greater resolution than taxonomic composition; a finding that is consistent with previous work showing that traits better reflect functional patterns and processes than taxonomic composition [52,53]. In our study, life history traits related to voltinism, development time and body size, along with feeding preferences and respiration contributed to the dissimilarity between buffered and unbuffered reaches.

We expected that basal resources would change with a shift from autochthonous to allochthonous food resources in buffered reaches as incident light and stream temperatures decreased. For example, Allan et al. [18] showed that the relative abundance of scrapers decreased with greater vegetation cover and Wallace et al. [8] showed experimentally that excluding leaf litter and woody debris from the streams resulted in a significant decrease in abundance of shredders, gatherers and predators in mixed substrate habitats. In buffered reaches, higher inputs of allochthonous leaf litter and woody debris originating from the adjacent riparian vegetation was expected to result in a higher diversity and proportion of shredders (e.g., [34]). However, although the cover of woody debris and CPOM was higher in buffered reaches, shredder abundances failed to track these changes. This result likely reflects the very high abundances of the crustacean *Gammarus pulex* in unbuffered reaches, which is often the dominant shredder in southern Swedish streams but feeds on other resources also, including other invertebrates [54].

We found significantly higher abundances of macrophytes in unbuffered reaches supporting the conjecture that lower shading resulted in increased primary production. However, our prediction that unbuffered reaches would also have higher algal coverage and consequently higher scraper abundances was not supported—scraper abundances were significantly higher in buffered reaches. This finding could be due to differences in substrata (e.g., unbuffered reaches were dominated by fine sediment, whilst buffered reaches were more characterized by gravels).

Combined, these findings suggest that much of the macrophyte-driven primary production found in unbuffered reaches is likely entering aquatic food webs through detrital pathways via high abundances of *G. pulex* feeding on decaying macrophytes. High densities of gammarids potentially divert energy from the aquatic food web into trophic "cul-de-sacs" [55], because cross-habitat connectivity predominantly relies on insects with an adult flying stage as the primary vector for transferring energy and nutrients from aquatic to terrestrial food webs [56].

As predicted, taxa with a preference for gravel substrates and active aerial dispersal were more abundant at buffered reaches. These included EPT taxa, well known for their sensitivity to pollution and their preference for flowing, oxygen-rich waters and hard-bottom substrata [30,32,57]. Three species, in particular, were more abundant: the glossosomatid caddisfly *Agapetus ochripes*, the rhyacophilid caddisfly *Ryacophila nubile* and the baetid mayfly *Baetis rhodani*. The abundances of *R. nubila* were potentially explained by their preference for areas with moderate to high current velocities, in addition to their known predation of the Simullidae, which are often present in large numbers in these habitats [58,59]. Increased abundances of *A. ochripes* likely reflected differences in benthic habitat, since Glossosomatidae build cases of stones and cling to boulders, cobbles and large wood in well-oxygenated, flowing stream reaches, feeding on attached algae and FPOM [34]. Contrary to our expectations, we found higher proportion of plastron breathing taxa at the buffered reaches, which is most likely associated with the high abundance of adult coleopterans (i.e., *Limnius volckmari*, *Hydraena* sp. and family Haliplidae).

Invertebrate traits associated with depositional habitats (e.g., preference for or tolerance of negligible flow conditions and CPOM substrate preferences) were more prevalent in unbuffered reaches. We observed high abundances of *Pisidium* which was expected given these filter-feeding molluscs are commonly found in agricultural streams with slow velocities and a predominance of fine sediments [60,61].

4.3. Taxonomic and Trait Response to Environmental Variables

Constrained ordination (RDA) and variation partitioning (pRDA) revealed that the strongest predictors of macroinvertebrate communities between our paired reaches were related to nutrient enrichment, instream productivity and benthic habitat quality (e.g., % gravel). Although riparian characteristics alone explained only a minor proportion of the variability in taxonomic composition between our study reaches and was for the most part a negligible predictor of trait composition, these findings do not imply that riparian characteristics are not robust predictors of changes in the macroinvertebrate communities. The shared variation of functional community composition explained by riparian and instream factors (7%) indicate the strong linkage between aquatic and terrestrial habitats at the reach scale in our study. For example, instream shading was highly correlated with riparian canopy cover and, although our analyses failed to detect a significant effect of shading on instream water temperatures, many other studies have shown shading effects on water temperatures, e.g., [26,46]. In particular, the presence of riparian vegetation has been shown to moderate seasonal and diel variations of instream temperatures, e.g., [26,62,63]. Consequently, reforestation of riparian habitats is consequently used in ecosystem management for reducing or mitigating the effects of climate warming on instream communities [64]. The abundance and type of riparian vegetation also strongly affects instream basal resources directly through inputs of allochthonous organic matter and indirectly by altering incident light and thereby autochthonous production [50,65]. Hence, both water temperatures as a strong physiological driver and basal resources are considered as important determinants of instream biodiversity and function [18].

We implicitly assumed that we would be able to detect strong effects of environmental filtering (i.e., species sorting; [66]) on invertebrate communities due to changes in riparian and stream habitat. However, metacommunity theory has emphasized that community structure is determined not only by local abiotic environmental conditions (i.e., environmental filtering leading to species sorting), but also by biotic interactions and dispersal [67]. In particular, mass effects (i.e., the presence of species in environmentally suboptimal reaches due to high dispersal rates from environmentally suitable reaches) may obscure changes in α-diversity [68]. We found that, contrary to our expectations, metrics of α-diversity (e.g., taxa richness) did not differ between buffered and unbuffered reaches. This result might reflect spatial proximities of our paired unbuffered and buffered sites, which could allow mass effects from upstream reaches could influence local diversity patterns. For instance, forested reaches further upstream from our reach pairs could lead to the presence of sensitive taxa at unbuffered, downstream reaches and the relatively short distances between the paired reaches meant that tolerant taxa from unbuffered reaches could also be present downstream even though habitat conditions improved. However, we did see changes in β-diversity patterns between buffered and unbuffered reaches, with abundances of key taxa changing due to environmental filtering. The relative abundances of species and function composition are increasingly used to describe differences in β-diversity patterns [69]. Our trait analyses provided evidence of this with increased abundances of taxa with a preference for gravel substrates matching the change in benthic habitat conditions (% gravel) in the buffered reaches. These changes likely contributed to the increased abundances of sensitive EPT taxa in reaches with a forested riparian buffer.

4.4. Implications for Cross-Ecosystem Connectivity

Active aerial dispersal is typically related to larger insect body sizes and greater development time (e.g., Odonata, Ryacophilidae) and, thus, could reflect differing sensitivities to disturbance and/or reliance on autochthonous and allochthonous food resources [17]. Larger organisms generally live longer and, thus, may be more at risk of environmental fluctuations [70]. This could include extreme events (e.g., pollution, heatwaves) or summer low flows that lead to sedimentation, reduced oxygen concentrations and increased stream temperatures. The increased abundances of caddisflies like *R. nubile* in buffered reaches could be particularly important as actively aerial dispersers. Ecological theory predicts that larger organisms have greater metabolic demands [71] and, thus, actively aerial dispersing insects could be constrained by food quality and quantity. Allochthonous inputs of terrestrial organic matter were significantly higher in buffered reaches and although macrophytes were more prevalent in unbuffered reaches, filamentous algae cover was not significantly different between reach types.

The higher abundances of aquatic insects with a relatively large body size and aerial dispersal in buffered reaches may have repercussions for the transfer of nutrients and energy into terrestrial food webs [72]. For example, the biomass of stream insects sharing these traits has been correlated with riparian spider biomass and abundances [73]. However, in addition to the higher abundances of invertebrates completely lacking an adult flying stage in unbuffered reaches, the greater prevalence of passively aerial dispersing insects may also dampen cross-habitat connectivity in our streams. We observed more chironomid midges at unbuffered reaches which are weak fliers and their dispersal has been shown to be restricted to the stream edges [74]. Therefore, higher abundances with active aerial dispersing taxa at the buffered reaches in our study indicate the potential for the reciprocal transfer of subsidies to terrestrial ecosystems [5,47,75–77].

5. Conclusions

We found evidence of positive influence with changes in stream habitat and increased abundances of sensitive EPT stream insects at buffered reaches. The strong effect of riparian forest on shading was notable because of its potential to help reduce the growths of nuisance aquatic vegetation (i.e., filamentous green algae and macrophytes) and moderate stream temperatures.

We also used invertebrate traits to explore how riparian vegetation structure potentially affects stream-riparian food webs. Some of our results were counter-intuitive (i.e., high abundances of gammarid amphipod *G. pulex* at unbuffered sites); however, we did see evidence of potentially more stable food webs in buffered reaches, where increased inputs of terrestrial organic matter were complemented by the greater presence of coarse benthic substrate helping to facilitate grazing and scraping invertebrates (i.e., caddisfly *A. ochripes*). The higher abundances of active aerial dispersing stream insects in the buffered reaches also suggested that cross-habitat connectivity with riparian food webs could be strengthened, reflecting an increased reciprocal transfer of nutrients and energy to terrestrial ecosystems. Future work will focus more on how riparian vegetation structure influences these connections by quantifying feeding linkages and looking at seasonal patterns in insect emergence and riparian predator communities. We contend that forest riparian buffers could be a valuable management tool as a nature-based solution enabling adaptation to climate change in agricultural areas. Our present study has contributed to the development of a general framework for implementing forested riparian buffers in human-impacted landscapes by demonstrating the mitigation of agricultural impacts by riparian forest patches. These improvements add to the broad portfolio of benefits that riparian forest buffers can provide in modified landscapes.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13081028/s1, Table S1. Environmental variables (mean ± 1 SD) at unbuffered and buffered reaches in 10 boreal streams; Table S2. List of 62 families and 6 higher taxonomic groups, arranged by the highest abundance at the unbuffered sites; Table S3. Average values of invertebrate taxa and biodiversity indices at the buffered and unbuffered reaches; Table S4. Analysis of similarity (SIMPER) of taxonomic and trait community differences between unbuffered (n = 10) and buffered (n = 10) reaches; Table S5. Macroinvertebrate taxa scores (n = 94) from the partial redundancy analysis model (pRDA) conditioning out confounding influences of instream properties as well as spatial structure and catchment characteristics; Table S6. Trait distribution scores (n = 39) from the partial redundancy analysis model (pRDA) conditioning out confounding influences of instream properties as well as spatial structure and catchment characteristics; Figure S1. Unconstrained ordination (NMDS) of reaches based on Hellinger transformed taxa (a) and community weighted means of traits (b); Figure S2. Venn diagram showing variation partitioning (VP) of taxonomic (a) and trait (b) composition of macroinvertebrate communities in 20 study reaches.

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Forested Riparian Buffers Change the Taxonomic and Functional Composition of Stream Invertebrate Communities in Agricultural Catchments

Supplementary material

Table S1. Environmental variables (mean ± 1 SD) at unbuffered and buffered reaches in 10 boreal streams. Variables showing significant difference between the reaches are shown in bold text (paired *t*-test, $p < 0.05$). Catchment land use was not included as the individual streams were nested in the same sub-catchments. Statistical significance: ** < 0.01, * < 0.05, †< 0.1.

VARIABLE	Unbuffered	Buffered	*t*-ratio	*p* value
Riparian buffer size (m^2)	**1321 ± 1473**	**45415 ± 38904**	**−6.37**	**
Length (m)	**38 ± 48**	**510 ± 321**	**−6.33**	**
Width (m)	**23 ± 18**	**85 ± 42**	**−6.04**	**
Tree density (trees/m^2)	0.04 ± 0.03	0.06 ± 0.02	−1.51	0.1644
Unmanaged grass (%)	**32±20**	**4.9±6.9**	**−4.27**	**
Herbs (%)	34.5±23.9	31.3±19.7	−0.13	0.9004
Moss/lichens (%)	**3.7±4.8**	**17.7±12.1**	**4.24**	**
Trees/shrubs (%)	**19±14**	**48±14.7**	**3.33**	**
Rocks/bedrock (%)	**6.4±5.7**	**16.8±9.5**	**2.58**	*
Plant litter (%)	**11.2±12.3**	**47±16.6**	**4.83**	**
Bare ground (%)	5.8±4.4	4.1±3.6	−0.93	0.3779
Managed grass (%)	**15.4±18.3**	**0.5±1.6**	**−3.08**	*
Bankfull width (m)	6.7 ± 3	7.3 ± 3.6	1.08	0.3098
Bankfull depth (cm)	64.2 ± 11.5	66.7 ± 14.5	0.54	0.6027
Channel width (m)	4.3 ± 2.7	4.5 ± 3.5	0.01	0.9934
Channel depth (cm)	**27 ± 11.7**	**20.3 ± 9.3**	**3.55**	**
Flow (m/s)	**0.1 ± 0.1**	**0.2 ± 0.1**	**−2.75**	*
Temperature (°C)	14.2 ± 1.1	13.8 ± 1.7	2.01	†
Instream shading (%)	**34 ± 19.5**	**69.2 ± 15.4**	**−4.04**	**
pH	**5.5 ± 2.7**	**5.9 ± 3.5**	**−2.55**	*
EC (mS/m^2)	43.1 ± 30.4	41.5 ± 30.6	0.83	0.4279
Total organic carbon (mg/l)	19.6 ± 2.3	18.8 ± 3	1.06	0.3177
NH_4 (µg/l)	113 ± 179.7	29.9 ± 20.8	1.62	0.1338
NO_3–N (µg/l)	1050 ± 1497	1063 ± 1452	−1.24	0.2446
Total P (µg/l)	68.7 ± 42.8	55.49 ± 28.8	1.07	0.3135
Algae (%)	3.3 ± 6.6	5.1 ± 8.2	−0.70	0.5044
Bedrock, boulders (%)	8.6 ± 6.4	15 ± 13.7	−2.00	†
Bryophytes (%)	6.0 ± 10.4	11 ± 12.9	-1.40	0.4163
CPOM (%)	**5.1 ± 4.7**	**9.6 ± 6.3**	**−2.83**	*
Large woody debris (%)	**3.1 ± 3**	**9 ± 6.9**	**−3.39**	**
FPOM (%)	4.1 ± 1.9	7 ± 8.5	−1.06	0.3149
Gravel (%)	39 ± 13.6	52 ± 19.8	-1.19	0.2648
Macrophytes (%)	**16 ± 11.7**	**1.1 ± 1.1**	**4.84**	**
Fine sediment (%)	**25 ± 7.4**	**5.9 ± 4.8**	**6.15**	**

Table S2. List of 62 families and 6 higher taxonomic groups, arranged by the highest abundance at the unbuffered sites.

	Unbuffered		Buffered	
	Mean	Std. Dev.	Mean	Std. Dev.
Pisidiidae	470.2	885.7	183.5	297.8
Gammaridae	448.7	542.7	303.1	360.2

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	Unbuffered		Buffered	
	Mean	Std. Dev.	Mean	Std. Dev.
Chironomidae	192.7	220.1	103.5	78.4
Oligochaeta	180.4	167.6	123.9	92.8
Asellidae	147.0	244.8	38.9	33.7
Elmidae	132.3	286.4	137.7	251.9
Simuliidae	94.1	194.0	46.8	84.8
Nemouridae	69.5	103.0	23.5	27.8
Baetidae	60.3	107.5	75.9	85.5
Limnephilidae	51.6	35.4	30.5	19.4
Ephemeridae	22.4	61.8	12.6	22.1
Nematoda	20.6	63.1	3.6	4.6
Hydropsychidae	13.3	28.2	32.4	59.2
Ceratopogonidae	9.8	9.8	12.7	25.0
Bithyniidae	6.8	21.5	0.6	1.9
Polycentropodidae	6.0	9.9	9.0	14.7
Hydroptilidae	5.9	18.7	5.8	15.2
Erpobdellidae	5.7	9.8	2.1	4.0
Glossosomatidae	5.6	9.5	25.7	34.3
Empididae	5.2	10.5	8.6	18.4
Psychodidae	5.2	15.1	0.6	1.3
Limoniidae	4.8	10.3	0.8	1.9
Zygoptera	3.8	10.1	0.0	0.0
Tricladida	3.6	5.4	0.7	1.3
Lymnaeidae	3.1	4.2	0.9	2.2
Calopterygidae	2.7	4.5	0.0	0.0
Caenidae	1.8	5.7	7.8	24.7
Sialidae	1.7	2.9	0.4	1.0
Corduliidae	1.5	3.2	0.2	0.6
Glossiphoniidae	1.5	3.8	0.7	1.6
Leptoceridae	1.5	4.4	6.0	15.0
Acroloxidae	1.4	2.5	0.6	1.9
Neritidae	1.4	4.4	1.8	5.7
Rhyacophilidae	1.3	2.7	5.0	6.1
Heptageniidae	1.2	3.8	1.2	2.5
Hydrachnidiae	1.2	2.5	1.7	2.5
Tabanidae	1.2	2.5	0.0	0.0
Leuctridae	0.9	2.0	1.1	2.3
Haemopidae	0.8	2.5	0.0	0.0
Dytiscidae	0.6	1.9	0.0	0.0
Nepidae	0.6	1.9	0.0	0.0
Pediciidae	0.6	1.3	3.8	6.0
Scirtidae	0.6	1.9	0.7	1.9
Chrysomelidae	0.5	1.6	0.0	0.0
Gyrinidae	0.5	1.6	0.0	0.0
Haliplidae	0.2	0.6	0.7	2.2
Astacidae	0.1	0.3	0.0	0.0
Corixidae	0.1	0.3	0.0	0.0
Hydraenidae	0.1	0.3	6.8	16.8
Anisoptera	0.0	0.0	0.2	0.6
Chaoboridae	0.0	0.0	0.0	0.0
Cordulegastridae	0.0	0.0	0.9	2.8
Dreissenidae	0.0	0.0	0.0	0.0
Ephemerellidae	0.0	0.0	3.0	9.5
Lepidoptera	0.0	0.0	0.2	0.6
Lepidostomatidae	0.0	0.0	1.6	3.5
Leptophlebiidae	0.0	0.0	0.0	0.0

	Unbuffered		Buffered	
	Mean	Std. Dev.	Mean	Std. Dev.
Molannidae	0.0	0.0	0.3	0.9
Muscidae	0.0	0.0	0.0	0.0
Philopotamidae	0.0	0.0	0.0	0.0
Physidae	0.0	0.0	0.0	0.0
Planorbidae	0.0	0.0	0.3	0.9
Psychomyiidae	0.0	0.0	0.7	1.9
Ptychopteridae	0.0	0.0	0.6	1.9
Sericostomatidae	0.0	0.0	0.9	2.8
Stratiomyidae	0.0	0.0	0.0	0.0
Tipulidae	0.0	0.0	0.2	0.4
Valvatidae	0.0	0.0	0.7	2.2

Table S3. Average values of invertebrate taxa and biodiversity indices at the buffered and unbuffered reaches.

	Unbuffered	**Buffered**
Taxa richness	24.2 ± 5.2	26.2 ± 6.3
EPT taxa richness	7.1 ± 2.9	9.3 ± 5
% EPT	14.1 ± 7.1	21.1 ± 16.5
Shannon-Wiener index (H′)	2.12 ± 0.4	2.23 ± 0.5
Simpson index (1-D)	0.79 ± 0.1	0.8 ± 0.1
Evenness (E)	0.36 ± 0.1	0.38 ± 0.1
Dominance	0.21 ± 0.1	0.20 ± 0.1

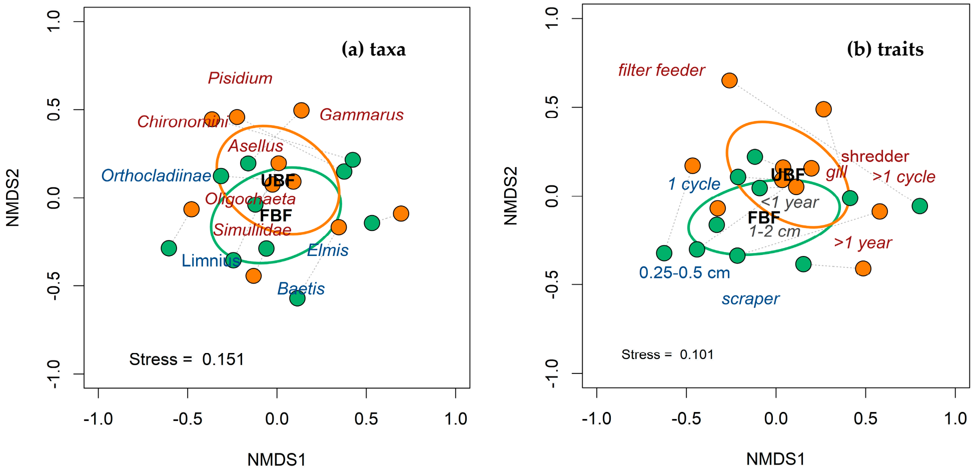


Figure S1. Unconstrained ordination (NMDS) of reaches based on Hellinger transformed taxa **(a)** and community weighted means of traits **(b).** Presented taxa and traits had the highest (top 10) contribution to average between group dissimilarity in SIMPER analysis (Table S4). The species and traits with are shown in dark brown color (associated to unbuffered reaches with orange ellipse and orange dots) and blue color (associated to buffered reaches with green ellipse and green dots) color. Traits shown in dark grey color (<1 year and 1–2 cm) had the same average community weighted means between the reaches.

Table S4. Analysis of similarity (SIMPER) of taxonomic and trait community differences between unbuffered (n = 10) and buffered (n = 10) reaches. Species contribution to average between group dissimilarity is shown in column “Contribution %”. Community composition is shown as average of Hellinger transformed taxa abundance, while traits are shown as average community weighted means.

Taxon	Contribution %	Cumulative %	Unbuffered	Buffered
		Community composition		
Gammarus pulex	3.9	7.1	0.41 ± 0.2	0.39 ± 0.3
Pisidium sp.	3.1	12.7	0.35 ± 0.2	0.29 ± 0.2
Limnius volckmari	2.5	17.3	0.11 ± 0.1	0.23 ± 0.2
Simuliidae	2.4	21.6	0.16 ± 0.2	0.15 ± 0.1
Elmis aenea	2.3	25.9	0.14 ± 0.1	0.22 ± 0.2
Asellus aquaticus	2.1	29.7	0.21 ± 0.2	0.15 ± 0.1
Chironomini	1.9	33.1	0.19 ± 0.1	0.09 ± 0.1
Baetis sp.	1.8	36.3	0.09 ± 0.1	0.10 ± 0.2
Orthocladiinae	1.7	39.5	0.12 ± 0.1	0.15 ± 0.1
Oligochaeta	1.6	42.5	0.30 ± 0.1	0.31 ± 0.1
		Trait composition		
# of cycles per year > 1	1.0	5.8	0.56 ± 0.1	0.48 ± 0.2
# of cycles per year = 1	1.0	11.6	0.41 ± 0.1	0.48 ± 0.2
shredder	1.0	17.4	0.39 ± 0.2	0.34 ± 0.1
filter-feeder	1.0	23.0	0.28 ± 0.2	0.20 ± 0.1
gill respiration	0.9	28.3	0.63 ± 0.1	0.59 ± 0.1
scraper	0.9	33.5	0.21 ± 0.1	0.33 ± 0.1
size 0.25–0.5 cm	0.8	38.4	0.24 ± 0.1	0.30 ± 0.1
life cycle > 1 year	0.7	42.7	0.45 ± 0.1	0.45 ± 0.1
life cycle < 1 year	0.7	47.0	0.55 ± 0.1	0.55 ± 0.1
size 1–2 cm	0.7	50.8	0.34 ± 0.1	0.27 ± 0.1

Table S5. Macroinvertebrate taxa scores (n = 94) from the partial redundancy analysis model (pRDA) conditioning out confounding influences of instream properties as well as spatial structure and catchment characteristics. Invertebrate taxa are sorted from highest to lowest scores on RDA1.

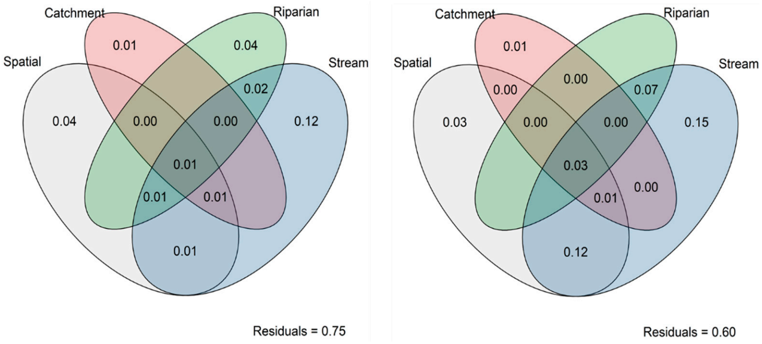
	RDA1	RDA2		RDA 1	RDA2
Gammarus pulex	0.3105	0.0211	Pisidium	−0.1504	−0.0500
Baetis sp.	0.1729	0.0482	Simuliidae	−0.1304	0.0519
Baetis rhodani	0.0972	-0.0199	*Limnius volckmari*	−0.1055	−0.0218
Elmis aenea	0.0418	−0.0402	*Amphinemura borealis*	−0.0848	0.0097
Oulimnius sp.	0.0361	0.0210	*Nemoura cinerea*	−0.0776	0.0069
Anabolia nervosa	0.0360	−0.0052	Chironomini	−0.0761	0.0724
Orthocladiinae	0.0263	−0.0981	*Asellus aquaticus*	−0.0668	0.0284
Nemoura avicularis	0.0238	−0.0186	Nematoda	−0.0555	−0.0282
Erpobdella octoculata	0.0212	0.0078	*Nemoura* sp.	−0.0442	0.0290
Hydroptila sp.	0.0211	−0.0226	Tanytarsini	−0.0386	0.0195
Agapetus ochripes	0.0161	−0.0297	Oligochaeta	−0.0343	−0.0006
Leuctra sp.	0.0157	0.0134	*Hydropsyche pellucidula*	−0.0327	0.0044
Tricladida	0.0155	0.0146	*Pericoma* sp.	−0.0318	−0.0051
Hydrachnidiae	0.0138	−0.0069	Limnephilidae	−0.0308	0.0460
Oulimnius tuberculatus	0.0137	0.0406	*Radix balthica*	−0.0268	0.0495
Rhyacophila sp.	0.0136	−0.0226	*Ephemera vulgata*	−0.0195	0.0103
Caenis rivulorum	0.0135	−0.0198	*Glossiphonia* sp.	−0.0170	0.0027
Lepidostoma hirtum	0.0131	−0.0143	*Tipula* sp.	−0.0159	−0.0059
Ceratopogonidae	0.0129	−0.0364	*Lype reducta*	−0.0133	−0.0031
Tanypodinae	0.0126	−0.0329	*Hydropsyche siltalai*	−0.0131	−0.0816
Polycentropus flavomaculatus	0.0121	0.0038	*Hydraena* sp.	−0.0109	−0.0530
Haliplus sp.	0.0111	−0.0020	Empididae	−0.0096	−0.0471

	RDA1	**RDA2**		**RDA 1**	**RDA2**
Hydropsyche angustipennis	0.0103	0.0457	*Haemopis sanguisuga*	−0.0091	0.0005
Somatochlora metallica	0.0097	0.0134	*Sialis lutaria*	−0.0078	−0.0006
Prodiamesinae	0.0096	−0.0337	*Baetis niger*	−0.0073	0.0024
Polycentropus irroratus	0.0086	0.0005	*Halesus* sp.	−0.0072	0.0079
Hydropsyche sp.	0.0084	−0.0129	Limoniidae	−0.0060	−0.0036
Cyrnus trimaculatus	0.0083	−0.0065	Dytiscidae	−0.0057	0.0020
Valvata piscinalis	0.0083	−0.0065	*Nepa cinerea*	−0.0055	0.0049
Helobdella stagnalis	0.0079	0.0050	*Theromyzon tessulatum*	-0.0055	0.0049
Bithynia tentaculata	0.0076	0.0005	*Calopteryx virgo*	−0.0053	0.0022
Theodoxus fluviatilis	0.0074	-0.0081	*Sialis lutaria* gr.	−0.0052	0.0069
Ephemerella mucronata	0.0070	−0.0145	*Rhyacophila fasciata*	−0.0045	−0.0122
Anisoptera	0.0065	−0.0015	*Calopteryx* sp.	−0.0044	0.0136
Athripsodes sp.	0.0060	−0.0234	Tabanidae	−0.0041	0.0143
Gyraulus acronicus	0.0055	−0.0043	*Centroptilum luteolum*	−0.0028	−0.0149
Molanna angustata	0.0055	−0.0043	*Heptagenia sulphurea*	−0.0026	−0.0137
Ceraclea sp.	0.0054	−0.0113	Zygoptera	−0.0024	0.0097
Eloeophila sp.	0.0052	−0.0008	*Dicranota* sp.	−0.0006	0.0049
Baetis vernus	0.0037	0.0290	*Plectrocnemia* sp.	0.0000	−0.0272
Oxyethira sp.	0.0035	0.0054	*Rhyacophila nubila*	0.0004	−0.0144
Amphinemura sp.	0.0028	0.0045	*Acroloxus lacustris*	0.0007	0.0161
Ptychoptera sp.	0.0027	−0.0120	*Donacia* sp.	0.0012	0.0019
Elodes sp.	0.0026	−0.0061	*Orectochilus villosus*	0.0012	0.0019
Astacidae	0.0024	0.0026	*Cordulegaster boltonii*	0.0015	−0.0059
Calopteryx splendens	0.0021	0.0032	*Sericostoma personatum*	0.0015	−0.0059
Lepidoptera	0.0021	−0.0064	*Sigara* sp.	0.0020	0.0032

Table S6. Trait scores (n = 39) from the partial redundancy analysis model (pRDA) conditioning out confounding influences of instream properties as well as spatial structure and catchment characteristics. Invertebrate taxa are sorted from highest to lowest scores on RDA1 axis. RDA2 is equivalent to PC1 axis, due to a one variable model (% Canopy).

	RDA1	**RDA2**
size 0.25–0.5 cm	0.0983	0.1001
1 cycle per year	0.0944	0.0448
filter feeder	0.0747	0.3239
tegument breathing	0.0455	0.0972
size 0.5–1 cm	0.0402	0.0502
aerial active dispersal	0.0356	−0.0671
plastron breathing	0.0263	−0.0465
sand substrate preference	0.0182	0.0423
mud substrate preference	0.0156	0.0974
aerial passive dispersal	0.0136	0.0810
predator	0.0134	0.0181
silt substrate preference	0.0121	0.0716
life cycle < 1 year	0.0112	−0.0085
gravel substrate preference	0.0071	−0.0540
scraper	0.0063	−0.2488
deposit feeder	0.0058	−0.0063
slow flow	0.0045	0.0497
parasite	0.0041	0.0109
null flow	0.0013	0.2102
size 4–8 cm	0.0009	−0.0008
<1 cycle per year	0.0008	0.0333
spiracle breathing	0.0001	−0.0067
size > 8 cm	−0.0002	−0.0001
size < 0.25 cm	−0.0002	0.0005

	RDA1	**RDA2**
fast flow	−0.0003	−0.1104
macrophytes substrate preference	−0.0040	−0.0159
flags/boulders/cobbles/pebbles substrate preference	−0.0042	−0.1157
medium flow	−0.0060	−0.1510
microphytes substrate preference	−0.0102	−0.0052
life cycle > 1 year	−0.0112	0.0085
organic/detritus/litter substrate preference	−0.0131	0.0248
aquatic active dispersal	−0.0171	−0.0713
twigs/roots substrate preference	−0.0224	−0.0463
aquatic passive dispersal	−0.0319	0.0593
size 2–4 cm	−0.0534	−0.0950
gill breathing	−0.0719	−0.0440
size 1–2 cm	−0.0857	−0.0549
>1 cycle per year	−0.0952	−0.0781
shredder	−0.1043	−0.1058



(a) taxa **(b) traits**

Figure S2. Venn diagram showing variation partitioning (VP) of taxonomic **(a)** and trait **(b)** composition of macroinvertebrate communities in 20 study reaches. Shown are total variations explained by set of predictors described as spatial (PCNM1, PCNM9, PCNM10), catchment (catchment area), riparian (% of canopy cover and rock/bedrock) and instream (nitrate, total phosphorus, % of algae and gravel) parameters. Values < 0 are not shown on the diagram.

Instream habitats alone, characterized by nutrients (nitrate, total phosphorus), substratum (gravel) and food resource (algae), explained 12–15% of the total variation in taxonomic ($F_{3,11}$=1.74, $p = 0.022$) and trait composition ($F_{4,11}$=1.75, $p = 0.065$), respectively (Figure 3). By contrast, riparian characteristics (% of canopy cover and rock/bedrock), catchment area nor spatial location were significant ($p > 0.05$). However, the shared variation between instream habitat and riparian characteristics explained 2% of the variability in taxonomic composition and 7% in trait composition. The independent and shared contribution of instream and riparian habitats significantly explained variability in taxonomic composition ($F_{5,11}$=2.52, $p = 0.003$) and trait composition ($F_{5,11}$=, $p = 0.035$).

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Stream–riparian meta-ecosystems are strongly connected through exchange of subsidies, but land use can disrupt ecological connectivity. This thesis explored the effects of forested riparian buffers, a common management practice in agricultural landscapes, and showed positive associations with ecological status, instream shading and the provision of suitable habitats for instream and riparian communities. Buffers strengthened cross-ecosystem connectivity and have the potential to affect a wide range of consumers in modified landscapes.

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