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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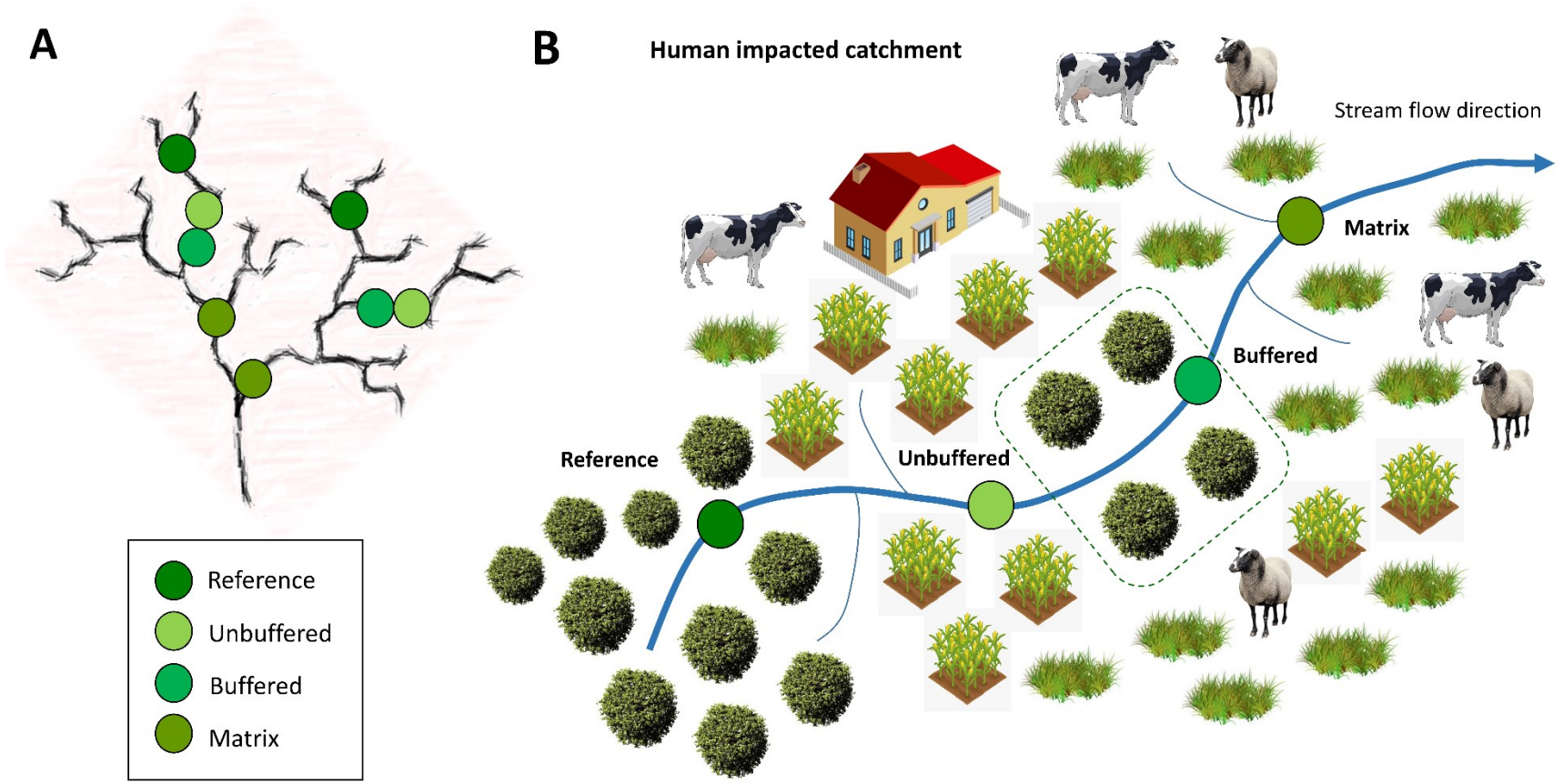

Figure S1. Four types of site were used in the CROSSLINK project: Pristine or least-impacted 'Reference' sites, site pairs with an 'Unbuffered' upstream site and a downstream 'Buffered' site with a woody riparian buffer on both banks, and 'Matrix' sites that were typically located further downstream 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.

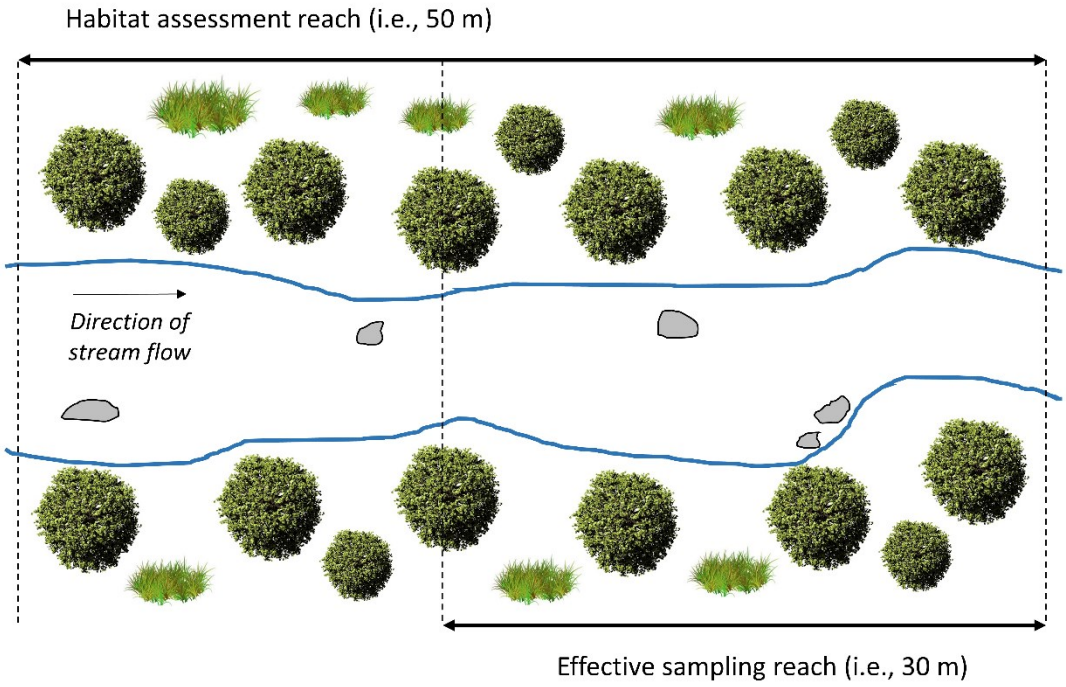

Figure S2. The effective sampling reach and the habitat assessment reach used in CROSSLINK. At each site, the different components of sampling for CROSSLINK were conducted over two reaches differing in length, with a shorter effective sampling reach nested in a longer habitat assessment reach.

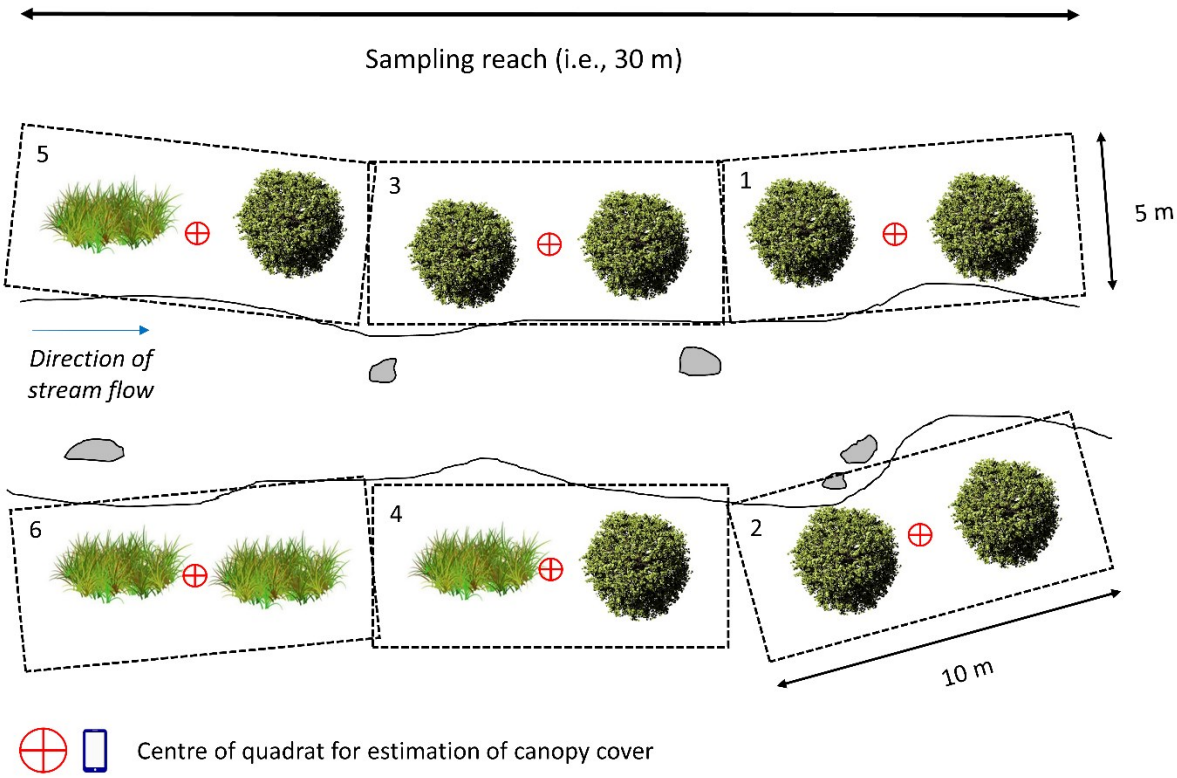

Figure S3. Riparian plots used to sample biodiversity and functional indicators (Protocols S3 and S4). We also measured aspects of riparian habitat within these plots (Protocol S2).

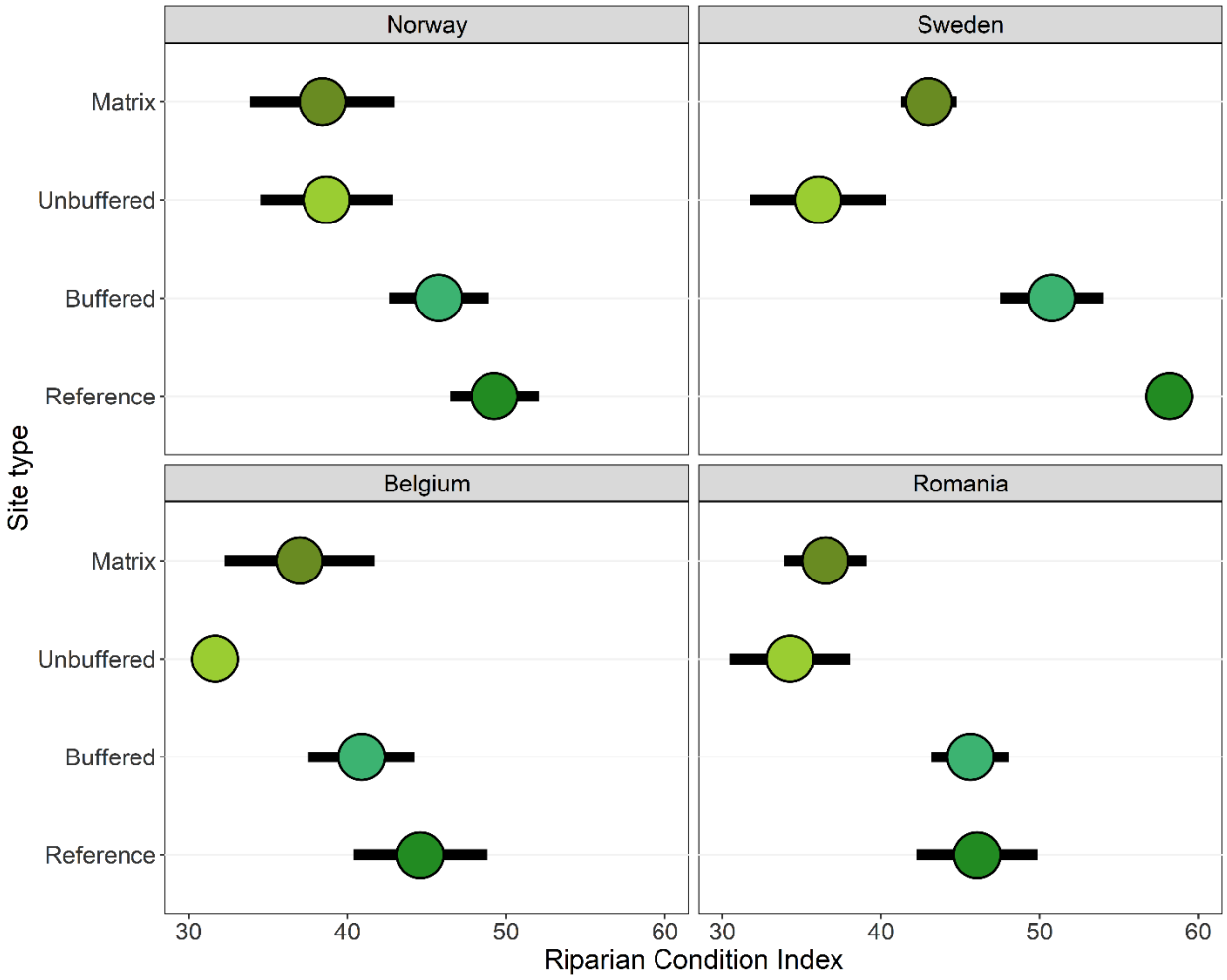

Figure S4. Mean values (± 95% CI) of the Riparian Condition Index for site types in each CROSSLINK case-study basin. The "Site type" refer to the sites used: pristine or least-impacted 'Reference' sites, site pairs with an 'Unbuffered' upstream site and a 'Buffered' downstream site with a woody riparian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

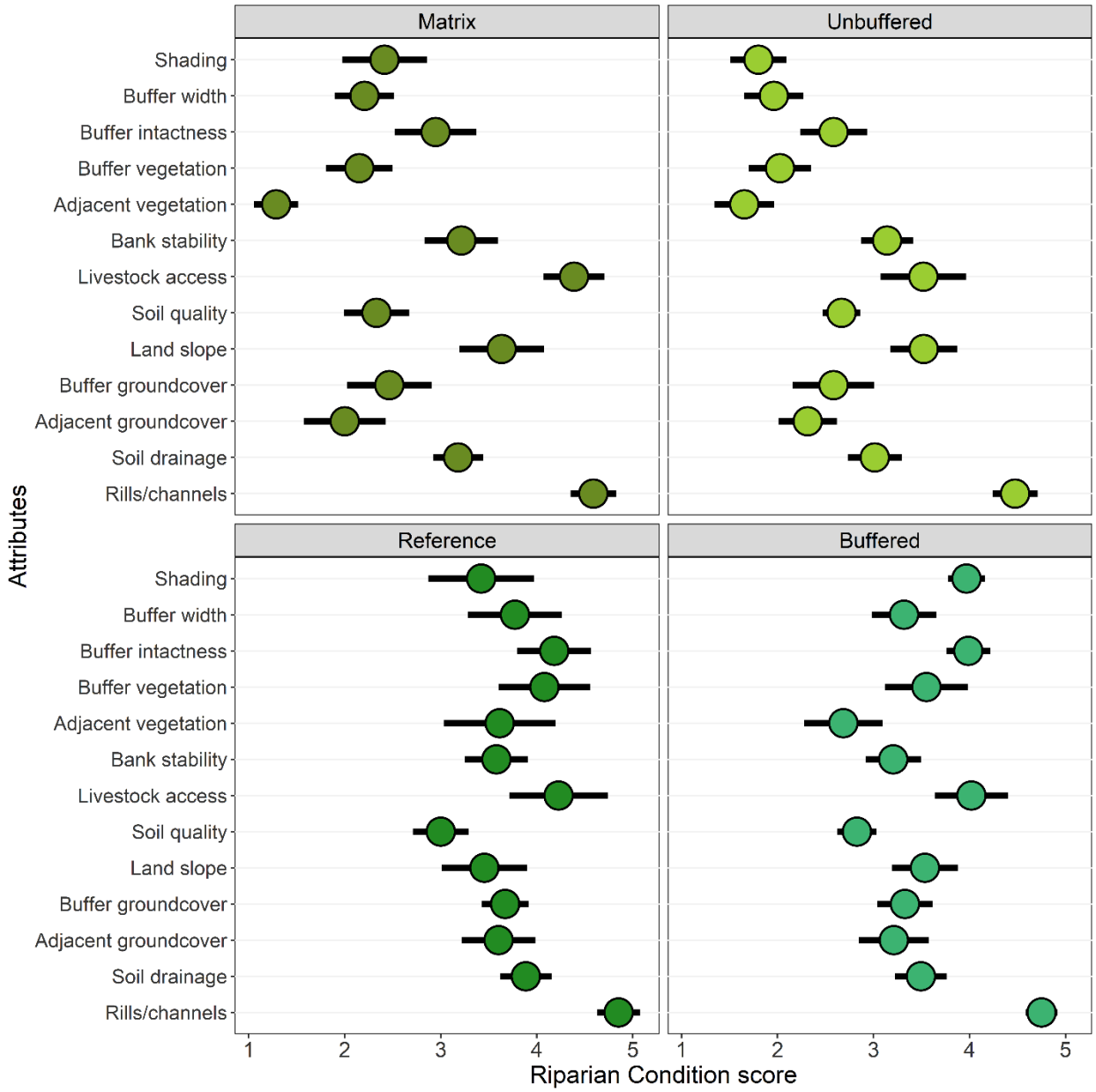

Figure S5. Mean values (± 95% CI) for each attribute used in the Riparian Condition Index for the four site types used in the CROSSLINK project. These plots include data from sites in Norway, Sweden, Belgium, and Romania. The "Site type" refer to the sites used: pristine or least-impacted 'Reference' 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):

$$d = \frac{C}{\pi} \quad (1)$$

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):

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

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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