Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Prioritizing conservation areas for Trichoptera in the Pyrenees using climate change vulnerability information at the taxonomic and genetic levels

Nieves López-Rodríguez

nievesbio95@gmail.com

Ecologia i Ciències Ambientals, Universitat de Barcelona (UB)

Julie Crabot

Institute of Environmental Assessment and Water Research

Raúl Acosta

Institute of Environmental Assessment and Water Research

Pello Isasi-Bea

Ecologia i Ciències Ambientals, Universitat de Barcelona (UB)

Miguel Cañedo-Arguelles

Institute of Environmental Assessment and Water Research

Mónica Lanzas

Ecologia i Ciències Ambientals, Universitat de Barcelona (UB)

Steffen Pauls

Senckenberg Research Institute and Natural History Museum Frankfurt/M

David Cunillera-Montcusí

Ecologia i Ciències Ambientals, Universitat de Barcelona (UB)

Virgilio Hermoso

Estación Biológica de Doñana

Oskar Schröder

Senckenberg Research Institute and Natural History Museum Frankfurt/M

R. G. Bina Perl

Senckenberg Research Institute and Natural History Museum Frankfurt/M

Julio Schneider

Senckenberg Research Institute and Natural History Museum Frankfurt/M

Romina Álvarez-Troncoso

Universidade de Vigo

Núria Bonada

Ecologia i Ciències Ambientals, Universitat de Barcelona (UB)

Research Article

Keywords: High Mountain Streams (HMS), Exact Sequence Variants (ESVs), Traits, Morphology, MARXAN

Posted Date: August 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10501311/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Identifying priority conservation areas is critical in high-mountain streams (HMS), which are highly vulnerable to climate change and biodiversity loss. However, systematic conservation planning rarely integrates species vulnerability with taxonomic and intraspecific genetic diversity, potentially overlooking important dimensions of biodiversity. We evaluated the climate change vulnerability of Trichoptera species using trait-based vulnerability values and assessed how different taxonomic and genetic diversity representations influence conservation priorities. We sampled Trichoptera across 17 streams, with three sampling sites per stream, and generated three complementary biodiversity datasets based on species-level identifications and Exact Sequence Variants (ESVs) obtained via COI metabarcoding. We developed species-specific climate change vulnerability values and incorporated them into the systematic conservation planning tool MARXAN, ensuring the representation of climate-sensitive communities across alternative conservation scenarios. Integrating functional and biogeographical traits into vulnerability values revealed differences in climate change sensitivity among taxa, identifying highly vulnerable species associated with restricted distributions and specific ecological requirements. MARXAN taxonomic scenarios selected fewer, more spatially clustered sites, whereas genetic scenarios maintained a more even spatial configuration and recovered western priority sites that would have been overlooked using taxonomic features alone. MARXAN genetic scenarios identified priority areas that preserved overall taxonomic diversity while capturing higher intraspecific diversity and maintaining vulnerability. Our findings demonstrate that integrating genetic diversity and species vulnerability into conservation planning improves the identification of climate-sensitive areas and provides a robust framework for biodiversity conservation in vulnerable high-mountain freshwater ecosystems.

Introduction

Global freshwater ecosystems are undergoing profound changes due to climate change, which is altering temperature regimes, hydrological cycles, and water quality at unprecedented rates (Dudgeon et al., 2006; IPCC, 2023). These changes pose significant threats to the biodiversity and ecological functioning of rivers, lakes, and streams, which provide essential ecosystem services such as water supply, nutrient cycling, and habitat provision (Jackson et al., 2001). The magnitude and pace of these transformations compromise the stability and resilience of freshwater ecosystems, putting biodiversity at risk and highlighting the urgent need to prioritize their conservation. To address these widespread threats, global biodiversity initiatives, such as the Kunming-Montreal Global Biodiversity Framework, set international targets for the conservation and representation of species, genetic, and ecosystem diversity, providing a benchmark for assessing the effectiveness of conservation strategies (CBD, 2022).

The establishment of priority sites for conservation has been proposed as an effective measure to halt biodiversity loss across different ecosystems (Brodie et al., 2023; Heino et al., 2009; Watson et al., 2014). For this purpose, systematic conservation planning emerges as a cost-effective approach to correctly represent biodiversity and promote its persistence in the long term through clear targets and strategic area selection (Margules & Pressey, 2000). Despite the continued expansion of protected areas

worldwide, this trend alone is not a reliable measure of conservation success, as biodiversity continues to decline globally (Rodríguez-Rodríguez & Martínez-Vega, 2022). Biodiversity decline also extends to reductions in functional and genetic diversity, which threaten ecosystem processes and adaptation capacity. However, conservation planning still relies mostly on species over other biodiversity dimensions (Hoban et al., 2020; Laikre et al., 2020). In this context, genetic tools enable the detection of intraspecific genetic diversity and population-level variation, improving the integration of adaptive potential into conservation planning (Nielsen et al., 2023; Pauls et al., 2013). Building on this perspective, recent contributions have highlighted multiple ways of integrating genetic data into systematic conservation planning, emphasizing either DNA barcoding, phylogenetic diversity, or population genomic approaches (Asmyhr et al., 2014; Coates et al., 2018; Nielsen et al., 2020; Paz-Vinas et al., 2018; Sheth & Thaker, 2017; Stark et al., 2025; Vasconcelos et al., 2018). Although these studies differ in the techniques used and the levels of diversity considered, they consistently demonstrate that integrating genetic data with traditional taxonomic assessments can enhance conservation planning by capturing evolutionary processes and population-level variation in conservation priorities.

While genetic approaches strengthen conservation planning by incorporating evolutionary processes and adaptive potential, they do not explicitly account for how biodiversity is expected to respond to future environmental change. In this regard, Climate Change Vulnerability Assessments (CCVAs) has become an increasingly important framework for evaluating species' susceptibility to climate change and guiding conservation priorities. CCVAs attempt to predict the degree to which climate change will impact species, subspecies, or populations (Foden, 2016). Overall, the vulnerability of the species depends on their ability to respond and adapt to changing conditions, which can be evaluated through trait-based approaches (Harper et al., 2022). Trait-based CCVAs provide a practical tool to approximate their loss, allowing the identification of critical traits for species resilience (Conti et al., 2014; Harper et al., 2022; Hershkovitz et al., 2015). By integrating species’ adaptive capacity and environmental sensitivity into CCVAs, conservation planners can prioritize areas where vulnerable species are most at risk, improving the coverage of management for species under future climate scenarios (Summers et al., 2012).

High-Mountain Streams (HMS), also known as alpine or high-elevation streams, are characterized by high species endemicity with a limited adaptability to climate change resulting from the specific local environmental conditions (Hotaling et al., 2017) and strong spatial isolation (Finn et al. 2011). HMS are projected to face decreasing winter precipitation alongside rising summer temperatures and more frequent droughts, thus accelerating snowmelt processes, glacial retreat, and disrupting hydrological regimes (Amblar-Francés *et al.*, 2020). In addition, some HMS, such as the ones in the Pyrenees (north-eastern Spain), also face climate-driven shifts in wind regimes that may influence species dispersal dynamics along longitudinal diversity patterns (López-Rodríguez, 2026), potentially modifying population connectivity for aquatic species with aerial stages. Overall, the cumulative changes of all these conditions will potentially foster shifts in species distribution ranges in mountain ecosystems, increasing extinction risk for narrowly distributed and specialized species (Brown et al., 2007; Guisan et al., 2019) or even intraspecific lineages (Bálint et al., 2011).

Trichoptera is an order of aquatic insects highly vulnerable to climate change (Kalaninová et al., 2014) and well represented in HMS, with some families and subfamilies (i.e., Rhyacophilidae, Drusinae), being particularly common. They exhibit high endemism, resulting from geographic isolation and evolutionary processes linked to historical glaciations, which have promoted local adaptation and genetic divergence (Domisch et al., 2011; Graf et al., 2008). In the Pyrenees, their distribution follows a pronounced longitudinal (west-east) gradient (López-Rodríguez, 2026; de Mendoza et al., 2015), combined with altitudinal temperature-related patterns associated with the cold and stable conditions typical of these ecosystems (Hering et al., 2009). The ecological specialization, endemism, and spatial structuring of Trichoptera makes them a key group to evaluate the potential of combining taxonomic, genetic, and trait-based CCVAs to guide conservation planning.

Here, we aimed to improve freshwater conservation prioritization in the Pyrenees by integrating species-specific climate change vulnerability with taxonomic and genetic biodiversity into systematic conservation planning. Specifically, we (1) assessed species-specific climate change vulnerability in Pyrenean Trichoptera by integrating functional and biogeographical traits into species-specific vulnerability values. We expected this species-specific vulnerability to provide complementary information to biodiversity patterns by identifying sensitive taxa whose conservation importance may not be captured by biodiversity representation alone. Furthermore, we (2) incorporated this vulnerability dimension into MARXAN to compare conservation scenarios based on taxonomic and genetic biodiversity datasets. We expected genetic diversity to capture finer-scale biodiversity variation not detectable through morphological identification (Paz-Vinas et al., 2018), providing additional information to conservation scenarios while maintaining the representation of taxonomic diversity.

Material and methods

Study area and sampling

We selected 17 pristine HMS on the southern slope of the Pyrenees across a wide range of altitudes (1128–2405 msl) and longitudes (2°19'3.6"E – 0°8'19.4"W) and located within national parks (Ordesa y Monte Perdido and Aigüestortes i Estany de Sant Maurici) or natural parks (Capçaleres del Ter i del Freser, Sorteny, Alt Pirineu, and Posets-Maladeta). We established three sampling sites along each stream (upper course, middle course, and lower course; López-Rodríguez, 2026). We selected these three sampling sites to obtain a broader characterization of the biodiversity of these HMS in the upper course versus downstream river sections. We also conducted field sampling in summer 2019 and spring 2020–2021 to reflect seasonal biodiversity patterns, with the second sampling campaign extending over two different years due to COVID-19 restrictions.

We measured several physicochemical parameters such as pH, dissolved oxygen (% and mg/L), water temperature, specific conductivity (SPC), and total suspended solids (TSS), using a multiparameter probe (YSI ProQuatro, Yellow Springs Instruments). We also obtained hydromorphological data, including channel width (mean, minimum, and maximum), channel depth (mean and maximum), and discharge,

which we measured using a flow velocity meter (MiniAir20, Schiltknecht). Finally, we assessed the Fluvial Habitat Index (IHF) following the protocol described by Pardo et al. (2002) to determine instream habitat heterogeneity (Supplementary material). We took a multihabitat macroinvertebrate sample along a 100-meter reach using a kicking net with 250 µm mesh size and sorted Trichoptera larvae. We collected successive samples until we found no new Trichoptera families in two consecutive samples. Additionally, we also collected adult Trichoptera by sweep-netting riparian vegetation during the field surveys whenever possible to improve taxonomic identification. We fixed all samples in 96% non-denatured ethanol, changing the ethanol after 24, 48, and 72 hours; and kept all samples in the freezer at -20 ºC until taxonomic identification to avoid DNA degradation.

Taxonomic and genetic diversity

We identified Trichoptera lavae under a stereoscope using specialised taxonomic keys (e.g., Décamps & Pujol, 1975; Coppa et al., 2012; Décamps, 1965, 1970; Graf et al., 2015; Sainz-Bariáin & Zamora-Muñoz, 2015; Vieira-Lanero, 2000; Vieira-Lanero et al., 1998, 2008; Wallace & Wallace, 1983; Waringer & Graf, 2019). We sent adult specimens to regional Trichoptera taxonomic experts (Prof. *Marcos González* and Dr. *Jesús Martínez*) for identification and used this information to refine larval identifications. We updated larval identifications to lower taxonomic levels only when adult records provided unambiguous support, such as when a taxon corresponded to a single known adult species in the study basin. We retained higher taxonomic levels whenever species-level assignment was uncertain.

We extracted DNA from pooled larval tissue per site using PowerSoil Pro DNA Extraction Kits (Qiagen) in a UV laminar flow cabinet inside a UV-sterilized room, using UV- and bleach-sterilized materials. To analyze intraspecific diversity, we used population genetic studies based on the Cytochrome C Oxidase subunit I (COI) gene, which showed congruent patterns with other intraspecific high-resolution genomic approaches (Weiss & Leese, 2025). We amplified a fragment of the COI gene using a two-step PCR protocol (Cruaud et al., 2017) and the primer pair fwhF2/ fwhR2n (GGDACWGGWTGAACWGTWTAYCCHCC / GTRATWGCHCCDGCTARWACWGG; Vamos et al., 2017). During the first PCR, we used 10 µl reaction volumes with 0.25 µM of each primer, 1X of the Multiplex PCR Master Mix (Qiagen Multiplex PCR Plus Kit, Qiagen), 2,75 µL of RNase-free water, and 1 µL of DNA. PCR conditions included an initial 5 min denaturation at 95°C, followed by 6 cycles of denaturation at 95°C, annealing at 68°C (decreasing by 1.5°C per cycle), and elongation at 72°C, followed by 24 cycles as before but with an annealing temperature of 58°C. The reactions ended with a final extension at 68°C for 10 minutes. For the second PCR, we used 10 µl reaction volumes containing 1x Q5 High Fidelity Polymerase Mix 2x (New England Biolabs), 0.25 µM of the universal forward and reverse primers, 1 µL of the PCR 1 products, and we filled the remaining volume with nuclease-free water. We run the second PCR with an initial 98°C for 30 seconds, followed by 10 cycles at 98°C for 10 seconds and 65°C for 75 seconds. We ended the reactions with a final extension at 65°C, 5 minutes. We verified amplification success via agarose gel electrophoresis. We pooled the libraries at equal DNA concentrations and purified them using 0.76X Ampure XP beads (Beckman). We quantified libraries with a Qubit 4.0 fluorometer (ThermoFisher) and checked for adapter dimers using a 2200 TapeStation with D1000 High

Sensitivity DNA Screen tapes (Agilent). Finally, we sequenced our library pools at Novogene using an Illumina NovaSeq system with 150 bp paired-end reads. We processed the sequence data using the APSCALE graphical interface (Buchner et al., 2022; Macher, 2022). It includes external tools VSEARCH (Rognes et al., 2016), CUTADAPT (Martin, 2011), and LULU filtering (Frøslev et al., 2017). Then, we applied quality filtering and assigned taxonomy using the BOLDigger pipeline (Buchner & Leese, 2020), retaining at species level when sequences showed ≥ 98% similarity to reference sequences and at genus level when similarity was ≥ 95%. We passed the quality control using TaxonTableTools (Macher et al., 2021). We subtracted potential contamination by removing, for each Exact Sequence Variant (ESVs), the maximum number of reads detected for these ESVs in any of the negative controls. Additionally, we applied a minimum relative abundance filter of 0.01% per sample to exclude low-read or potentially spurious sequences from the final dataset.

Vulnerability metric

We developed species-specific climate change vulnerability values for the identified Trichoptera species based on Conti et al. (2014), Graf et al. (2008), Hering et al. (2009), Hershkovitz et al. (2015), and Sarremejane et al. (2020). We selected 12 traits particularly relevant in HMS and categorized them into two groups: biogeographical (endemicity and persistence) and ecological + biological (stream zonation preference, temperature preference, altitude preference, microhabitat/substrate preference, feeding type, forewing female length max, emergence/flight period, duration of emergence period, life span, and reproductive cycle). The selected vulnerability traits reflect multiple functional angles, with a special emphasis on factors such as dispersal potential and habitat preferences (Isasi-Bea, 2020). For each trait, we scaled each trait category from 1 to 4 according to its relative contribution to climate change vulnerability (1 low, 2 medium, 3 high, and 4 very high) (Table 1). We obtained biogeographical traits using the “rgbif” package (Chamberlain et al. 2025) to retrieve species distribution data from the GBIF database (https://www.gbif.org/). While relying solely on GBIF may not always provide fully robust estimates due to gaps in sampling or reporting, in our study, GBIF allowed us to capture expected species distribution patterns. We reviewed all GBIF-derived distributions based on expert knowledge to ensure consistency with known species ranges (see supplementary material). We categorized endemisms into three levels based on the species’ known geographic distribution: non-endemic, for species with broad distributions beyond the Pyrenees; Pyrenean endemic, for species restricted to the Pyrenees; and Pyrenean micro-endemic, for species with extremely narrow distributions within specific areas of the Pyrenees. We also categorized species’ persistence based on their temporal occurrence in our samples. In this case, we defined four levels: Last year only, for species detected on GBIF exclusively in the most recent year; between 2 and 3 years, for species detected in the last two or three consecutive years; between 4 and 5 years; and + 5 years, for species consistently detected across historical records, but not recently detected. For biological and ecological traits, we scored each species on a 10-point scale for each trait modality (Supplementary Material). Finally, we scored each species based on data from the Freshwater Ecology database (www.freshwaterecology.info; Schmidt-Kloiber, & Hering, 2015) and specific bibliographic references, reflecting species' affinity to each trait category (see further information in Supplementary Material).

The species' vulnerability value represents how sensitive the species is to climate change, and we calculated it as the average of all traits values. We analyzed the potential association of vulnerable species with altitudinal position using an Indicator Value Analysis (IndVal) and computed corrected point-biserial correlation coefficients (Phi) using the "IndVal" package (Cáceres & Legendre, 2009; de Cáceres et al., 2010). For each group, we calculated Phi coefficients and their 95% confidence intervals via bootstrapping (n = 1000). We considered taxa whose confidence intervals did not overlap zero as significant indicator species. We performed all statistical analyses using R version 4.4.1 (R Core Team, 2024), and generated graphs using the "ggplot2" package (Wickham, 2016; Wickham *et al.*, 2024).

Prioritization of conservation sites

We identified priority sites for conservation using the MARXAN software (Ball et al., 2009). MARXAN is a widely used decision-support tool in systematic conservation planning that identifies sets of sites that achieve biodiversity representation targets at minimum cost, providing transparent and repeatable prioritization scenarios (Ball et al., 2009). In MARXAN, the objective function as defined in Hermoso et al. (2014) and Serra et al. (2020) includes:

$$Objective\ function = \sum_{i \in sites} Cost_i + \sum_{j \in especies} Shortfall\ Penalty_j\ x\ SPF_j$$¶

Where:

Objective function = the value MARXAN seeks to minimize, with lower values indicating more optimal site selections, considering the cost and the conservation targets.

Cost = the sum of the costs of the selected sites; in our study, we kept the cost constant for all sites (Cost = 10) to ensure that prioritization reflected only species vulnerability. So the total cost simply increased with the number of sites selected.

Shortfall Penalty = the penalty applied when a conservation target is not met, calculated as the shortfall (the difference between the target and the proportion actually represented). For a given conservation feature (e.g. a species), the conservation target was the proportion of its total distribution area to be conserved in the final solution.

SPF = the Species Penalty Factor applied to the penalty. The higher it is, the more strongly solutions that fail to meet the conservation target are penalized. We set SPF = 50 for all species.

We carried out MARXAN scenarios independently for the taxonomic and genetic data. First, we computed a MARXAN scenario for the taxonomic data using species as conservation features. We defined the target as a proportion of the distribution of a given species, and calculated its proportion as a function of the species vulnerability value, scaled between 0 and 50%. This means that, for the most vulnerable species, MARXAN aimed to include 50% of the sites where we detected the species in the

final solution. Meanwhile, the target for the least vulnerable species was 0, which means that their distribution had no weight in the site selection. We chose the 50% threshold because higher values led to an over-selection effect, in which MARXAN tended to keep all sampling sites in the solution to meet the targets for the different vulnerable taxa. Conversely, setting the minimum to 0 ensured that the least vulnerable species did not influence the selection outcome, although they could be covered in sites prioritized for other vulnerable species. This parameterization allowed us to balance the weight given to species vulnerability while avoiding artifacts in the prioritization results.

Secondly, we ran a MARXAN scenario on the genetic data using the ESVs as conservation features. We defined the proportion of the distribution to conserve for each ESVs the same way as in the taxonomic scenarios, as a function of vulnerability scaled between 0 and 50%. We transferred species-level vulnerability values to corresponding ESVs when species-level identification was available. For ESVs resolved only to the genus level, we averaged the species-level vulnerability of all species present in the study area (observed or likely to be observed, see Supplementary Table 06 – 2 for details). All ESVs of a given species or genus contributed equally to site prioritization.

However, the genetic and taxonomic MARXAN scenarios differed substantially in the number of conservation features considered, with 46 taxa included in the taxonomic scenario versus 135 ESVs scenario. Because MARXAN must achieve conservation targets for each feature individually, the larger number of ESVs likely increased the number of sites required to meet all targets. This effect may have been further reinforced by the narrower spatial distributions of some ESVs compared to morphologically defined taxa. To avoid this effect, we aggregated ESVs according to the lowest available taxonomic level to generate a second dataset representing genetic diversity (ESVs-aggregated scenario). Whenever possible, ESVs were aggregated at the species level; otherwise, they were aggregated at the genus level. This intermediate scenario included 52 conservation features and allowed comparison between taxonomic and fine-scale genetic approaches while reducing the disparity in the number of conservation features among scenarios. Therefore, we evaluated three MARXAN prioritization scenarios (all including species vulnerability values): a taxonomic scenario, an ESVs scenario, and an ESVs-aggregated scenario.

Given the limited number of sites and high taxonomic turnover, identifying priority sites for all taxa simultaneously would have resulted in the selection of most sites across all scenarios. To address this, following previous studies (Bruno et al., 2022; Fernández-Calero et al., 2024), we performed 100 bootstrap iterations, in which we identified priority sites for 50% of the conservation features for each MARXAN scenario (i.e., species, ESVs, and ESVs-aggregated). We then calculated the site selection frequency across the best solutions from all bootstrap iterations to identify consistently prioritized areas. Finally, we compared the frequency of selection in MARXAN solutions with the average community vulnerability to explore whether sites with higher community vulnerability were consistently prioritized.

Results

Upper course sites were characterized by the presence of vulnerable species, while tolerant species were associated with lower courses. The IndVal analysis consistently revealed vulnerable species in upper course sites, including *Rhyacophila intermedia* McLachlan, 1868, *Micrasema vestitum* Navás, 1918, *Polycentropus* cf. *kingi* McLachlan, 1881, or *Plectrocnemia laetabilis* McLachlan, 1880 (Fig. 1). Conversely, less vulnerable species, such as *Hydropsyche dinarica* (Marinkovic-Gospodnetic, 1979), *Hydropsyche instabilis* (Curtis, 1834), *Allogamus auricollis* (Pictet, 1834), *Halesus digitatus* (Schrank, 1781), or *Glossosoma boltoni* (Curtis, 1834), were negatively associated with upper course sites. In downstream sites, the pattern was reversed: species such as *H. instabilis, Agapetus fuscipes* (Curtis, 1834), *G. conformis* (Neboiss, 1963), and *Beraea maurus* (Curtis, 1834) showed positive associations and low vulnerability, whereas highly vulnerable species, especially *Synagapetus placidus* (Navás, 1918) and *R. angelieri* (Decamps, 1965), were negatively associated with them. In middle-stream sites, a mixture of species from both upper course and downstream communities, including some exclusive to these transitional sites, was consistently found; reflecting the gradual replacement of upper course-associated species by downstream-associated species.

MARXAN identified different priority sites for conservation under the three scenarios (i.e., taxonomic, ESVs, and ESVs-aggregated), resulting in differences not only in the location of the sites that were repeatedly selected across bootstraps but also in the number of sites selected and in their ecological characteristics (Fig. 2; Table 02). The ESVs scenario showed a wide spatial coverage (Fig. 2a, 2c), selecting at least one site in almost every basin and including sites not selected in the other scenarios. This scenario represented the highest taxonomic (67%) and genetic (72%) diversity covered and an average vulnerability of 2.48 (SD = 0.20). However, it required the highest number of sites (n = 21) among those selected in at least 50% of the 100 MARXAN iterations. Meanwhile, the taxonomic scenario selected the fewest sites (n = 8), with an average vulnerability of 2.49 (SD = 0.13); and achieved a diverse coverage of 64% of species and 41% of ESVs. It also showed good overlap with the ESVs-aggregated scenario following a bimodal selection structure (Fig. 2b), with low selection frequency on western HMS and high selection frequency of sites located in central and eastern HMS. However, The ESVs-aggregated scenario preserved higher ESVs diversity (59%) and showed the highest mean vulnerability score among scenarios (2.51; SD = 0.16).

Discussion

Here, we found that integrating species-specific vulnerability with taxonomic and genetic diversity reveals complementary conservation patterns that would remain overlooked when considering each biodiversity dimension independently. This result reflects four main contributions: (i) integrating biogeographical, biological and ecological traits into a species-specific vulnerability values revealed differences in climate change sensitivity among taxa, identifying highly vulnerable species associated with restricted distributions and specific ecological requirements; these sensitivity patterns were spatially structured across the river network, with vulnerable taxa concentrated in headwater habitats; (ii) incorporating this vulnerability dimension into MARXAN resulted in priority scenarios that consistently represented areas supporting vulnerable communities, as all scenarios selected sites with comparable

levels of community vulnerability and altitudinal distribution despite differences in biodiversity representation; (iii) genetic diversity revealed additional intraspecific variation that generated conservation scenarios different from, and requiring larger spatial networks than those based solely on taxonomy; and (iv) aggregating genetic diversity at the taxonomic level provided a balanced scenario that incorporated additional information from intraspecific diversity while maintaining comparability with traditional taxonomy, resulting in a more integrated representation of multiple biodiversity dimensions.

Species vulnerability reveals hidden conservation priorities under climate change

Our species vulnerability values were primarily driven by altitudinal gradients, highlighting the importance of environmental filtering in shaping species sensitivity (Brown et al., 2007; Leathers et al., 2023). High-altitude streams consistently harbored the most vulnerable communities, likely reflecting the strong filtering imposed by the cold, stable, and oxygen-rich conditions characteristic of headwater habitats, which favor taxa with narrow ecological requirements and limited ecological plasticity (Finn et al., 2013; Kuemmerlen et al., 2025). Species such as *R. intermedia, R. angelieri*, *M. vestitum*, and *Silo graellsii* Pictet, 1865, among others, display high vulnerability values, consistent with their dependence on these specialized environmental conditions typical of western Pyrenean headwaters (Cauvy-Fraunié et al., 2015; Graf et al., 2008). These patterns highlight the particular sensitivity of high-mountain stream ecosystems to climate change and anthropogenic pressures, as alterations in temperature, hydrological regimes, and habitat stability may disproportionately affect species adapted to narrow environmental conditions (Dudgeon et al., 2006; IPCC, 2023; Amblar Francés *et al.*, 2020). Therefore, preserving climatic refugia in headwater habitats is essential for maintaining highly specialized and range-restricted taxa (Martini et al., 2024), as even minor thermal changes can shift or eliminate species with narrow climatic niches (Domisch et al., 2011; Isaak & Rieman, 2013).

Integrating species vulnerability into conservation planning

Trait-based vulnerability assessments identify the species most sensitive to environmental change, but incorporating this information into spatial conservation planning remains challenging. By including species vulnerability values in MARXAN, all prioritization scenarios consistently represented high-altitude headwaters supporting the most vulnerable communities, despite substantial differences in the biodiversity features used for prioritization. This result indicates that integrating species vulnerability into conservation planning can safeguard climate-sensitive taxa while maintaining flexibility in biodiversity representation across alternative conservation solutions. Rather than favouring widespread and tolerant taxa, this approach ensures that species with restricted distributions and narrow ecological requirements remain incorporated into priority networks, improving the capacity of conservation planning to anticipate future climate change (Martini et al., 2024).

Integrating genetic diversity balances biodiversity resolution and conservation feasibility

Prioritization tools are essential when human and financial resources are limited, as they improve spatial efficiency and provide a more comprehensive representation of ecosystem complexity (Ball et al., 2009).

However, the outcomes of these approaches strongly depend on the nature of the conservation features used to define prioritization targets. Taxonomic scenarios selected fewer, more spatially clustered sites than genetic-based approaches, whereas ESVs and ESVs-aggregated scenarios identified a broader network of priority areas across the Pyrenees. The greater overlap between taxonomic and genetic scenarios in the eastern Pyrenees may reflect the west-to-east climatic gradient, where lower elevations and higher habitat heterogeneity promote macroinvertebrate diversity and increase the probability that sites are prioritized regardless of the biodiversity feature considered (de Mendoza & Catalan, 2010; Cuadrat et al., 2024; López-Rodríguez, 2026). Differences were particularly evident in the western region, where genetic diversity highlighted additional priority sites that were not recovered by taxonomy alone. This pattern likely reflects the ability of genetic data to capture intraspecific variation and evolutionary processes that remain hidden when species are treated as homogeneous conservation units (Paz-Viñas *et al.*, 2018; Nielsen et al., 2023), consistent with previous studies showing that genetic approaches reveal complementary conservation priorities (Asmyhr et al., 2014; Rosauer et al., 2017). Although representing genetic diversity generally requires selecting more sites and therefore increases conservation costs (Nielsen et al., 2023), our results show that aggregating ESVs at the taxonomic level provides a practical compromise between biodiversity resolution and conservation feasibility. This approach retains information on intraspecific diversity while avoiding the substantial increase in spatial requirements associated with using all ESVs individually in our study, complementing previous proposals to integrate genetic data into conservation planning (Balint *et al.*, 2011; Carvalho et al., 2019; Hermoso et al., 2016; Paz-Viñas *et al.*, 2018). By combining species vulnerability, taxonomic and genetic biodiversity within MARXAN, our prioritization framework identifies not only where biodiversity is concentrated, but also where its most vulnerable evolutionary and ecological components are represented.

Methodological considerations and future perspectives

Despite these advances, several limitations should be acknowledged. First, our genetic analyses were based on a single mitochondrial marker (COI), which captures only a subset of genetic variation within species and may overlook population structure revealed by higher-resolution nuclear markers (Weiss et al., 2024). Integrating genome-wide or adaptive genetic information could further improve the identification of populations with greater evolutionary potential under climate change (Barratt et al., 2024). Second, the species vulnerability values obtained in our study represent an evidence-based approximation of species sensitivity derived from available ecological, biogeographical, and biological trait information (Kuemmerlen et al., 2025). However, this framework does not incorporate species' adaptive genetic variation, which may influence their capacity to respond to future environmental changes (Barratt et al., 2024). As knowledge of species ecology, genetics, and trait databases improves, vulnerability assessments could be refined and incorporated into future prioritization exercises. Third, our prioritization focused on taxonomic and genetic diversity representation and do not consider landscape connectivity or dispersal dynamics to maintain population linkages and ecological corridors (Hermoso et al., 2020), which are also critical for long-term population persistence under changing climatic conditions (Daigle et al., 2020). Fourth, we did not account for functional diversity but acknowledge that our species vulnerability approach includes traits that might reflect ecological

functions (e.g., reproductive cycle could be related to secondary production). Finally, our analyses were restricted to Trichoptera communities inhabiting Pyrenean high-mountain streams, many of which are already included within protected areas. Further evaluations across other freshwater taxa, mountain systems, and conservation contexts will be necessary to assess the broader applicability of this approach. Therefore, our results should be interpreted as a framework for improving biodiversity representation within existing conservation networks rather than as a replacement for current protection schemes.

Conclusions

Towards multiscale conservation planning under climate change This study exemplifies how the multiscale conservation principles of the Kunming-Montreal Global Biodiversity Framework (CBD, 2022) can be implemented by integrating species-specific climate change vulnerability with taxonomic and genetic biodiversity, expanding traditional taxonomic-based prioritization towards more inclusive conservation scenarios. Vulnerability assessments identified sensitive taxa and environmentally vulnerable sites associated with key environmental gradients, particularly altitude. By incorporating species vulnerability values to climate change and ESVs-aggregated diversity into MARXAN prioritization, we identified conservation scenarios that captured complementary biodiversity dimensions while maintaining spatial feasibility. These results highlight the importance of moving beyond only taxonomic prioritization towards multiscale conservation strategies that account for species sensitivity, community composition, and intraspecific diversity. Such integrative approaches provide a foundation for adaptive conservation planning and contribute to the implementation of biodiversity targets under global environmental change.

Declarations

Acknowledgments & funding:

We would like to thank Florian Leese for his review of the manuscript and the Freshwater Ecology, Hydrology, and Management research group (FEHM-lab). This study was supported by ERANET-LAC Project 01DN19023 "GLOBIOS - Global observatory network for freshwater biodiversity in high mountain streams; -PCI2019-103495- the Spanish Ministry of Science, Innovation, and Universities – Agencia Estatal de Investigación and co-financed by ERDF (A Way of Making Europe). With the support of the Industrial Doctorates Plan of the Department of Research and Universities of the Generalitat de Catalunya (DI21-0001), awarded to Nieves López-Rodríguez, Núria Bonada, and the company Eurofins-Cavendish S.L.U. Núria Bonada is supported by ICREA-Academia 2021 funded by the Catalan Institution for Research and Advanced Studies. Also, this paper was co-funded by Sonja Jähnig (DFG) JA 1827/6-2). David Cunillera-Montcusí has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 101062388. Virgilio Hermoso acknowledges funding received from the Agencia Estatal de Investigación (PID2023-150039NB-I00). Miguel Canedo-Arguelles has been seconded to the ERC Executive Agency. The views

expressed in this paper are purely those of the author. They do not necessarily reflect the views or official positions of the European Commission, the ERC Executive Agency or the ERC Scientific Council.

Author's contribution

Article Conceptualization (NLR, JC, RA, MCA, NB); Data Curation (NLR, JC); Formal Analysis (NLR, JC, PIB, ML, DCM); Funding Acquisition (RA, MCA, SP, NB); Investigation (NLR, JC, RA); Methodology (NLR, JC, RA, MCA, PIB, ML, VH); Project Administration (RA, NB); Resources (NLR, RA, PIB, SP, OS, BP, JS, RAT); Supervision (NB); Visualization (NLR, JC); Writing-Original Draft (NLR); Writing-review & editing (NLR, JC, RA, PIB, MCA, ML, DCM, VH, SP, OS, BP, JS, RAT, NB)

Data availability

The datasets generated and analysed during the current study have been deposited in the Dryad Digital Repository. During peer review, they are available through the private Dryad review link: (http://datadryad.org/share/LINK_NOT_FOR_PUBLICATION/NCYl0aYeQOD_o9jy-6AlMNhyDxWVenSE9e9ASCWRN8w). Upon acceptance of the manuscript, the dataset will be made publicly available through Dryad.

The metabarcoding sequence data have been deposited in the European Nucleotide Archive (ENA) under BioProject **PRJEB106080** (Title: *Global observatory network for freshwater biodiversity in high mountain streams*).

References

1. Amblar-Francés MP, Ramos-Calzado P, Sanchis-Lladó J, Hernanz-Lázaro A, Peral-García MC, Navascués B, Dominguez-Alonso M, Pastor-Saavedra MA, Rodríguez-Camino E (2020) High resolution climate change projections for the Pyrenees region. Adv Sci Res 17:191–208. https://doi.org/10.5194/asr-17-191-2020
2. Asmyhr MG, Linke S, Hose G, Nipperess DA (2014) Systematic Conservation Planning for Groundwater Ecosystems Using Phylogenetic Diversity. PLoS ONE 9(12):e115132. https://doi.org/10.1371/journal.pone.0115132
3. Bálint M, Domisch S, Engelhardt CHM, Haase P, Lehrian S, Sauer J, Theissinger K, Pauls SU, Nowak C (2011) Cryptic biodiversity loss linked to global climate change. Nat Clim Change 1(6):313–318. https://doi.org/10.1038/nclimate1191
4. Ball IR, Possingham HP, Watts ME (2009) Marxan and relatives: Software for spatial conservation prioritization. In: Moilanen A, Wilson KA, Possingham HP (eds) Spatial Conservation Prioritization: Quantitative Methods and Computational Tools. Oxford University Press, Oxford, UK, pp 185–195. https://doi.org/10.1093/oso/9780199547760.003.0014
5. Barratt CD, Onstein RE, Pinsky ML, Steinfartz S, Kühl HS, Forester BR, Razgour O (2024) Life on the edge: A new toolbox for population-level climate change vulnerability assessments. Methods Ecol

Evol 15(11):2038–2058. https://doi.org/10.1111/2041-210x.14429

6. Brodie JF, Mohd-Azlan J, Chen C, Wearn OR, Deith MCM, Ball JGC, Slade EM, Burslem DFRP, Teoh SW, Williams PJ, Nguyen A, Moore JH, Goetz SJ, Burns P, Jantz P, Hakkenberg CR, Kaszta ZM, Cushman S, Coomes D, Helmy O, Reynolds E, Rodríguez G, Jetz JP, W., &, Luskin MS (2023) Landscape-scale benefits of protected areas for tropical biodiversity. Nature 620(7975):807–812. https://doi.org/10.1038/s41586-023-06410-z
7. Brown LE, Hannah DM, Milner AM (2007) Vulnerability of alpine stream biodiversity to shrinking glaciers and snowpacks. Glob Change Biol 13(5):958–966. https://doi.org/10.1111/j.1365-2486.2007.01341.x
8. Bruno D, Hermoso V, Sánchez-Montoya MM, Belmar O, Gutiérrez-Cánovas C, Cañedo-Argüelles M (2022) Ecological relevance of non-perennial rivers for the conservation of terrestrial and aquatic communities. Conserv Biol 36(6):e13982. https://doi.org/10.1111/cobi.13982
9. Buchner D, Leese F (2020) BOLDigger – a Python package to identify and organise sequences with the Barcode of Life Data systems. Metabarcoding Metagenomics 4:e53535. https://doi.org/10.3897/mbmg.4.53535
10. Buchner D, Macher T-H, Leese F (2022) APSCALE: Advanced pipeline for simple yet comprehensive analyses of DNA metabarcoding data. Bioinformatics 38(20):4817–4819. https://doi.org/10.1093/bioinformatics/btac588
11. Carvalho SB, Torres J, Tarroso P, Velo-Antón G (2019) Genes on the edge: a framework to detect genetic diversity imperiled by climate change. Glob Change Biol 25(12):4034–4047. https://doi.org/10.1111/gcb.14740
12. Cauvy-Fraunié S, Espinosa R, Andino P, Jacobsen D, Dangles O (2015) Invertebrate Metacommunity Structure and Dynamics in an Andean Glacial Stream Network Facing Climate Change. PLoS ONE 10(8):e0136793. https://doi.org/10.1371/journal.pone.0136793
13. Chamberlain S, Barve V, McGlinn D, Oldoni D, Desmet P, Geffert L, Ram K (2025) rgbif: Interface to the Global Biodiversity Information Facility API. R package version x.x.x. https://CRAN.R-project.org/package=rgbif
14. Coates DJ, Byrne M, Moritz C (2018) Genetic Diversity and Conservation Units: Dealing With the Species-Population Continuum in the Age of Genomics. *Frontiers in Ecology and Evolution, 6*. https://doi.org/10.3389/fevo.2018.00165
15. Conti L, Schmidt-Kloiber A, Grenouillet G, Graf W (2014) A trait-based approach to assess the vulnerability of European aquatic insects to climate change. Hydrobiologia 721(1):297–315. https://doi.org/10.1007/s10750-013-1690-7
16. Convention on Biological Diversity (CBD) (2022) Kunming-Montreal Global Biodiversity Framework. CBD/COP/DEC/15/4. Montreal: Convention on Biological Diversity. 14. https://www.cbd.int/doc/decisions/cop-15/cop-15-dec-04-en.pdf
17. Coppa G, Graf W, Tachet H (2012) A revised description of the larvae of three species of the Rhyacophila tristis group: Rhyacophila aquitanica, Rhyacophila pubescens and Rhyacophila tristis

(Trichoptera: Rhyacophilidae). Ann de Limnologie - Int J Limnol 48(2):215–223. https://doi.org/10.1051/limn/2012014

18. Cruaud P, Rasplus J-Y, Rodriguez LJ, Cruaud A (2017) High-throughput sequencing of multiple amplicons for barcoding and integrative taxonomy. Sci Rep 7(1):41948. https://doi.org/10.1038/srep41948
19. Cuadrat JM, Serrano-Notivoli R, Prohom M, Cunillera J, Tejedor E, Saz MÁ, De Luis M, Llabrés-Brustenga A, Soubeyroux J (2024) Climate of the Pyrenees: Extremes indices and long-term trends. Sci Total Environ 933:173052. https://doi.org/10.1016/j.scitotenv.2024.173052
20. Daigle RM, Metaxas A, Balbar AC, McGowan J, Treml EA, Kuempel CD, Possingham HP, Beger M (2020) Operationalizing ecological connectivity in spatial conservation planning with Marxan Connect. Methods. Ecol Evol 11(4):570–579. https://doi.org/10.1111/2041-210x.13349
21. Cáceres MD, Legendre P (2009) Associations between species and groups of sites: Indices and statistical inference. Ecology 90(12):3566–3574. https://doi.org/10.1890/08-1823.1
22. De Cáceres M, Jansen F, Dell N (2010) indicspecies: Relationship Between Species and Groups of Sites (Version 1.7.15). https://cran.r-project.org/package=indicspecies de Mendoza & Catalan, 2010
23. De Mendoza G, Ventura M, Catalan J (2015) Environmental factors prevail over dispersal constraints in determining the distribution and assembly of Trichoptera species in mountain lakes. Ecol Evol 5(13):2518–2532. https://doi.org/10.1002/ece3.1522
24. Décamps H (1965) Larves pyrénéennes du genre Rhyacophila [Trichoptères]. Ann Limnol 1(1):51–72. https://doi.org/10.1051/limn/1965022
25. Décamps H (1970) Les larves de Brachycentridae (Trichoptera) de la faune de France. Taxonomie et écologie. Ann Limnol 6(1):51–73. https://doi.org/10.1051/limn/1970009
26. Décamps H, Pujol JY (1975) Les larves de Drusinae des Pyrénées (Trichoptères, Limnephilddae). Ann Limnol 11(2):157–167. https://doi.org/10.1051/limn/1975006
27. Domisch S, Jähnig SC, Haase P (2011) Climate-change winners and losers: Stream macroinvertebrates of a submontane region in Central Europe. Freshw Biol 56(10):2009–2020. https://doi.org/10.1111/j.1365-2427.2011.02631.x
28. Dudgeon D, Arthington AH, Gessner MO, Kawabata Z-I, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard A-H, Soto D, Stiassny MLJ, Sullivan CA (2006) Freshwater biodiversity: Importance, threats, status and conservation challenges. Biol Rev 81(2):163–182. https://doi.org/10.1017/S1464793105006950
29. Fernández-Calero JM, Cunillera-Montcusí D, Hermoso V, Quevedo-Ortiz G, Fortuño P, Acosta R, Gomà J, Cid N, Vinyoles D, Ruhí A, Cañedo-Argüelles M, Bonada N (2024) Integrating spatiotemporal hydrological connectivity into conservation planning to protect temporary rivers. Aquat Conservation: Mar Freshw Ecosyst 34(3):e4139. https://doi.org/10.1002/aqc.4139
30. Finn DS, Khamis K, Milner AM (2013) Loss of small glaciers will diminish beta diversity in Pyrenean streams at two levels of biological organization. Glob Ecol Biogeogr 22(1):40–51. https://doi.org/10.1111/j.1466-8238.2012.00766.x

31. Finn DS, Bonada N, Múrria C, Hughes JM (2011) Small but mighty: headwaters are vital to stream network biodiversity at two levels of organization. J North Am Benthological Soc 30(4):963–980. https://doi.org/10.1899/11-012.1
32. Foden WB (2016) IUCN SSC guidelines for assessing species' vulnerability to climate change [Resource]. https://iucn.org/resources/publication/iucn-ssc-guidelines-assessing-species-vulnerability-climate-change
33. Frøslev TG, Kjøller R, Bruun HH, Ejrnæs R, Brunbjerg AK, Pietroni C, Hansen AJ (2017) Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nat Commun 8(1):1188. https://doi.org/10.1038/s41467-017-01312-x
34. Graf W, Murphy J, Dahl J, Zamora-Munoz C, Lopez-Rodriguez MJ (2008) Distribution and ecological preferences of European freshwater organisms. Volume 1. Trichoptera (A. Schmidt-Kloiber & D. Hering, Eds.; Vol. 1). Pensoft Publishing. http://www.pensoft.net/newreleases/14279-toc.html
35. Graf W, Vitecek S, Previšić A, Malicky H (2015) New species of Limnephilidae (Insecta: Trichoptera) from Europe: Alps and Pyrenees as harbours of unknown biodiversity. Zootaxa 3911(3):381–395. https://doi.org/10.11646/zootaxa.3911.3.5
36. Guisan AA, Broennimann O, Buri A, Cianfrani C, D'Amen M, Di Cola V, Fernandes R, Gray SM, Mateo RG, Pinto E, Pradervand J-N, Scherrer D, Vittoz P, Von Däniken I, Yashiro E (2019) Climate Change Impacts on Mountain Biodiversity. In T. E. Lovejoy & L. Hannah (Eds.), Biodiversity and Climate Change (pp. 221–233). *Yale University Press*. https://doi.org/10.2307/j.ctv8jnzw1.29
37. Harper J, Van Wilgen N, Turner A, Tolley K, Maritz B, Clusella-Trullas S, da Silva J, Cunningham S, Cheney C, Villiers A, Measey J, Foden W (2022) Application of a trait-based climate change vulnerability assessment to determine management priorities at protected area scale. Conserv Sci Pract 4(8):e12756. https://doi.org/10.1111/csp2.12756
38. Heino J, Virkkala R, Toivonen H (2009) Climate change and freshwater biodiversity: Detected patterns, future trends and adaptations in northern regions. Biol Rev 84(1):39–54. https://doi.org/10.1111/j.1469-185X.2008.00060.x
39. Hering D, Schmidt-Kloiber A, Murphy J, Lücke S, Zamora-Muñoz C, López-Rodríguez MJ, Huber T, Graf W (2009) Potential impact of climate change on aquatic insects: A sensitivity analysis for European caddisflies (Trichoptera) based on distribution patterns and ecological preferences. Aquat Sci 71(1):3–14. https://doi.org/10.1007/s00027-009-9159-5
40. Hermoso V, Kennard MJ, Schmidt DJ, Bond N, Huey JA, Mondol RK, Jamandre BW, Hughes JM (2016) Species distributions represent intraspecific genetic diversity of freshwater fish in conservation assessments. Freshw Biol 61(10):1707–1719. https://doi.org/10.1111/fwb.12810
41. Hermoso V, Kennard MJ, Linke S (2014) Evaluating the costs and benefits of systematic data acquisition for conservation assessments. Ecography 38(3):283–292. https://doi.org/10.1111/ecog.00792
42. Hermoso V, Vasconcelos RP, Henriques S, Filipe AF, Carvalho SB (2020) Conservation planning across realms: Enhancing connectivity for multi-realm species. J Appl Ecol 58(3):644–654.

https://doi.org/10.1111/1365-2664.13796

43. Hershkovitz Y, Dahm V, Lorenz AW, Hering D (2015) A multi-trait approach for the identification and protection of European freshwater species that are potentially vulnerable to the impacts of climate change. Ecol Ind 50:150–160. https://doi.org/10.1016/j.ecolind.2014.10.023
44. Hoban S, Bruford M, D'Urban Jackson J, Lopes-Fernandes M, Heuertz M, Hohenlohe PA, Paz-Vinas I, Sjögren-Gulve P, Segelbacher G, Vernesi C, Aitken S, Bertola LD, Bloomer P, Breed M, Rodríguez-Correa H, Funk WC, Grueber CE, Hunter ME, Jaffe R, Liggins L, Mergeay J, Moharrek F, O'Brien D, Ogden R, Palma-Silva C, Pierson J, Ramakrishnan U, Simo-Droissart M, Tani N, Waits L, Laikre L (2020) Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. Biol Conserv 248:108654. https://doi.org/10.1016/j.biocon.2020.108654
45. Hotaling S, Finn DS, Giersch J, Weisrock J, D. W., Jacobsen D (2017) Climate change and alpine stream biology: Progress, challenges, and opportunities for the future. Biol Rev 92(4):2024–2045. https://doi.org/10.1111/brv.12319
46. Intergovernmental Panel on Climate Change (IPCC) (2023) Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland. https://doi.org/10.59327/IPCC/AR6-9789291691647
47. Isaak DJ, Rieman BE (2013) Stream isotherm shifts from climate change and implications for distributions of ectothermic organisms. Glob Change Biol 19(3):742–751. https://doi.org/10.1111/gcb.12073
48. Isasi Bea P (2020) Vulnerability to climate change of Trichoptera species in Sierra Nevada and Pyrenees (Spain & Andorra) [Tesis de máster, Universitat de Barcelona]. Recuperado de https://www.fehm.cat/wp-content/uploads/2025/03/2020_TFM_Pello-Isasi.pdf
49. Jackson RB, Carpenter SR, Dahm CN, McKnight DM, Naiman RJ, Postel SL, Running SW (2001) Water in a Changing World. *Ecological Applications, 11*(4), 1027–1045. https://doi.org/10.1890/1051-0761(2001)011%255B1027:WIACW%255D2.0.CO;2
50. Kalaninová D, Bulánková E, Šporka F (2014) Caddisflies (Trichoptera) as good indicators of environmental stress in mountain lotic ecosystems. Biologia 69(8):1030–1045. https://doi.org/10.2478/s11756-014-0405-5
51. Kuemmerlen M, Graf W, Waringer J, Vitecek S, Kučinić M, Previšić A, Keresztes L, Bálint M, Pauls SU (2025) Spring-dwellers emerge as indicators of climate change vulnerability. ZooKeys 1263:289–315. https://doi.org/10.3897/zookeys.1263.148253
52. Laikre L, Hoban S, Bruford MW, Segelbacher G, Allendorf FW, Gajardo G, Rodríguez AG, Hedrick PW, Heuertz M, Hohenlohe PA, Jaffé R, Johannesson K, Liggins L, MacDonald AJ, OrozcoterWengel P, Reusch TBH, Rodríguez-Correa H, Russo I-RM, Ryman N, Vernesi C (2020) Post-2020 goals overlook genetic diversity. Science 367(6482):1083–1085. https://doi.org/10.1126/science.abb2748
53. Leathers K, Herbst D, Safeeq M, Ruhi A (2023) Dynamic, downstream-propagating thermal vulnerability in a mountain stream network: Implications for biodiversity in the face of climate

change. Limnol Oceanogr 68:S101–S114. https://doi.org/10.1002/lno.12264

54. López-Rodríguez N (2026) Advancing fundamental and applied river ecology through molecular analysis of aquatic invertebrates in the Iberian Peninsula. Universitat de Barcelona. https://hdl.handle.net/10803/697913
55. Macher T-H, Beermann AJ, Leese F (2021) TaxonTableTools: A comprehensive, platform-independent graphical user interface software to explore and visualise DNA metabarcoding data. Mol Ecol Resour 21(5):1705–1714. https://doi.org/10.1111/1755-0998.13358
56. Macher T-H (2022) TillMacher/apscale_gui [Python]. (Original work published 2021). https://github.com/TillMacher/apscale_gui
57. Margules CR, Pressey RL (2000) Systematic conservation planning. Nature 405(6783):243–253. https://doi.org/10.1038/35012251
58. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17(1):10–12. https://doi.org/10.14806/ej.17.1.200
59. Martini J, Brighenti S, Vanek M, Schwingshackl T, Vallefuoco F, Scotti A, Lencioni V, Bottarin R (2024) Rock glacier springs: cool habitats for species on the edge. Biodivers Conserv 33(14):4017–4042. https://doi.org/10.1007/s10531-024-02937-3
60. Nielsen ES, Beger M, Henriques R, von der Heyden S (2020) A comparison of genetic and genomic approaches to represent evolutionary potential in conservation planning. Biol Conserv 251:108770. https://doi.org/10.1016/j.biocon.2020.108770
61. Nielsen ES, Hanson JO, Carvalho SB, Beger M, Henriques R, Kershaw F, von der Heyden S (2023) Molecular ecology meets systematic conservation planning. Trends Ecol Evol 38(2):143–155. https://doi.org/10.1016/j.tree.2022.09.006
62. Pardo I, Alvarez M, Casas J, Moreno JL, Vivas S, Bonada N, Alba-Tercedor J, Jaimez-Cuéllar P, Moya G, Prat N, Robles S, Suárez Alonso M, Toro M, Vidal-Abarca M (2002) El hábitat de los ríos mediterráneos. Diseño de un índice de diversidad de hábitat. Limnetica 21:115–133. https://doi.org/10.23818/limn.21.21
63. Pauls SU, Nowak C, Bálint M, Pfenninger M (2013) The impact of global climate change on genetic diversity within populations and species. Mol Ecol 22(4):925–946. https://doi.org/10.1111/mec.12152
64. Paz-Vinas I, Loot G, Hermoso V, Veyssière C, Poulet N, Grenouillet G, Blanchet S (2018) Systematic conservation planning for intraspecific genetic diversity. *Proceedings of the Royal Society B: Biological Sciences, 285*(1877), 20172746. https://doi.org/10.1098/rspb.2017.2746
65. R Core Team (2024) *R: A language and environment for statistical computing*. (Version 4.4.1). R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
66. Rodríguez-Rodríguez D, Martínez-Vega J (2022) Effectiveness of Protected Areas in Conserving Biodiversity: A Worldwide Review. Springer International Publishing. https://doi.org/10.1007/978-3-030-94297-7

67. Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: A versatile open source tool for metagenomics. PeerJ 4:e2584. https://doi.org/10.7717/peerj.2584
68. Rosauer DF, Pollock LJ, Linke S, Jetz W (2017) Phylogenetically informed spatial planning is required to conserve the mammalian tree of life. *Proceedings of The Royal Society B Biological Sciences*, *284*(1865), 20170627. https://doi.org/10.1098/rspb.2017.0627
69. Sainz-Bariáin M, Zamora-Muñoz C (2015) Larval descriptions of Annitella esparraguera (Schmid 1952) and Annitella iglesiasi González & Malicky 1988 (Trichoptera: Limnephilidae), two endemic species from Southern Europe. Zootaxa 4006:347–360. https://doi.org/10.11646/zootaxa.4006.2.6
70. Sarremejane R, Cid N, Stubbington R et al (2020) DISPERSE, a trait database to assess the dispersal potential of European aquatic macroinvertebrates. Sci Data 7:386. https://doi.org/10.1038/s41597-020-00732-7
71. Schmidt-Kloiber A, Hering D (2015) www.freshwaterecology.info – An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. Ecol Ind 53:271–282. https://doi.org/10.1016/j.ecolind.2015.02.007
72. Serra N, Kockel A, Game ET, Grantham H, Possingham HP, McGowan J (2020) Marxan User Manual: For Marxan version 2.43 and above. The Nature Conservancy (TNC), Arlington, Virginia, United States and Pacific Marine
73. Sheth BP, Thaker VS (2017) DNA barcoding and traditional taxonomy: An integrated approach for biodiversity conservation. Genome 60(7):618–628. https://doi.org/10.1139/gen-2015-0167
74. Stark JS, Schröder O, Müller J, Seifert L, Pauls SU (2025) Temporal monitoring of genetic diversity in aquatic insects: A pilot study in the Bavarian Forest National Park. ZooKeys 1263:499–518. https://doi.org/10.3897/zookeys.1263.147797
75. Summers DM, Bryan BA, Crossman ND, Meyer WS (2012) Species vulnerability to climate change: Impacts on spatial conservation priorities and species representation. Glob Change Biol 18(7):2335–2348. https://doi.org/10.1111/j.1365-2486.2012.02700.x
76. Vamos EE, Elbrecht V, Leese F (2017) Short COI markers for freshwater macroinvertebrate metabarcoding. Metabarcoding Metagenomics 1:e14625. https://doi.org/10.3897/mbmg.1.14625
77. Vasconcelos R, Razgour O, Tarroso P, Fasola M, Carranza S, Alves PC (2018) Combining molecular and landscape tools for targeting evolutionary processes in reserve design: An approach for islands. PLoS ONE 13(7):e0200830. https://doi.org/10.1371/journal.pone.0200830
78. Vieira-Lanero R, González M, Cobo F (1998) Larval description of Micrasema servatum (Navás, 1918) (Trichoptera, Brachycentridae). Graellsia 54:3–8. https://doi.org/10.3989/graellsia.1998.v54.i0.336
79. Vieira-Lanero R (2000) Las larvas de los tricópteros de Galicia (Insecta Trichoptera). https://www.semanticscholar.org/paper/Las-larvas-de-los-tric%C3%B3pteros-de-Galicia-(Insecta-)-Lanero/0e4176bfae5cf836652e3e0623c629b4b14199fa
80. Vieira-Lanero R, González M, Cobo F, Servia M (2008) The larva of Micrasema cenerentola Schimid, 1952 (Insecta: Trichoptera: Brachycentridae). Ferrantia 55:133–150

81. Wallace ID, Wallace B (1983) A revised key to larvae of the genus Plectrocnemia (Polycentropodidae: Trichoptera) in Britain, with notes on Plectrocnemia brevis McLachlan. Freshw Biol 13(1):83–87. https://doi.org/10.1111/j.1365-2427.1983.tb00659.x
82. Waringer J, Graf W (2019) The larva and pupa of Apatania stylata stylata Navás 1916, with an updated key to larvae of the European species of genus Apatania Kolenati 1848 (Apataniidae, Trichoptera). Freshw Sci 38(1):203–212. https://doi.org/10.1086/701800
83. Watson JEM, Dudley N, Segan DB, Hockings M (2014) The performance and potential of protected areas. Nature 515(7525):67–73. https://doi.org/10.1038/nature13947
84. Weiss M, Leese F (2025) Population genetic data (COI, ddRAD) of Sialislutaria (Insecta, Megaloptera) from the Emscher catchment (Germany). Biodivers Data J 13:e141997. https://doi.org/10.3897/BDJ.13.e141997
85. Weiss M, Lorenz AW, Feld CK, Leese F (2024) Strong Small-Scale Differentiation but No Cryptic Species Within the Two Isopod Species Asellus aquaticus and Proasellus coxalis in a Restored Urban River System (Emscher, Germany). Ecol Evol 14(11):e70575. https://doi.org/10.1002/ece3.70575
86. Wickham H (2016) Data Analysis. In H. Wickham (Ed.), Ggplot2: Elegant Graphics for Data Analysis (pp. 189–201). Springer International Publishing. https://doi.org/10.1007/978-3-319-24277-4_9
87. Wickham H (2024) *ggplot2: Elegant Graphics for Data Analysis*. Ggplot2. https://ggplot2.tidyverse.org/

Tables

Table 01: Traits used to assess species vulnerability based on biogeographic, biological and ecological characteristics. The scoring system reflects the level of climate change vulnerability associated with each trait category, ranging from low (1) to high (4).

Category	Trait	Trait category	Scoring
Biogeographical	Endemicity	Non endemic	1
		Pyrenean endemic	3
		Pyrenean micro-endemic	4
	Persistance	Last year	1
		Between 2 and 3 years	2
		Between 4 and 5 years	3
		Older	4
Biological and Ecological	Stream zonation preference	Other	1
		Metarhithral (mrh)	2
		Epirhithral (erh)	3
		Eu/Hypocrenal (euc/hyc)	4
	Temperature preference	Warm (was)	1
		Moderate (cos)	2
		Cold (eut)	4
	Altitude preference	Other	1
		Montane (sal)	2
		Subalpine (alp)	3
		Alpine (niv, sni)	4
	Microhabitat/substrate preference	≥ 4 microhabitats	1
		3 microhabitats	2
		2 microhabitats	3
		1 microhabitat	4
	Feeding type	≥ 4 feeding strategies	1
		3 feeding strategies	2
		2 feeding strategies	3
		1 feeding strategy	4
	Forewing female length max	≥ 15 mm	1

	10-15 mm	2
	5-10 mm	3
	< 5 mm	4
Emergence/flight period	All seasons	2
	Winter dominant	4
	Spring & Autumn dominant	3
	Summer dominant	4
Duration emergence period	Long duration	1
	Short duration	4
Life span	< 1 year	2
	≥ 1 year	4
Reproductive cycle	Multivoltine (sup1)	1
	Bivoltine (eq1)	3
	Univoltine (inf1)	4

Table 02. Summary of MARXAN prioritization results for taxonomic, ESVs, and ESVs-aggregated analyses. The table shows the number of targets considered in MARXAN, the number of sites that had been selected in more than half of the 100 iterations, the number of taxa and ESVs across these selected sites (and the percentage it represents from the total diversity observed in the study area), and the mean and standard deviation of the altitude and the average community vulnerability across selected sites.

	MARXAN selection		**Taxonomic diversity**		**ESVs diversity**		**Altitude (m)**		**Vulnerability**	
Model	Nº targets	Nº sites	Nº taxa	% total	Nº ESVs	% total	Mean	SD	Mean	SD
Taxonomic	46	8	41	64	75	41	1851	268	2.49	0.13
ESVs	135	21	43	67	131	72	1901	321	2.48	0.20
ESVs-aggregated	52	13	40	62	107	59	1982	251	2.51	0.16

Figures

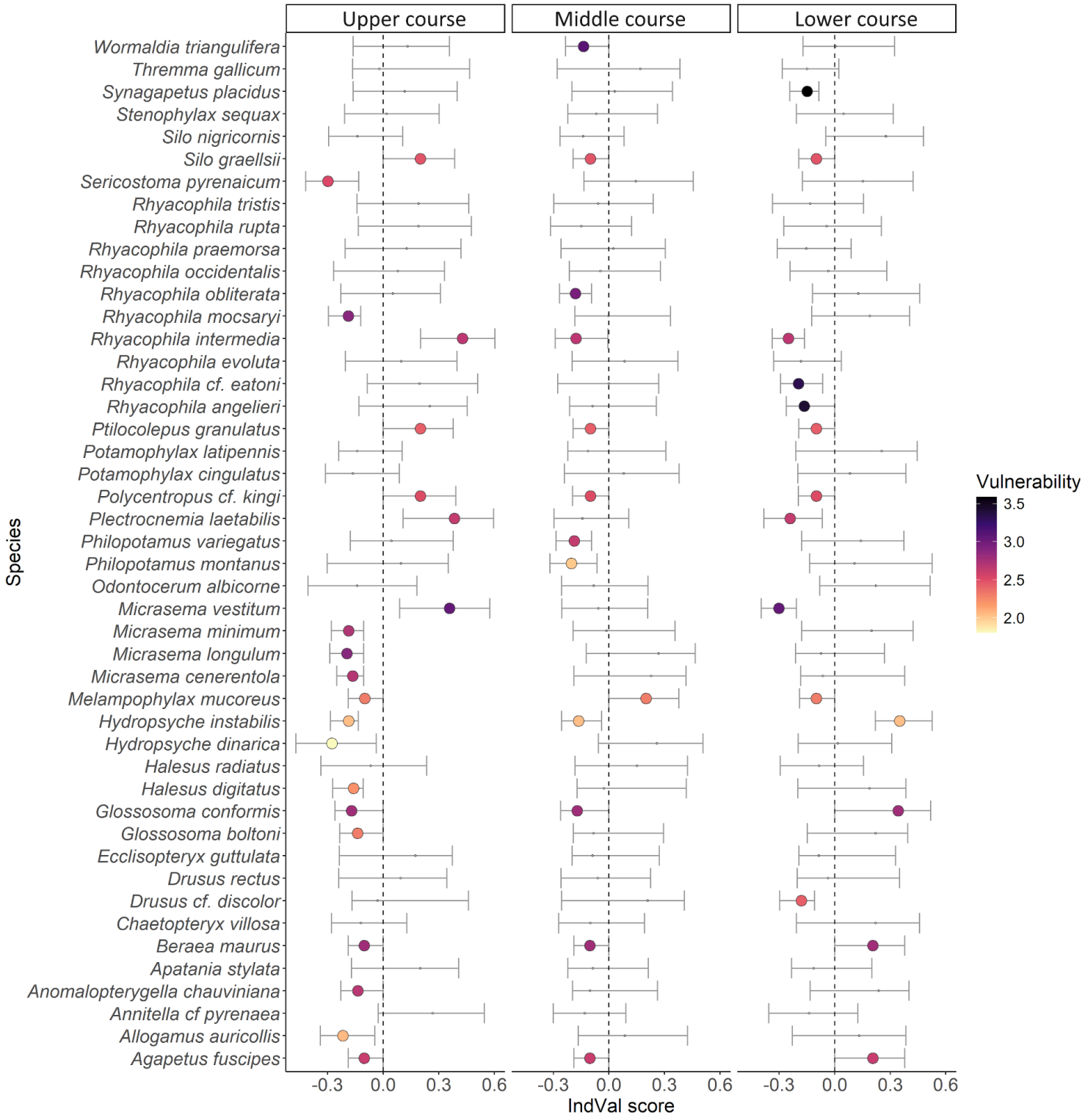


Figure 1

Species vulnerability patterns. Strength of the association of a given species (mean ± SE) with each altitudinal position (upper course, middle course y lower course). IndVal score represents the Indicator Value for each species with respect to each group of sites, combining specificity and fidelity. Positive

values indicate a strong association with the group, while negative values indicate a weak or absent association. When the IndVal statistic was significant, the corresponding point was colored according to species vulnerability

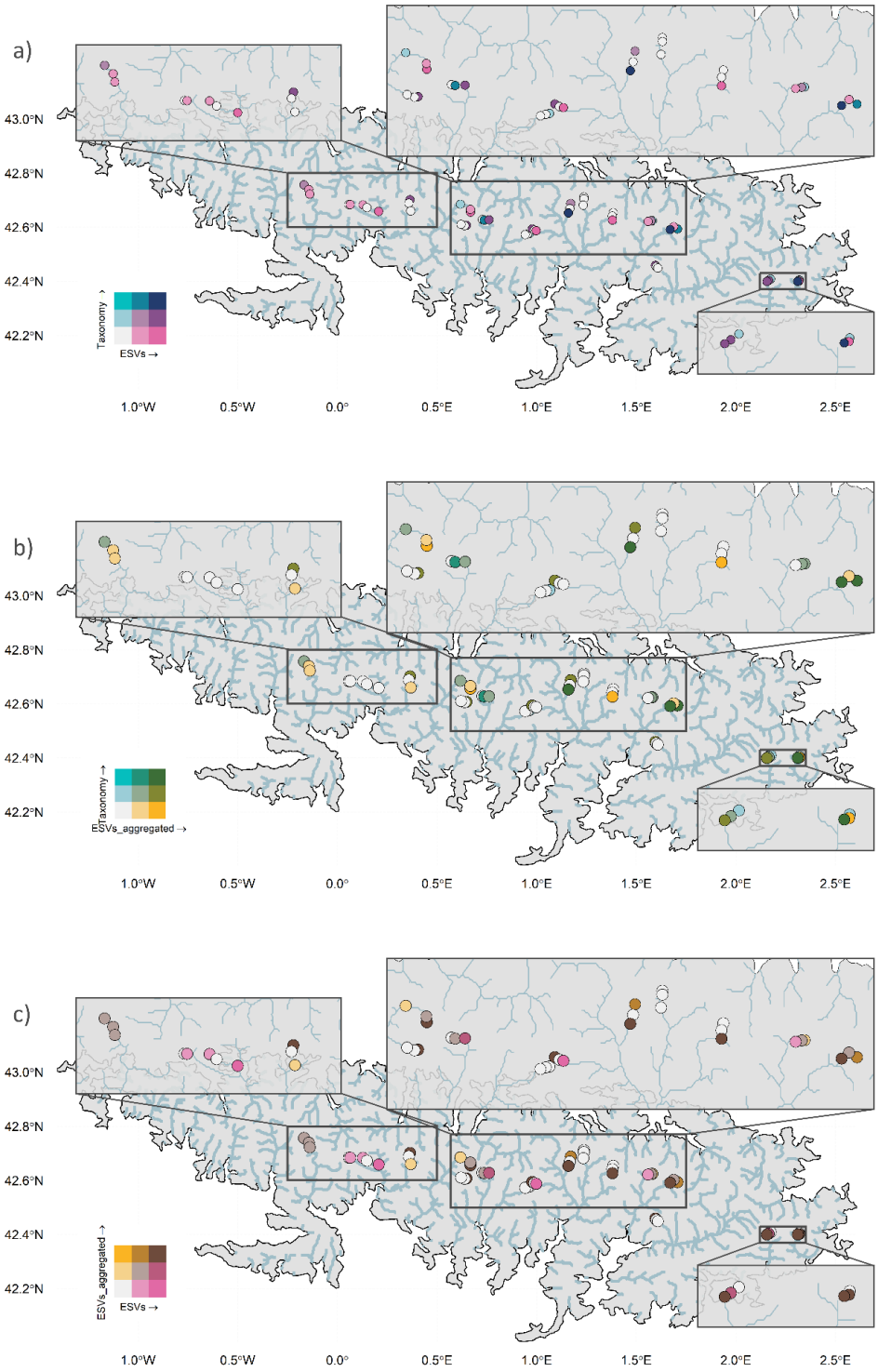


Figure 2

Comparison of selection frequency (100 MARXAN runs) across different biodiversity metrics. (**a**) the relationship between the taxonomic approach and ESVs scenarios, (**b**) the relationship between the taxonomic approach and aggregated ESVs scenarios, (**c**) the relationship between ESVs and ESVs-aggregated scenarios. In each panel, the two metrics are represented by their corresponding colors (blue for the taxonomic approach, pink for ESVs, and orange for ESVs-aggregated). White indicates no selection, pure colors represent selection by a single metric, and intermediate tones indicate varying degrees of overlap in selection between the two approaches.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuplementaryMaterial.docx