Genetic diversity affects ecosystem functions across trophic levels as much as species diversity, but in an opposite direction

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ABSTRACT

Understanding the relationships between biodiversity and ecosystem functioning stands as a cornerstone in ecological research. Extensive evidence now underscores the profound impact of species loss on the stability and dynamics of ecosystem functions. However, it remains unclear whether the loss of genetic diversity within key species yield similar consequences. Here, we delve into the intricate relationship between species diversity, genetic diversity, and ecosystem functions across three trophic levels —primary producers, primary consumers, and secondary consumers— in natural aquatic ecosystems. Our investigation involves estimating species diversity and genome-wide diversity -gauged within three pivotal species- within each trophic level, evaluating seven key ecosystem functions, and analyzing the magnitude of the relationships between biodiversity and ecosystem functions (BEFs). We found that, overall, the absolute effect size of genetic diversity on ecosystem functions mirrors that of species diversity in natural ecosystems. We nonetheless unveil a striking dichotomy: while genetic diversity was positively correlated with various ecosystem functions, species diversity displays a negative correlation with these functions. These intriguing antagonist effects of species and genetic diversity persists across the three trophic levels (underscoring its systemic nature), but were apparent only when BEFs were assessed within trophic levels rather than across them. This study reveals the complexity of predicting the consequences of genetic and species diversity loss under natural conditions, and emphasizes the need for further mechanistic models integrating these two facets of biodiversity.

Introduction

Diversity *within* and *among* species are both important to ensure and stabilize ecosystem functions (Cardinale *et al.* 2012; Raffard *et al.* 2019). Studies on the links between biodiversity and ecosystem functioning (BEFs) have primarily focused on the interspecific (species) facet of biodiversity (Balvanera *et al.* 2006; Hooper *et al.* 2005). However, the intraspecific (genetic) facet of biodiversity has also recently been shown to have substantial effects on ecosystem functions (Crutsinger *et al.* 2006; Hughes & Stachowicz 2004; Reusch *et al.* 2005). Recent meta-analyses have shown that genetic diversity of plant and animal populations affect ecosystem functions, and that the magnitude (and shape) of intraspecific BEFs is similar to that of species diversity (Raffard *et al.* 2019; Wan *et al.* 2022).

Although natural assemblages encompass both intra- and interspecific diversity, most studies investigating BEFs are considering each biodiversity facet separately (but see, Fridley & Grime 2010; Prieto *et al.* 2015; Grele *et al.* 2024). This makes it difficult to differentiate the relative role of genetic and species diversity in ecosystem functions, impeding general predictions regarding the consequences of biodiversity loss as a whole on ecosystem functions (Blanchet *et al.* 2023). For instance, we are currently unaware whether the loss of genetic diversity within a few species in an assemblage is as detrimental for ecosystem functions as a species loss, or whether the combined loss of genetic and species diversity may have non-additive consequences for ecosystem dynamics. Although these biodiversity loss scenarios are realistic, our knowledge on the relative role of genetic *vs*. species diversity in ecosystem functions are still too scarce to provide reliable predictions.

The few studies investigating the combined effects of genetic and species diversity on ecosystem functions were all conducted experimentally by manipulating the genetic and species diversity of assemblages under controlled conditions (Fridley & Grime 2010; Hargrave *et al.* 2011; Prieto *et al.* 2015; but see Grele *et al.* 2024). Our understanding of genetic (intraspecific) and species (interspecific) BEFs therefore relies on simplified ecosystems that often lack variation in other factors (including spatial scales, abiotic factors…), and in which

82 feedbacks between ecosystem functions and biodiversity are limited (Duffy *et al.* 2017; Prunier
83 *et al.* 2023). However, knowledge acquired from BEFs at the interspecific level reveals that
84 environmental variation can either reduce or enhance the effects of biodiversity on ecosystem
85 functions, hence generating large variance in the magnitude and direction of BEFs measured
86 in the wild (Hagan *et al.* 2021; Van Der Plas 2019). One can therefore predict that, under
87 natural conditions, the relative influence of genetic and species diversity on ecosystem
88 functions may deviate from what has been quantified under controlled conditions, although it
89 is difficult to predict the direction of this deviation as field studies (in particular for genetic
90 BEFs) are too scarce to generate clear predictions. Therefore, we need further realistic field
91 studies of BEFs, embracing the whole diversity of life forms (from genes to species) and
92 across realistic environmental gradients to test whether -under natural conditions- species and
93 genetic BEFs are of similar magnitude.

94 BEF studies often consider a single trophic level, despite accumulating evidence that
95 biodiversity at a given trophic level can propagate across trophic levels, generating "multi-
96 trophic BEFs" (Lefcheck *et al.* 2015; Soliveres *et al.* 2016, Seibold *et al*. 2018). In particular,
97 studies testing the joint effects of genetic and species diversity on ecosystem functions have
98 mostly considered the effect of primary producer diversity on their own productivity ("*within-*
99 *trophic level BEFs*", e.g., Hargrave *et al.* 2011; Prieto *et al.* 2015). However, genetic and
100 species diversity within a given trophic level may have propagating effects on the ecosystem
101 at other trophic levels (hereafter, "*between-trophic level BEFs*"). Indeed, it is predicted that a
102 genetically-diverse predator population shares their resources more efficiently than a
103 genetically-poor predator population, which might permit a higher prey species coexistence
104 and hence a larger prey biomass (*between-trophic level* BEFs due to genetic diversity, e.g.,
105 Raffard *et al.* 2021). Alternatively, a species-rich community of primary producers likely
106 exhibits higher primary production, as organisms in species-rich communities share basal
107 resources more efficiently than in species-poor communities (*within-trophic level BEFs due to*
108 *species diversity,* Balvanera *et al.* 2006; Hooper *et al.* 2005). Similarly, the relative impact of

genetic and species diversity should be inconsistent across trophic. At higher trophic levels (e.g., predators), species richness is generally lower, which should increase the likelihood for genetic diversity (of a few species) to have strong effects on functions. A simple prediction might therefore be that the relative impact of genetic diversity on ecosystem functions should increase with increasing trophic levels (Blanchet *et al.* 2020). Studies considering genetic and species BEFs under a realistic multitrophic scenario may thus help understanding the trophic contexts under which either genetic or species diversity is more impactful on ecosystem functions than the other, and to test whether genetic and species effects can propagate across trophic levels or not (Seibold *et al*. 2018, Li *et al*. 2020, Moi *et al*. 2021).

Here, we conducted a field study to test the relative importance of genetic and species diversity for ecosystem functions across multiple trophic levels in a natural landscape. We focused on three trophic levels from river ecosystems; riparian trees (primary producers), macroinvertebrate shredders (primary consumers) and fish (secondary consumers). For each trophic level, we quantified the species diversity of each community, as well as the genetic diversity of a single target and dominant species (*Alnus glutinosa*, *Gammarus* sp. and *Phoxinus dragarum* respectively). We further estimated several ecosystem functions, including leave decomposition of riparian trees, biomass (as productivity estimates) of each target species and total biomass of each community within each trophic level. We relied on causal analyses, taking into account the direct and indirect effects of the environment (through biodiversity) on ecosystem functions (Duffy *et al.* 2016) to test i) whether BEFs measured at the genetic level (*genetic* BEFs) are similar in magnitude and direction to BEFs measured at the species level (*species* BEFs); and ii) whether *within-trophic level* BEFs are similar in magnitude than *between-trophic level* BEFs. We also tested whether the relative effects of species and genetic diversity on ecosystem functions (within or between trophic levels) are consistent across the three trophic levels (primary producers, primary consumers and secondary consumers), in order to generalize findings along the trophic chain. We predicted that -contrary to what has been observed under controlled conditions- *genetic* BEFs and

136 *species* BEFS will not be similar in magnitude, especially because environmental variation
137 may modulate each of them differentially. We further expected that significant genetic and
138 species BEFs will be observed both *within*- and *between*-trophic levels, leading to *within*- and
139 *between*-trophic levels of similar magnitude. Finally, we predicted that the magnitude of
140 *genetic* BEFs will be higher (than that of *species* BEFs) at the highest trophic level (secondary
141 consumers) than at the lowest trophic level (primary producers), mainly because species
142 richness at higher trophic levels presents a lower gradient that at the lowest trophic levels.

143

144 **Materials and methods**

145 *Sampling sites and trophic chain*

146 We sampled 52 sites in Southern France from the Adour-Garonne watershed, and
147 distributed along an east-west gradient in the Pyrenees Mountains (**Figure 1a**). We acquired
148 data on species diversity, genetic diversity and ecosystem functions at three trophic levels
149 (primary producers, primary consumers and secondary consumers) (**Figure 1b**). Riparian
150 trees (57 species in the sampled area) provide organic matter in the form of fallen leaves as
151 a food source for decomposers. We selected the common alder *Alnus glutinosa* for acquisition
152 of genetic data due to its dominance at most sites and its functional relevance, as its roots
153 serve as shelters for many aquatic species and are involved in nitrogen fixation.
154 Macroinvertebrate shredders (101 genera in the sampled area) are primary consumers using
155 leaves as resources, and converting them into accessible organic matter for other species.
156 We focused on the most abundant Gammarid (Crustacean) species for genetic data
157 acquisition, referred to as *Gammarus* sp. This species has not yet been formally named
158 although it is phylogenetically distinct from its closest relative, *Gammarus fossarum* (Carnevali
159 2022; Piscart, unpublished data). This species is particularly efficient at decomposing tree
160 leaves, in particular those from *Alnus* (Macneil *et al.* 1997). Fish (20 species in the sampled
161 area) are secondary consumers feeding on invertebrates (amongst others). We used the

minnow *Phoxinus dragarum* as the fish target species as it is an abundant and important predator strongly impacting invertebrate communities (Raffard *et al.* 2021).

Biodiversity estimates

Species datasets. At each site, we collected data on the abundance of all species within each trophic level, at one occasion for trees (July-August 2021) and two occasions for invertebrates (July and November 2020) and fishes (mid-July to mid-August 2020 and 2021), to obtain accurate biodiversity estimates. We identified tree species along a 200 m transect of each river bank, excluding trees with trunk smaller than 2 cm in diameter and more than one meter away from the bank. The abundances of trees were estimated as the total number of individuals per species and per site. For invertebrates, we identified shredders to the genus level (or to the family level for some groups such as chironomids) sampled from two types of standardized traps installed in four micro-habitats distributed along the 200m transect used to identify trees: natural coconut brushes (15*5.5 cm, bristles length 7.5 cm) recovered after 1.5 month of colonization, and litter bags (15*11 cm, 0.8 cm mesh size) filled with senescent *Alnus* leaves from each site and recovered after nine days of colonization (see below). We calculated abundances of each genus by summing the number of individuals per genus found in the coco brushes and the litter bags, and we averaged the abundances over the two sampling occasions to get a single estimate per genus per site. For fish, we collected all specimens during single-pass electric fishing sessions over a mean area of ~ 469.9 m^2 (± 174 m^2) distributed along the 200 m transect. We anesthetized, identified and counted individuals at the species level. We calculated fish abundances as the number of individuals per species and per m^2, and we averaged the abundances over the two sampling occasions as for invertebrates. Fish species number varies from 1 to 11, invertebrate genus number varies from 15 to 42 and the tree species number varies from 7 to 20 (see Fargeot *et al*. 2023 for details).

188 *Genetic datasets*. At each site, we collected tissue from up to 32 individuals of each of the
189 three target species, a sample size having found sufficient for estimating the genomic diversity
190 of populations (Hale *et al*. 2012). We collected fresh leaves of *A. glutinosa* in May 2020,
191 specimens of *Gammarus* sp. in February 2020, and a piece of pelvic fin from *P. dragarum*
192 individuals in summer 2020. The DNA of these samples was extracted using commercial kits
193 for *Alnus* and *Gammarus sp.* and a salt-extraction protocol for *P. dragarum* (see Fargeot *et al.*
194 2023 for details). For each specimen, DNA concentrations were measured using Qubit 3.0
195 fluorometer (Life Technologies®, USA). Sequencing was performed based on equimolar pools
196 of DNA ("pool-seq" approach, Schlötterer *et al.* 2014) from each population and each species.
197 For *Gammarus sp*., we also obtained a ~600 bp mitochondrial sequence from the COI
198 mitochondrial gene from each individual to ensure identification and avoid mixing individuals
199 from different species. *Gammarus* sp. was found allopatric in most sites, but for a few sites
200 from the eastern part of the area in which two species were identified (Carnevali 2022). In this
201 latter case, we conserve only the target species for creating the DNA pools. We created one
202 DNA pool per site per species (52 pools for *A. glutinosa*, 47 pools for *Gammarus* sp. and 44
203 pools for *P. dragarum*) and performed double-digest restriction-site associated DNA
204 sequencing (ddRAD-seq) for *A. Glutinosa* and *Gammarus* sp. (respectively, PstI/MseI and
205 Pst/HindIII enzymes) and normalized Genotyping-by-Sequencing (nGBS) for *P. dragarum*
206 (MsII enzyme). Library preparation and pool-sequencing were executed by LGC Genomics
207 (Biosearch Technologies®, Germany) on an Illumina NovaSeq® (2x150 pb). Data processing
208 was performed following De Kort *et al.* (2018), except that read mapping was performed on
209 reference genomes. The genome of *A. glutinosa* was already available (Griesmann *et al.*
210 2018), and we assembled reference genomes from Illumina short-read sequencing and
211 PacBio long-read sequencing for *Gammarus. sp.* (available upon request) and *P. dragarum*
212 (accession number on DDBJ/ENA/GenBank: JARPMJ000000000), respectively. SNP calling
213 was performed with (i) filtering of raw sequencing files; (ii) indexing of reference genomes; (iii)
214 mapping reads to the reference; (iv) filtering for unpaired and badly/non-mapped reads; (v)
215 assembling all read information in a single file per population and per species and (vi)

216 calculating SNP allelic frequencies (De Kort *et al.* 2018). The total numbers of SNPs retrieved
217 were 583 862 for *A. glutinosa*, 331 728 for *Gammarus* sp. and 414 213 for *P. dragarum* (see
218 Fargeot *et al.* 2023 for details).

219 *Species and genetic diversity estimates*. We calculated *α*- diversity per site using the Shannon
220 entropy from the "hillR" R package for both species and genetic diversity. The Shannon
221 entropy is a metric of evenness that takes into account the distribution of allele or species
222 abundances within each site (Chao *et al.* 2014) by weighting each species/allele by its
223 proportional abundance (q = 1). Results were similar when using the Simpson's diversity index
224 (q = 2, results not shown). It is noteworthy that -given the spatial extent of the sampling area
225 and the number of sampling sites- genetic and species diversity estimated in this study
226 constitutes a fair representation of the biodiversity found in the rivers from the Pyrenean
227 Piedmont, covering a wide range of biological complexity.

228

229 *Ecosystem function measurements*

230 At each site, we measured seven ecosystem functions. We collected biomass
231 production data of all species at each trophic level (hereafter "total biomass") and the biomass
232 production of each target species as estimates of productivity, as well as the decomposition
233 rate of *Alnus* leaves. Productivity -as we quantified it- is obviously affected by local
234 environmental characteristics, and for this reason, we took into account these potential
235 environmental effects (see hereafter). For riparian tree biomass, we used the trunk diameter
236 of each single tree as a proxy of individual tree biomass, and we summed the trunk diameters
237 of all trees found along the transect (divided by the length of the transect) to estimate the total
238 tree biomass per site and per meter of bank. The same approach was used to estimate *A.*
239 *glutinosa* biomass. For macroinvertebrate shredders, we estimated the total invertebrate
240 biomass by drying all individuals for 24 h at 60 °C before weighing them (10^{-4} g precision). The
241 same procedure was used to estimate the biomass of *Gammarus* sp. For both estimates, we

242 averaged biomasses over the two sampling sessions. For fish, (fresh) total fish biomass was
243 estimated as the total weight of all individuals (0.01 g precision) per site, whereas *P. dragarum*
244 biomass was the mass of all *P. dragarum* specimens per site. Fish biomasses were averaged
245 over the two sampling sessions.

246 For the decomposition rate, we quantified leaf mass loss in litter bags placed in four
247 micro-habitats per site twice (July and November 2020). We gathered and dried senescent
248 leaves during fall 2019 from five *Alnus* trees per site to limit individual-specific effects on
249 decomposition. Litter bags were 15 cm x 11 cm pockets of plastic-wire mesh (mesh size; 8
250 mm to allow invertebrates colonization) in which we introduced 4g of dried leaves before
251 closing the bags with staples. We installed three bags per micro-habitat (12 per site) that we
252 removed sequentially after ~9 days, ~18 days and ~27 days respectively to estimate
253 decomposition rates. Bags were brought back to the laboratory, the remaining leaves were
254 cleaned, dried and weighed. Decomposition rate was estimated as the slope of leaf mass loss
255 over time (obtained from a linear model) that we averaged across replicates and temporal
256 sessions (Raffard *et al.* 2021).

257

258 *Environmental data*

259 A major challenge for inferring BEFs from empirical data is to take into account the
260 direct and indirect (through biodiversity) effects of environmental factors on ecosystem
261 functions (Duffy *et al.* 2016, 2017). Failing to do this may result in overestimated and/or
262 artefactual BEFs, especially if the same environmental factor simultaneously affects
263 biodiversity and ecosystem processes (Grace *et al.* 2016). For each site, we measured
264 thirteen variables related to river topography and physico-chemical characteristics that likely
265 influence biodiversity and ecosystem processes (Altermatt 2013). *River bed width* (m) was
266 averaged from five measurements per site. *Connectivity* was calculated as the "closeness
267 centrality", *i.e.,* the inverse of the sum of the distances of a node to all other nodes along the

268 shortest paths possible (Altermatt 2013), using QGIS and the "RiverDist" R package. *Altitude*
269 (m), *distance from the outlet* (m) and *east-west gradient* (longitudinal position along the
270 Pyrenees chain) were measured using QGIS; *oxygen concentration* ($mg.L^{-1}$), *oxygen*
271 *saturation* (%), *water temperature* (°C), *specific conductivity* (µS/cm) and *pH* were measured
272 (and averaged) in summers 2020 and 2021 using a multi-parameter probe (Aqua TROLL 500,
273 In-Situ Inc.). Concentration of NO_{3-}, NO_{2-}, NH_4^+ and PO_4^{3-} were estimated (and averaged)
274 during summers 2020 and 2021 from a filtered water volume (100 mL) using the Alpkem Flow
275 Solution Iv Autoanalyzer (OI Analytical®).

276 A Principal Component Analysis combining all thirteen variables was performed using
277 the R package "ade4" (Dray & Dufour 2007), and coordinates of each site on the two first axes
278 (38.03% of the total variance, see **Table 1**) were used as two synthetic environmental
279 variables for further analyses. We kept only these two first axes to avoid collinearity and over-
280 parameterization of subsequent models. The first axis is defined by a strong contribution of (in
281 decreasing order) oxygen concentration and altitude (**Table 1**). The second axis is defined by
282 a strong contribution of east-west gradient and connectivity (**Table 1**).

283

284 *Statistical analyses*

285 *BEF relationships*. To quantify the magnitude of association between biodiversity estimates
286 and ecosystem functions (BEFs), we performed piecewise Structural Path Models (pSEM,
287 "piecewiseSEM" package, Lefcheck 2016). pSEM allows modelling direct and indirect causal
288 relationships among a set of response variables and predictors (Shipley 2009). Further, pSEM
289 uses local estimates of each linear structural equation separately (*i.e.*, parameters are
290 estimated from a series of independent models forming a general causal graph), which allows
291 the inclusion of a large number of parameters despite modest sample sizes (Shipley 2009).
292 We ran a pSEM for each ecosystem function separately (*i.e.*, seven pSEMs, see an example
293 in **Figure 2**). In each pSEM, the ecosystem function was the dependent variable whereas the

294 six biodiversity estimates (species and genetic diversity estimated for each trophic level) and
295 the two synthetic environmental variables were the predictors. In each model, environmental
296 predictors were allowed to explain each biodiversity estimate (indirect effects of environmental
297 variables through their influence on biodiversity, see **Figure 2**). For some functions (in
298 particular those associated with plant biomass), irrelevant biodiversity-functions links were not
299 included (e.g., the impact of fish or invertebrate diversity on tree biomasses), which results in
300 34 BEFs (out of the 42 possible links) having been included in the meta-regression (see
301 hereafter).

302 From each pSEM model, we retrieved the local parameter (standardized estimate, an
303 equivalent to a coefficient of correlation) associated with the direct effect of each biodiversity
304 estimate (six per function, but for some functions for which ecology-irrelevant BEFs were
305 excluded) on the function (coloured arrows in **Figure 2**), which provides both the magnitude
306 and the direction of each BEF. To smoothen comparison, we calculated a standardized effect
307 size for each BEF by applying the Fisher's Z transformation (*Zr*) to the standardized estimates.
308 Positive *Zr* indicate positive associations between biodiversity and ecosystem functions,
309 whereas negative *Zr* indicate negative relationships. The higher the absolute value of Zr, the
310 higher the strength of the association. *Zr* therefore indicate both the direction (positive or
311 negative) and the magnitude of the associations. Our seven measures of ecosystem functions
312 were not correlated one to each other (all $r_{pearson}<|0.39|$).

313

314 *Direction and magnitude of all types of BEFs*. We used a linear mixed-model to test (i) whether
315 the magnitude and direction of *genetic* BEFs are similar to those of *species* BEFs, and (ii)
316 whether *within-trophic level* BEFs are similar in effect size to *between-trophic level* BEFs. In
317 this model, *Zr* (providing the direction and magnitude of each BEF, n=34) was the dependent
318 variable, and the predictors were the diversity facets used to measure biodiversity (genetic or
319 species diversity) and the type of BEF (*within-trophic* or *between trophic* levels, triangles vs.
320 dots in **Figure 2**). We included the two-term interaction between diversity facet and type of

321 BEF to test whether the magnitude and direction of *genetic* and *species* BEFs are consistent
322 across *within-trophic level* and *between-trophic level* BEFs. We further included in this model
323 the type of ecosystem function as a random term (to take into account that each ecosystem
324 function was associated with several biodiversity estimates) as well as the inverse of the
325 asymptotic variance (v_z=n-3) associated with each effect size as a weighting parameter for
326 each case study (Balvanera *et al.* 2006; Raffard *et al.* 2019).

327 We ran an additional linear mixed-model similar to the previous one, except that we
328 added as a fixed effect the trophic level at which biodiversity was measured to estimate BEFs
329 (primary producers, primary consumers or secondary consumers) as well as all interaction
330 terms. Interaction terms allow testing the consistency of major conclusions across trophic
331 levels, thereby determining the extent to which our findings can be generalized along the
332 trophic chain. Models were run using the lmer function ("lme4" package) and significance of
333 fixed effects was determined using type III ANOVA with Wald chi-square tests (function Anova
334 from the "car" R package, α=0.05).

335

336 **Results**

337 Details of causal models linking environmental parameters, species and genetic
338 diversity and ecosystem functions are graphically depicted in **Figure 3a-g**. Note that only
339 relationships for which p-values were below 0.20 are shown on these graphs. This threshold
340 was chosen arbitrarily to provide readable causal graphs and to highlight only on the most
341 biologically relevant relationships.

342 The percentage of variance in ecosystem function explained by the environment and
343 biodiversity varies from 10% (invertebrate biomass, **Figure 3e**) to 55% (*Phoxinus* biomass,
344 **Figure 3f**) and was moderate overall. For all functions but the three biomass, part of the
345 variance was (directly) explained by at least one out of the two environmental PCA axes. For
346 some functions (e.g., *Phoxinus* biomass, Figure 3f), there was a combined effect of several

347 biodiversity estimates, whereas for other functions (e.g., *Alnus* biomass, Litter decomposition,
348 **Figure 3a, 3c**) the effect of a single biodiversity estimate predominates. Overall, direct
349 environmental effects on ecosystem functions did not predominate, and environmental effect
350 sizes were similar (in strength) to that of biodiversity effects, showing the non-negligible role
351 of biodiversity for ecosystem functions in the wild.

352

353 Individual effect sizes (*Zr*) measured between biodiversity estimates and ecosystem
354 functions were weak to moderate, irrespectively of the considered ecosystem function and of
355 the type of BEFs (*genetic/species BEFs*, *within-trophic level/ between-trophic level* BEFs)
356 (**Figure 4a, 4b, Table S2**). As expected under natural conditions (Hagan *et al.* 2021), BEFs
357 ranged from negative to positive, and their distribution were centred around 0, although we
358 observed a slight tendency for *genetic* BEFs toward positive values (**Figure 3b**). Only four out
359 of the 34 BEFs were strong and significant; two significant BEFS concerned *species* BEFs
360 (negative relationship between the biomass of *A. glutinosa* and the diversity of trees, *Zr* = -
361 0.446, 95% CI [-0.695, -0.143]; negative relationship between the biomass of *P. dragarum* and
362 the diversity of fish, *Zr* = -0.529, 95% CI [-0.802, -0.166]) and two concerned *genetic* BEFs
363 (negative relationship between the biomass of *P. dragarum* and the diversity of *A. glutinosa*,
364 *Zr* = -0.321, 95% CI [-0.602, -0.019]; positive relationship between the biomass of *Gammarus*
365 *sp.* and the diversity of *P. dragarum*, *Zr* = 0.446, 95% CI [0.001, 0.829]) (**Figure 4a**).
366 Noteworthily, for *within-trophic* BEFs, most case studies fall into the category whereby genetic
367 BEFs tend to be positive and species BEFs tend to be negative (grey bottom-right square in
368 **Figure 4a**).

369 We confirmed this visual tendency by summarizing all individual *Zr* through a meta-
370 regression. Indeed, we found a significant interaction between the facet at which biodiversity
371 is measured (genetic or species diversity), and the type of BEF that was measured (within- or
372 between trophic levels; **Table 2**). This interaction indicates (i) that -overall- *within-trophic level*
373 BEFs were significantly negative when considering species diversity ($Zr_{\text{Within*Species}}$ = -0.185,

374 95% CI [-0.343, -0.027]), whereas *within-trophic level* BEFs were significantly positive when
375 considering genetic diversity ($Zr_{Within*Genetic}$ = 0.168, 95% CI [0.010, 0.326], see **Figure 5a**), and
376 (ii) that this pattern was not observed for *between-trophic levels* BEFs, where no particular
377 trend was observed (**Figure 5a**). Although most individual *Zr* were weak to moderate (and not
378 significant), their consistency (in term of magnitude and direction) resulted in a significant
379 pattern whereby species and genetic diversity have opposite effects on ecosystem functions
380 for *within-trophic level* BEFs; species diversity is negatively associated, whereas genetic
381 diversity is positively associated with ecosystem functions, but only when the influence of
382 biodiversity on ecosystem functions is measured within the same trophic level.

383 When including the trophic level at which biodiversity is measured, we found no
384 significant interaction terms between trophic levels and other fixed effects nor any additive
385 effect of trophic levels (see **Table S1**). This indicates that our main findings were consistent
386 across trophic levels, *i.e.*, the respective negative and positive effects on ecosystem functions
387 of species and genetic diversity hold statistically true across all trophic levels (**Figure 5b**).

388

389 **Discussion**

390 We provide empirical evidence that, in natural ecosystems, the effect sizes of genetic
391 and species diversity on multi-trophic ecosystem functions are of similar magnitude, but
392 operate in opposite directions. Indeed, for BEFs measured within the same trophic level, the
393 effects of species diversity across multiple ecosystem functions were moderately negative on
394 average, whereas the effects of genetic diversity were moderately positive. This suggests an
395 antagonistic effect between the genetic and the species components of biodiversity in the
396 modulation of ecosystem functions within one trophic level. This antagonistic effect was not
397 identified for BEFs measured across trophic levels, since in these cases the influence of both
398 genetic diversity and species diversity across multiple ecosystem functions was generally not
399 different from zero. These conclusions hold true across three trophic levels (plants,

invertebrates and fish), indicating that the relative effects of genetic and species diversity on ecosystem functions are not limited to a specific trophic level.

Our study is one of the few field-based study revealing BEFs across an entire (riverine) food chain spanning from primary producers to secondary consumers. Indeed, most previous BEF studies in the field focused on a single trophic level, and predominantly on terrestrial primary producers (Duffy *et al.* 2017; Van Der Plas 2019, but see e.g., Li et al. 2020, Moi et al. 2021). This permitted encompassing a broad range of ecosystem functions that depict the overall functioning of a riverine ecosystem (rather than focusing on a single compartment). Moreover, we focused both on the effects of genetic and species diversity on these ecosystem functions, which has rarely (if not ever) been evaluated so far and which provides an exhaustive overview of BEFs in the wild. Our causal analyses also statistically took into account the direct (and indirect) effect of environmental factors on ecosystem functions, which is a prerequisite to isolate biodiversity effects. Nonetheless, causal relationships obtained from observational data (rather than from experimental data) are notoriously difficult to infer and must therefore be interpreted with care (Duffy *et al*. 2017). As a result, the BEFs we estimated display strong variability (ranging from negative to positive values) and a very few of them (4 out of 34) were statistically significant according to conventional tresholds. Although the statistical inferences made in this study are based on a large sample size, it is noteworthy that the general patterns we will describe hereafter (and their interpretation) have to be considered with care, as we can not rule out the possibility that some patterns might arise because of statistical biases rather than biological reality. Nonetheless, we -as ecologists- feel important to provide such a general picture from field data (even if partially distort by statistical limits), as this represents basic patterns that we have to understand.

We revealed that direct environmental effects on ecosystem functions were (in average) not stronger in intensity than biodiversity effects, which is coherent with previous syntheses on species BEFs in the wild (Duffy *et al*. 2017). Furthermore, environmental factors used to describe sampling sites in this study were not strong predictors of species and genetic

427 biodiversity. Two non-exclusive hypotheses may explain this observation: (i) using PCA axes
428 to resume environmental gradients may blur some specific environment-biodiversity links, and
429 (ii) as shown and explained in a companion paper (Fargeot *et al*. 2023), the East-West
430 gradient used in this study (rather than a classical upstream-downstream gradient) intrinsically
431 limits the potential for strong environmental effects on biodiversity (which was the purpose of
432 this sampling design). Nonetheless, after accounting for these environmental covariates, we
433 found that most individual BEFs (either *genetic* or *species*, *within-trophic levels* or *between-*
434 *trophic levels* BEFs) were weak to moderate in magnitude, and that they operated almost
435 equally in both direction (i.e., positive and negative association between biodiversity and
436 ecosystem functions). As such, the distribution of individual effect sizes was centred around
437 0, for both *genetic* and *species* BEFs. Accordingly, there were only four individual BEFs that
438 were significant, three out of them were negative and one was positive (**Table S2**). This
439 general pattern (low to moderate BEFs with both positive and negative direction) is actually
440 consistent with the most exhaustive meta-analysis having synthesized the magnitude and
441 direction of *species* BEFs in the wild (Van Der Plas 2019) and with recent conceptual works
442 (Hagan *et al.* 2021) concluding that strong and positive BEFs should not be the norm in natural
443 ecosystems, but rather that a mix of positive, neutral and negative BEFs are expected. Our
444 empirical findings are consistent with this conclusion.

445 We focused both on *within-trophic level* and *between-trophic level* BEFs, which likely
446 encompasses a broad array of mechanisms sustaining potential associations between
447 biodiversity and ecosystem functions. For instance, two out of the four significant BEFs we
448 reveal are negative association between species diversity (fish or tree species diversity
449 respectively) and the biomass production of one of the target species (*Phoxinus sp*. and *Alnus*
450 *sp*. respectively). These *within-trophic level* BEFs can -for instance- arise either because, if
451 resources are limited, increased number of species within a patch limit the biomass production
452 of each individual species, or because of a poorer competitive ability of the target species
453 under some environmental conditions, which favors the settlement of additional species.

454 Teasing apart these two hypotheses is difficult and further studies are needed to isolate
455 underlying mechanisms. The other two significant BEFs concerned the association (either
456 positive or negative) between the genetic diversity of a target species (*Alnus sp.* or *Phoxinus*
457 *sp.*) with the biomass production of another target species (*Phoxinus sp.* or *Gammarus sp.,*
458 respectively). These *between-trophic level* BEFs likely arise through indirect effects implying
459 the diversity and availability of (prey) resources. An obvious limit of this field-based study is
460 the impossibility to tease out these mechanisms. Another limit is associated with the fact that,
461 although environmental covariates were taken into account in causal models, they were
462 synthetized by two PCA axes, and we cannot ensure that all potential environmental
463 covariates have been taken into account (see above). This can influence the actual estimates
464 of BEFs in the wild (Duffy *et al*. 2017). Nonetheless, it is noteworthy that we have previously
465 shown that -in this dataset- species and genetic diversity were not correlated one to each other
466 and that each biodiversity facet was sustained by different environmental predictors (Fargeot
467 *et al*. 2023, see also **Figure 3**). This implies that environmental and biodiversity effects inferred
468 in this study should not be strongly distorted by collinearities and can -in theory- be interpreted
469 independently one from each other (e.g., the positive effect *Phoxinus* genetic diversity on
470 *Phoxinus* biomass is independent from the negative of fish species diversity as the two
471 estimates of biodiversity do not co-vary, see **Figure 3f**).

472 Keeping limitations associated with field-based studies into account (see above),
473 revealing associations between ecosystem functions and species and genetic biodiversity (or
474 the lack of) in natural ecosystems is an important step forward to set theoretical and
475 experimental approaches aiming at understanding this complex biological reality. Beyond
476 individual BEF case studies (that were not the main aim of this study), their aggregation across
477 trophic levels and biodiversity facets revealed a clear (and statistically supported) pattern
478 whereby, within trophic levels, genetic and species diversity display antagonistic association
479 with ecosystem functions; the global effect of species diversity across multiple ecosystem
480 functions was negative, whereas the global effect of genetic diversity was positive. This pattern

481 emerges from the “cumulative” effects of weak to moderate associations between biodiversity
482 and ecosystem functions that consistently point toward the same direction (positive for genetic
483 diversity, negative for species diversity), emphasizing the meaningfulness of meta-regressions
484 (and more generally approaches based on effect sizes rather than on p-values) to reveal
485 biological patterns. We hereafter discuss the ecological relevance of this general pattern.

486 Our results confirm a previous meta-analysis demonstrating that genetic and species
487 diversity modulates ecosystem functions with a similar magnitude (Raffard *et al.* 2019), and
488 results from few experimental studies that manipulated both the genetic and species
489 components of biodiversity under controlled conditions (e.g., Jiang *et al.* 2022; Prieto *et al.*
490 2015). Indeed, within trophic levels, the absolute mean effect size of genetic and species
491 diversity across ecosystem functions were of the same magnitude ($|Zr| = 0.168$ and 0.185 for
492 genetic and species diversity effects respectively), and slightly greater than the effect sizes
493 reported under controlled conditions ($|lnRR| = 0.132$ and 0.134 for genetic and species
494 diversity effects respectively, Raffard *et al.* 2019) and those more generally reported for
495 *species* BEFs ($|Zr| = 0.101$, Balvanera *et al.* 2006). Although comparing effect sizes among
496 studies that strikingly differ in their spatial coverage (small or large spatial scale), their
497 taxonomic focus (e.g., primary producers *vs*. predators, species *vs*. genetic diversity…) and/or
498 their approaches (experimental *vs*. observational studies) is questionable (especially given
499 the non-linear nature of BEFs), our findings suggests for the first time that under natural
500 conditions, the effects of genetic and species components of biodiversity on ecosystem
501 functions are comparable. However, our study goes two steps further as (i) it extends the
502 conclusion made by Raffard *et al.* (2019) to multiple trophic levels and (ii) it suggests that the
503 effects of genetic and species BEFs can actually operate in opposite directions.

504 As pointed out by Raffard *et al.* (2019), the vast majority (91% of 23 reviewed studies
505 by 2019, see also Wan *et al.* 2022) of studies investigating the effects of genetic diversity on
506 ecosystem functions have focused on primary producers, and all of them were based on
507 experiments, which is also the case for most studies manipulating both genetic and species

508 diversity. These trends strongly hamper any generalization. On the contrary, our findings
509 provide a solid support for broadening the conclusion that both genetic and species diversity
510 can influence ecosystem functions in the wild. More strikingly, our results suggests that,
511 although the absolute effect sizes of genetic and species BEFs are of similar magnitude, for
512 *within-trophic level* BEFs, the direction of their effects are opposite; species diversity (in
513 general) reduces the rate of ecosystem functions, whereas genetic diversity enhances the
514 same functions. For instance, all other things being equal, higher fish species diversity is
515 associated with a lower productivity (biomass) in *P. dragarum* (see above for potential
516 explanations), whereas its own genetic diversity tends to be associated with a higher
517 productivity (**Table S2**). In this specific case, genetic and species diversity of the same trophic
518 group (fish) tended to have opposite effects on the same function (productivity of *P. dragarum*).
519 However, in most cases this was not the case as genetic diversity was positively associated
520 with some functions, whereas species was negatively associated with other functions. The
521 distinction between these two patterns is important as in the latter case (genetic and species
522 diversity are associated with different functions) managing/conserving the intra- and
523 interspecific diversity of a single trophic group (e.g., trees) can alter more than one ecosystem
524 function, and sometimes functions that are even not directly associated to the managed trophic
525 group. Moreover (and importantly), as genetic and species diversity have been found to be
526 uncorrelated spatially in this landscape (Fargeot *et al.* 2023), covariation among diversity
527 estimates cannot explain these patterns. These antagonistic effects of genetic and species
528 diversity on ecosystem functions parallel previous experimental findings on plants (Hazard *et*
529 *al.* 2017; Tang *et al.* 2022). It is now essential to understand the mechanisms sustaining these
530 antagonistic effects as a step forward.

531 S*pecies* BEFs were on average negative (see **Table S2** for individual estimates), which
532 contrasts with the general view that species biodiversity favours ecosystem functions,
533 although it is not that surprising (Dee *et al.* 2023; Hagan *et al.* 2021). Indeed, the net effect of
534 species biodiversity on ecosystem functions results from the combined effects of both negative

535 factors, arising from antagonistic interactions such as negative complementarity or negative
536 selection effect, and positive factors, arising from beneficial interactions such as niche
537 complementarity or facilitation (Loreau & Hector 2001). We can speculate that, in our case,
538 the net effect of interspecific interactions mostly results from negative complementarity among
539 species (or strong negative selection effect), whereas the net effect of intraspecific interactions
540 may result from facilitative interactions and/or improved niche complementarity with increased
541 genetic diversity. Intraspecific competition is generally stronger than interspecific competition
542 (Connell 1983), and intraspecific interactions could be expected to lead more frequently to
543 negative complementarity (and hence negative *genetic* BEFs) than interspecific interactions.
544 Since we observe the opposite, we can hypothesize that genetic diversity is essential to
545 increase niche complementarity within species (Bolnick *et al.* 2003) and hence to reduce the
546 pervasive effects of intraspecific interactions (Hughes *et al.* 2008; Prunier *et al.* 2023). Given
547 the empirical nature of our study and the fact that our meta-regressive approach includes
548 several types of BEFs (e.g., species richness acting either on the biomass of a single focal
549 species or on the biomass of an entire focal community), it is hard to tease apart specific and
550 underlying mechanisms. Theoretical approaches, modelling simultaneously the genetic and
551 species components of biodiversity, would be extremely useful to reveal the mechanisms
552 sustaining opposite effects of intra- and interspecific diversity on ecosystem functions.

553 These antagonistic effects were observed only for BEFs measured *within* trophic
554 levels, not for those measured *between* trophic levels. An overall *between-trophic level* BEF
555 not different from zero suggests that biodiversity at a trophic level has only limited impact on
556 ecosystem functions at another trophic level. For example, the biomass of *P. dragarum* was
557 primarily influenced by genetic and species diversity in fishes, rather than the diversity of their
558 preys (**Table S2**). However, for both genetic and species estimates of biodiversity, there was
559 a substantial variation in effect sizes for *between-trophic level* BEFs that ranged from negative
560 to positive BEFs (**Figure 5**). This suggests that biodiversity effects across trophic levels may
561 be more variable in their direction than within-trophic level BEFs, which appear as more

562 constrained. Variability in the magnitude and direction of effect sizes for *between-trophic level*
563 BEFs likely blur a more general trend, but this variation is actually expected under natural
564 conditions in which interactions involve multiple prey and predator species, fostering co-
565 adaptation among communities from different trophic levels (Aubree *et al.* 2020; Poisot *et al.*
566 2013). In these cases, trophic complementarity between two trophic levels (i.e., the originality
567 of a species based on the identity of the species it interacts with) might be a stronger
568 determinant of ecosystem functions than complementarity measured at either one of the two
569 trophic levels (Poisot *et al.* 2013). Quantifying trophic complementarity among our three target
570 species (and communities) using stable isotope or gut content analyses for instance would be
571 extremely valuable to assess whether this complexity can better explain BEFs between trophic
572 levels than diversity measured at one of the trophic level (Aubree *et al.* 2020).

573 The empirical patterns we revealed here were all extremely consistent across the three
574 trophic levels, hence allowing generalization. It is noteworthy that, although statistically strong
575 and consistent, these patterns must be interpreted with care as field-based approaches are
576 limited in properly taking into account the environmental heterogeneity of natural ecosystems
577 (Hagan *et al.* 2021). BEFs were not particularly stronger at any specific trophic level and the
578 relative effects of genetic and species diversity were not dependent on the trophic level at
579 which the function was estimated. We may have expected a stronger top-down regulation (i.e.,
580 biodiversity of predators has more effects than biodiversity of preys) of ecosystem functions
581 since previous studies showed that biodiversity loss should have greater consequences for
582 multi-functionality when it occurs at higher trophic levels (Lefcheck *et al.* 2015, Seibold *et al.*
583 2018). For instance, increased genetic diversity within a predatory fish species has
584 experimentally been shown to indirectly increase the rate of litter decomposition by increasing
585 the diversity of shredders (Raffard *et al.* 2021). Similarly, the relative effects of genetic and
586 species diversity on functions may have varied among trophic levels, and in particular the
587 relative importance of genetic diversity may have been higher for species-poor trophic levels
588 (i.e., fish community) because of a “compensatory effect”. We found no evidence for these

potential trophic-level dependencies, but instead found extremely consistent patterns, which, from a broader perspective, reveal the importance of integrating both multi-trophic and multi-faceted approaches in predicting the overall consequences of biodiversity loss on ecosystem functioning.

To conclude, we found that the genetic (intraspecific) and species (interspecific) facets of biodiversity are both important drivers of multiple ecosystem functions in a natural and multi-trophic context. In the wild, these two facets of biodiversity can, as expected, generate low to moderately high impacts on ecosystem functions measured across three trophic levels, and they can operate in opposite directions (but on different functions; genetic diversity is positively associated with some functions, species diversity is negatively associated with other functions). This shows the importance for managers to develop integrative conservation plans spanning the entire diversity of life (from genes to species). For instance, genetic diversity loss often precedes species loss, and our results suggest that -in mountain streams- losing genes may actually be particularly detrimental for the performance of ecosystem functions. As such, it appears essential to maintain populations with high levels of genetic diversity in these ecosystems. Future studies should (i) extend these findings to other ecosystems and by quantifying natural genetic variation in more than a single species per trophic level, (ii) generate theoretical predictions regarding the mechanisms sustaining the antagonistic effects of genetic and species diversity on functions we revealed, and (iii) use a broader integrative approach for estimating biodiversity across facets (*inclusive* biodiversity) by using either a trait-based approach or a genetic-based approach as recently proposed by Blanchet *et al.* (2023) and Loreau *et al.* (2023).

AUTHOR CONTRIBUTION

Laura Fargeot: Conceptualization (Supporting); Methodology (Equal); Software (Equal); Validation (Equal); Formal analysis (Lead); Investigation (Lead); Data curation (Lead); Writing - original draft (Lead); Writing - review & editing (Equal); Visualization (Lead); Supervision (Equal); Project administration (Lead). **Camille Poesy:** Methodology (Equal); Validation (Equal); Investigation (Lead); Data curation (Supporting); Supervision (Equal); Project administration (Supporting). **Maxim Lefort:** Methodology (Supporting); Investigation (Lead); Data curation (Supporting); Supervision (Equal); Project administration (Supporting). **Jérôme G. Prunier:** Methodology (Supporting); Software (Equal); Formal analysis (Supporting); Investigation (Supporting); Resources (Equal); Data curation (Supporting); Writing - original draft (Supporting); Writing - review & editing (Supporting). **Madoka Krick:** Methodology (Supporting); Investigation (Equal). **Rik Verdonck**: Methodology (Supporting); Software (Equal); Investigation (Supporting); Writing - review & editing (Supporting). **Charlotte Veyssière:** Methodology (Supporting); Investigation (Equal). **Murielle Richard:** Methodology (Supporting); Investigation (Supporting); Writing - review & editing (Supporting). **Delphine Legrand:** Validation (Equal); Writing - review & editing (Equal); Visualization (Supporting). **Géraldine Loot:** Validation (Equal); Investigation (Supporting); Writing - review & editing (Supporting); Visualization (Supporting). **Simon Blanchet:** Conceptualization (Lead); Methodology (Lead); Software (Supporting); Validation (Equal); Formal analysis (Supporting); Investigation (Lead); Resources (Equal); Data curation (Supporting); Writing - original draft (Supporting); Writing - review & editing (Lead); Visualization (Lead); Supervision (Lead); Project administration (Lead); Funding acquisition (Lead).

REFERENCES

Altermatt, F. (2013). Diversity in riverine metacommunities: a network perspective. *Aquatic Ecology*, 47, 365–377.

Aubree, F., David, P., Jarne, P., Loreau, M., Mouquet, N. & Calcagno, V. (2020). How community adaptation affects biodiversity–ecosystem functioning relationships. *Ecol Lett*, 23, 1263–1275.

Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D., *et al.* (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services: Biodiversity and ecosystem functioning/services. *Ecol Lett*, 9, 1146–1156.

Blanchet, S., Fargeot, L. & Raffard, A. (2023). Phylogenetically-conserved candidate genes unify biodiversity–ecosystem function relationships and eco-evolutionary dynamics across biological scales. *Mol Ecol*, 32, 4467–4481.

Blanchet, S., Prunier, J.G., Paz-Vinas, I., Saint-Pé, K., Rey, O., Raffard, A., *et al.* (2020). A river runs through it: The causes, consequences, and management of intraspecific diversity in river networks. *Evol Appl*, 13, 1195–1213.

Bolnick, D.I., Svanback, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D., *et al.* (2003). The ecology of individuals: Incidence and implications of individual specialization. *Am Nat*, 161, 1–28.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., *et al.* (2012). Biodiversity loss and its impact on humanity. *Nature*, 486, 59–67.

Carnevali, M. (2022). Etude phylogénétique des populations de Gammares (Gammarus sp.) du piedmont Pyrénéen. Master thesis. University of Toulouse, France.

Chao, A., Chiu, C.-H. & Jost, L. (2014). Unifying Species Diversity, Phylogenetic Diversity, Functional Diversity, and Related Similarity and Differentiation Measures Through Hill Numbers. *Annu Rev Ecol Evol Syst*, 45, 297–324.

Connell, J.H. (1983). On the prevalence and relative importance of interspecific competition : evidence from field experiments. *Am Nat*, 122, 661–696.

Crutsinger, G.M., Collins, MD, Fordyce, J.A., Gompert, Z., Nice, C. & Sanders, N.J. (2006). Plant Genotypic Diversity Predicts Community Structure and Governs an Ecosystem Process. *Science*, 313, 966–968.

De Kort, H., Baguette, M., Prunier, J.G., Tessier, M., Monsimet, J., Turlure, C., *et al.* (2018). Genetic costructure in a meta-community under threat of habitat fragmentation. *Mol Ecol*, 27, 2193–2203.

Dee, L.E., Ferraro, P.J., Severen, C.N., Kimmel, K.A., Borer, E.T., Byrnes, J.E.K., *et al.* (2023). Clarifying the effect of biodiversity on productivity in natural ecosystems with longitudinal data and methods for causal inference. *Nat Commun*, 14, 2607.

Dray, S. & Dufour, A.-B. (2007). The **ade4** Package: Implementing the Duality Diagram for Ecologists. *J Stat Soft*, 22.

Duffy, J.E., Godwin, C.M. & Cardinale, B.J. (2017). Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature*, 549, 261–264.

Duffy, J.E., Lefcheck, J.S., Stuart-Smith, R.D., Navarrete, S.A. & Edgar, G.J. (2016). Biodiversity enhances reef fish biomass and resistance to climate change. *P Natl Acad Sci*, 113, 6230–6235.

Fargeot, L., Poesy, C., Lefort, M., Prunier, J.G., Krick, M., Veyssière, C., *et al.* (2023). Genomic and species diversity patterns across multiple trophic levels in riverscapes. *Oikos*, 2023, e10015.

Fridley, J.D. & Grime, J.P. (2010). Community and ecosystem effects of intraspecific genetic diversity in grassland microcosms of varying species diversity. *Ecology*, 91, 2272–2283.

Grace, J.B., Anderson, T.M., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., *et al.* (2016). Integrative modelling reveals mechanisms linking productivity and plant species richness. *Nature*, 529, 390–393.

688 Griesmann, M., Chang, Y., Liu, X., Song, Y., Haberer, G., Crook, M.B., *et al.* (2018).
689 Phylogenomics reveals multiple losses of nitrogen-fixing root nodule symbiosis.
690 *Science*, 361, eaat1743.
691 Grele, A., Massad, T.J., Uckele, K.A., Dyer, L.A, Antonini, Y., Braga, L., Forister, M.L., Sulca, L., Kato, M.,
692 Lopez, H.G., Nascimento, A.R., Parchman, T., Simbaña, W.R., Smilanich, A.M., Stireman, J.O.,
693 Tepe, E.J., Walla, T. & Richards, L.A. (2024). Intra- and interspecific diversity in a tropical plant
694 clade alter herbivory and ecosystem resilience. *eLife*, 12, RP86988
695 Hale, M.L., Burg, T.M. & Steeves, T.E. (2012). Sampling for microsatellite-based population genetic
696 studies: 25 to 30 individual per population is enough to accurately estimate allele frequencies.
697 *PloS ONE*, 7, e45170
698 Hagan, J.G., Vanschoenwinkel, B. & Gamfeldt, L. (2021). We should not necessarily expect
699 positive relationships between biodiversity and ecosystem functioning in observational
700 field data. *Ecol Lett*, 24, 2537–2548.
701 Hargrave, C.W., Hambright, K.D. & Weider, L.J. (2011). Variation in resource consumption
702 across a gradient of increasing intra- and interspecific richness. *Ecology*, 92, 1226–
703 1235.
704 Hazard, C., Kruitbos, L., Davidson, H., Taylor, A.F.S. & Johnson, D. (2017). Contrasting
705 effects of intra- and interspecific identity and richness of ectomycorrhizal fungi on host
706 plants, nutrient retention and multifunctionality. *New Phytologist*, 213, 852–863.
707 Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., *et al.* (2005).
708 Effects of biodiversity on ecosystem functioning: A consensus of current knowledge.
709 *Ecol Monogr*, 75, 3–35.
710 Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N. & Vellend, M. (2008). Ecological
711 consequences of genetic diversity: Ecological effects of genetic diversity. *Ecol Lett*, 11,
712 609–623.
713 Hughes, A.R. & Stachowicz, J.J. (2004). Genetic diversity enhances the resistance of a
714 seagrass ecosystem to disturbance. *Proc Natl Acad Sci U.S.A.*, 101, 8998–9002.

Jiang, M., He, L., Fan, B., Wang, T., Yang, N., Liu, Y., *et al.* (2022). Intraspecific more than interspecific diversity plays an important role on Inner Mongolia grassland ecosystem functions: A microcosm experiment. *Sci Total Env* 826, 154134.

Lefcheck, J.S. (2016). PIECEWISESEM : Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods Ecol Evol*, 7, 573–579.

Lefcheck, J.S., Byrnes, J.E.K., Isbell, F., Gamfeldt, L., Griffin, J.N., Eisenhauer, N., *et al.* (2015). Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. *Nat Commun*, 6.

Li, F., Altermatt, F., Yang, J., An, S., Li, A., & Zhang, X. (2020). Human activities' fingerprint on multitrophic biodiversity and ecosystem functions across a major river catchment in China. Global change biology, 26, 6867-6879.

Loreau, M. & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412, 72–76.

Loreau, M., Jarne, P. & Martiny, J.B.H. (2023). Opportunities to advance the synthesis of ecology and evolution. *Ecol Lett*, 26.

Macneil, C., Dick, J.T.A. & Elwood, R.W. (1997). THE TROPHIC ECOLOGY OF FRESHWATER *GAMMARUS* SPP. (CRUSTACEA:AMPHIPODA): PROBLEMS AND PERSPECTIVES CONCERNING THE FUNCTIONAL FEEDING GROUP CONCEPT. *Biol Rev*, 72, 349–364.

Moi, D. A., Romero, G. Q., Antiqueira, P. A., Mormul, R. P., Teixeira de Mello, F., & Bonecker, C. C. (2021). Multitrophic richness enhances ecosystem multifunctionality of tropical shallow lakes. *Funct Ecol*, 35, 942-954.

Poisot, T., Mouquet, N. & Gravel, D. (2013). Trophic complementarity drives the biodiversity–ecosystem functioning relationship in food webs. *Ecol Lett*, 16, 853–861.

Prieto, I., Violle, C., Barre, P., Durand, J.-L., Ghesquiere, M. & Litrico, I. (2015). Complementary effects of species and genetic diversity on productivity and stability of sown grasslands. *Nat Plants*, 1, 15033.

Prunier, J.G., Chevalier, M., Raffard, A., Loot, G., Poulet, N. & Blanchet, S. (2023). Genetic erosion reduces biomass temporal stability in wild fish populations. *Nat Commun*, 14, 4362.

Raffard, A., Cucherousset, J., Montoya, J.M., Richard, M., Acoca-Pidolle, S., Poésy, C., *et al.* (2021). Intraspecific diversity loss in a predator species alters prey community structure and ecosystem functions. *PLoS Biol*, 19, e3001145.

Raffard, A., Santoul, F., Cucherousset, J. & Blanchet, S. (2019). The community and ecosystem consequences of intraspecific diversity: a meta-analysis: The ecological effects of intraspecific diversity. *Biol Rev*, 94, 648–661.

Reusch, T.B.H., Ehlers, A., Hämmerli, A. & Worm, B. (2005). Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proc Natl Acad Sci U.S.A.*, 102, 2826–2831.

Schlötterer, C., Tobler, R., Kofler, R. & Nolte, V. (2014). Sequencing pools of individuals — mining genome-wide polymorphism data without big funding. *Nat Rev Genet*, 15, 749–763.

Seibold, S., Cadotte, M. W., MacIvor, J. S., Thorn, S., & Müller, J. (2018). The necessity of multitrophic approaches in community ecology. *Trends Ecol Evol*, 33, 754-764.

Shipley, B. (2009). Confirmatory path analysis in a generalized multilevel context. *Ecology*, 90, 363–368.

Soliveres, S., van der Plas, F., Manning, P., Prati, D., Gossner, M.M., Renner, S.C., *et al.* (2016). Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature*, 536, 456–459.

Tang, T., Zhang, N., Bongers, F.J., Staab, M., Schuldt, A., Fornoff, F., *et al.* (2022). Tree species and genetic diversity increase productivity via functional diversity and trophic feedbacks. *eLife*, 11, e78703.

Van Der Plas, F. (2019). Biodiversity and ecosystem functioning in naturally assembled communities. *Biological Reviews*, 94, 1220–1245.

Wan, N.-F., Fu, L., Dainese, M., Hu, Y.-Q., Pødenphant Kiær, L., Isbell, F., *et al.* (2022). Plant genetic diversity affects multiple trophic levels and trophic interactions. *Nat Commun*, 13, 7312.

Table 1. Characteristics of the two first principal components identified by the Principal Component Analysis (PCA) ran on the thirteen environmental variables. The part of the total environmental variance (%) and the contribution of each variable on each component are shown. The variables that contributed significantly to the axis are highlighted in bold.

	Component 1	Component 2
Part of total Variance (%)	21.66	16.37
River width	0.596	0.320
Connectivity	-0.155	**0.646**
Altitude	**0.648**	-0.385
Distance from outlet	0.528	-0.331
East-west gradient	0.105	**-0.795**
Oxygen concentration	**0.738**	0.343
Oxygen saturation	0.266	-0.509
Water temperature	-0.594	-0.012
Specific conductivity	-0.463	-0.045
pH	0.573	0.398
Concentration in $NO_3^+NO_2$	-0.369	-0.286
Concentration in NH_4+	-0.019	-0.207
Concentration in PO_4^{3-}	-0.279	0.236
Global characteristic	Low altitude, poorly oxygenated site - High altitude, highly oxygenated sites	Poorly connected east site - Highly connected west site

Table 2. ANOVA table for the linear mixed model testing whether the relationships between biodiversity and ecosystem functions measured in a riverine trophic chain differ between the biodiversity facets (species or genetic diversity) and the types of BEF (*within-* or *between-trophic levels*). A Wald chi-quare test is used to test the significance of each fixed effect.

	Degree of freedom	Chisq-value	P-value
(Intercept)	1	0.287	0.595
Biodiversity facet	1	0.232	0.630
Type of BEF	1	5.393	**0.020**
Biodiversity facet*Type of BEF	1	5.567	**0.018**

Figure captions

Figure 1. Location of sampling sites and illustration of the trophic chain. **(a)** Distribution of the 52 sampling sites (black dots) spanning an East-West gradient at the foothills of the Pyrenees Mountains (France). Each site is denoted by a six-letter code, with the three first letters indicating the river name and the three last letters indicating the closest city or village. **(b)** Our study focused on a tri-trophic food chain commonly found in mountain rivers, consisting of riparian trees, macroinvertebrate shredders and fishes (from bottom to top). Within each trophic level, we measured two facets of biodiversity: genetic diversity in a single target species within each trophic level (specifically *Phoxinus dragarum*, *Gammarus* sp. and *Alnus glutinosa*), and species diversity from communities.

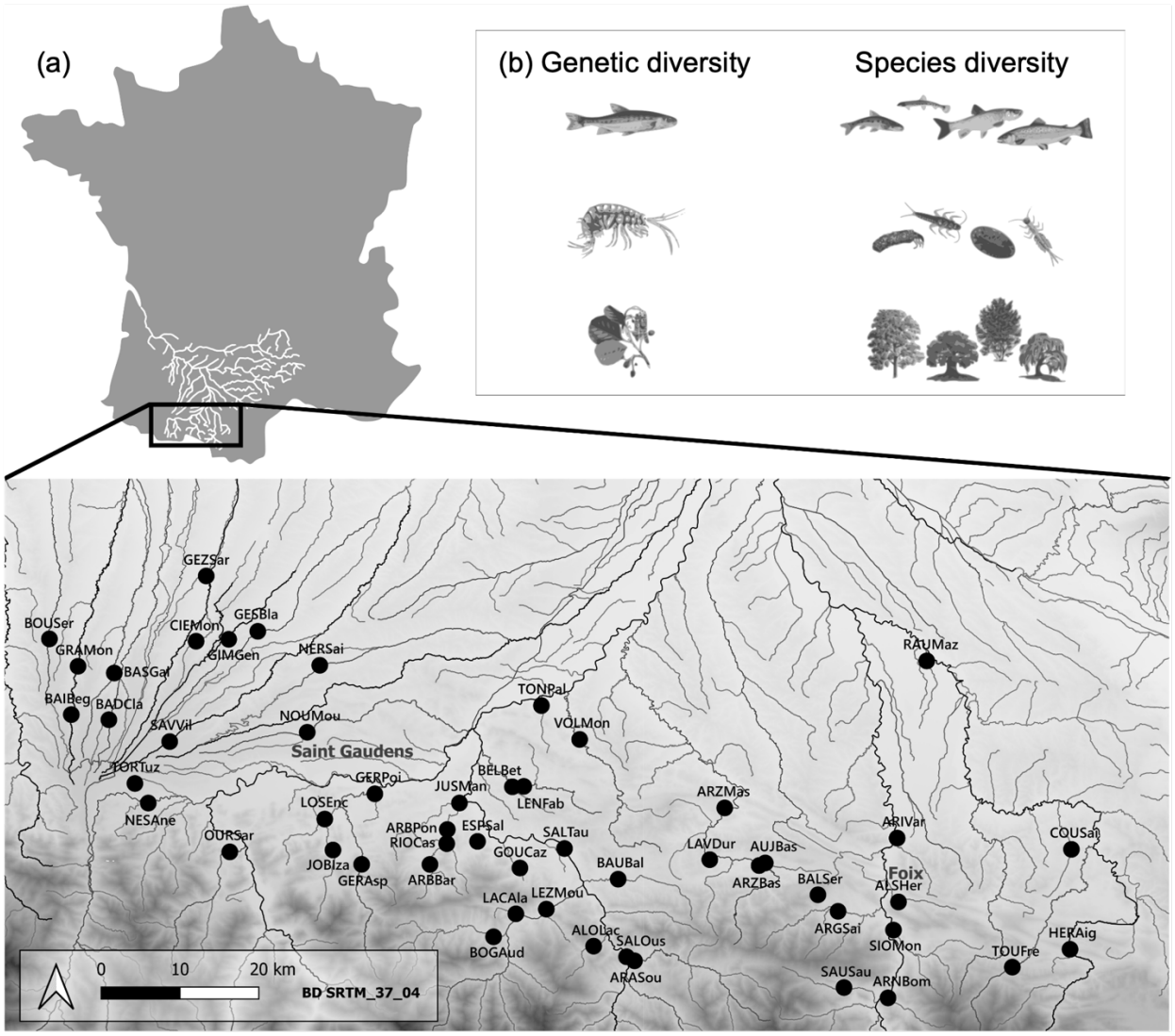
Figure 2. Example of one of the seven causal models used to quantify the relationships between (species and genetic) diversity and ecosystem functions. We focused on seven ecosystem functions associated with genetic and species diversity at three trophic levels (green for primary producer, orange for primary consumer and blue for secondary consumer). Each relationship between biodiversity and ecosystem functions (n = 6 values per function, but for some functions for which irrelevant links were not considered, see the text, n = 32 values in total) was measured at the same trophic level (triangles) or at another trophic level (dots).

Figure 3. Details of the seven causal models linking abiotic parameters, species and genetic diversity and ecosystem functions. Each causal graph (a to g) represents a simplified illustration of the relationships between the two PCA axes synthetizing the environmental parameters of each sampling site (Environmental axis 1 and 2), the species diversity estimated at each trophic level (boxes “Fish”, “Shredders” and “Trees”), the genomic diversity estimated from each focal species at each trophic level (boxes “*Phoxinus*”, “*Gammarus*” and “*Alnus*”), and each ecosystem function (one model per function). Only the relationships for which the p-value was inferior to 0.20 are indicated for visual simplification. Full arrows indicated positive effects, whereas dotted arrows indicated negative effects. The width of the arrows is proportional to the size of their effects. The percentage of variance explained by environmental and biodiversity effects on ecosystem functions (r^2) is indicated for each function.

Figure 4. General description of individual effect sizes measured between biodiversity estimates and ecosystem functions (BEFs) in a riverine trophic chain. (a) The magnitude and direction of individual effect sizes (*Zr*) of biodiversity is shown for each ecosystem functions as a biplot between *Zr* associated with genetic diversity (y-axis, *genetic* BEFs) measured for one of three target species (*Alnus glutinosa*, *Gammarus sp.* and *Phoxinus dragarum*) and *Zr* associated with species diversity (x-axis, *species* BEFs) measured for one of three trophic levels (trees, invertebrates and fish). For each ecosystem function (but the biomass of trees and of *A. glutinosa*), a total of six *Zr* are depicted in the biplot; four of them are associated with biodiversity measured at another trophic level than the one of the target functions (red symbols, e.g., effect of fish diversity on invertebrate biomass) and two of them are associated with biodiversity measured at the same trophic level than the one of the target functions (blue symbols, e.g., effect of fish diversity on fish biomass). The arrows indicate significant *Zr* (95% confidence intervals excluded 0, see Table S2); vertical arrows are for significant *genetic* BEFs, horizontal arrows are for *species* BEFs. White quadrats stand for situation in which genetic and species BEFs are in the same direction, whereas grey quadrats indicate situation in which genetic and species BEFs are in the opposite direction. Within each quadrat, sub-quadrats indicate the relative magnitude of BEFs, i.e., whether *genetic* BEFs are stronger, weaker or equal in magnitude than *species* BEFs. (b) Density plots displaying the distribution of individual *Zr* for *species*- and *genetic* BEFs (dotted and full lines respectively).

Figure 5. Magnitude and direction of the mean effects sizes estimated from the relationships between biodiversity and ecosystem functions (BEFs) measured in a riverine trophic chain. (a) The magnitude and direction of BEFs are expressed as effect sizes (*Zr*) and are displayed according to the facet used to measured biodiversity (genetic or species diversity, light grey and white boxplots respectively) and to the type of BEFs (*within-trophic level* BEFs or *between-trophic level* BEFs, triangles and dots respectively). Red colour and stars indicate global effect sizes that are significantly different from zero (p-value <0.05). Large symbols are mean ± 95%IC estimated as marginal effects from the meta-regressions. Small symbols are raw estimates. (b) Same representation as (a) but with details at each trophic level (mean ± 95%IC estimated as marginal effects from the meta-regressions, green for primary producer, orange for primary consumer and blue for secondary consumer). The trophic level at which BEFs are measured is coherent across all trophic levels.

Figure 1


(a)
(b) Genetic diversity
Species diversity
GEZSar
BOUSer
GRAMon
CIEMon
GESBla
GIMGen
NERSai
BASGal
BAIBeg
BADCla
SAVVil
NOUMou
Saint Gaudens
TORTuz
NESAne
GERPoi
JUSMan
BELBet
LENFab
TONPal
VOLMon
RAUMaz
ARZMas
LOSEnc
OURSar
ARBPon
RIOCas
ESPSal
SALTau
GOUCaz
JOBIza
GERAsp
ARBBar
LACAla
LEZMou
BAUBal
LAVDur
AUJBas
ARZBas
BALSer
ARIVar
Foix
ALSHer
COUSal
ARGSai
SIOMon
HERAig
TOUFre
BOGAud
ALOLac
SALOus
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SAUSau
ARNBom
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10
20 km
BD SRTM_37_04

854 **Figure 2**

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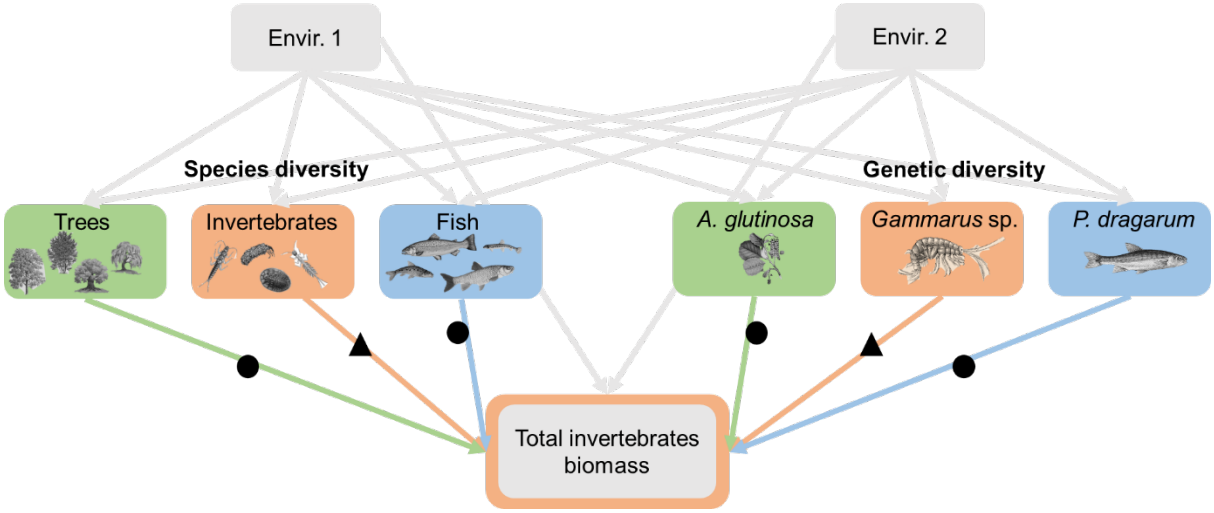

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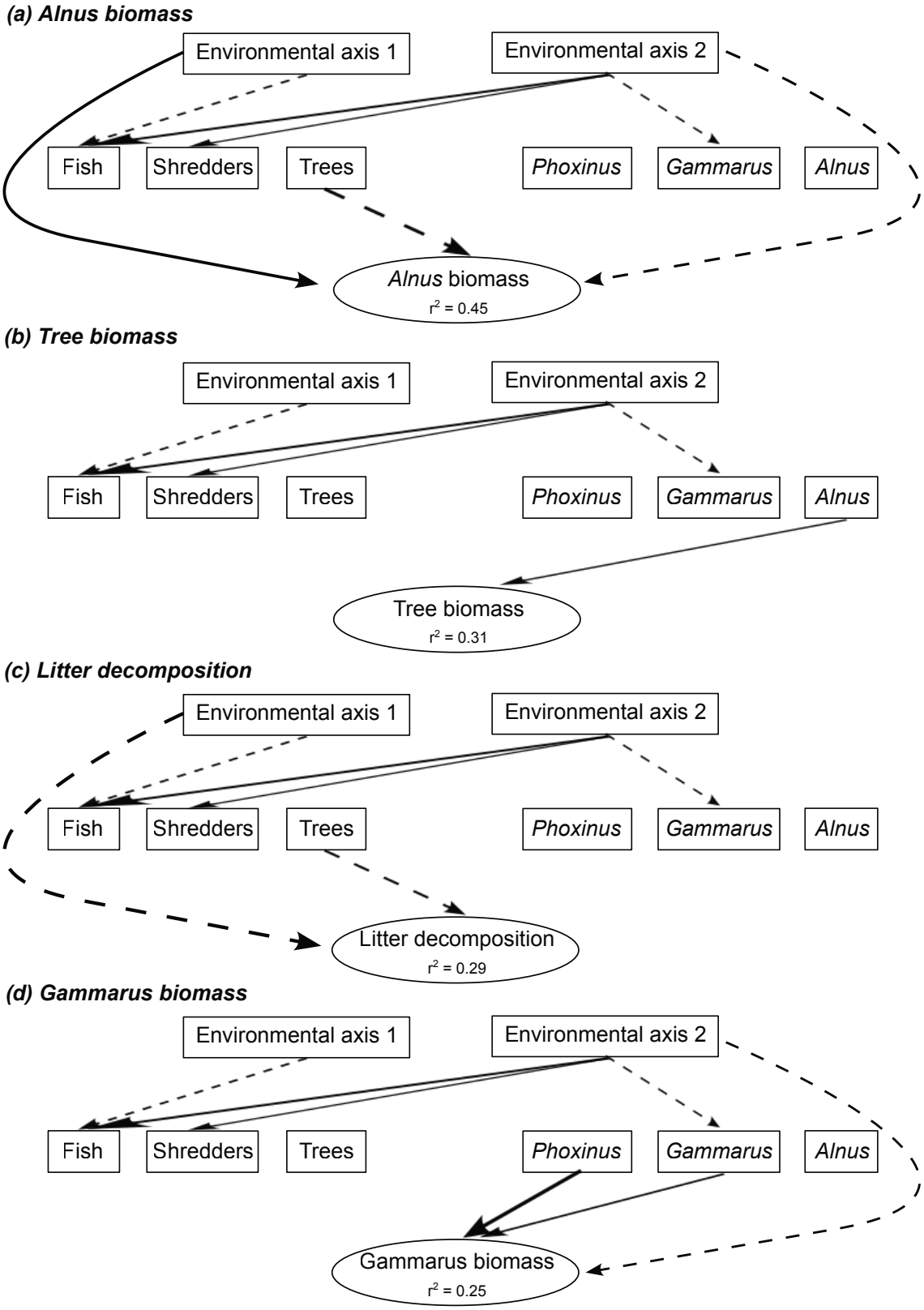
859 **Figure 3**



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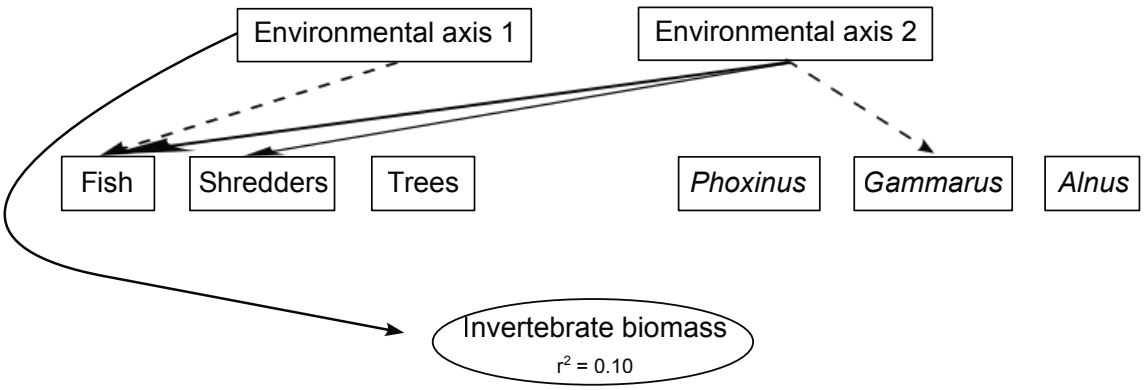
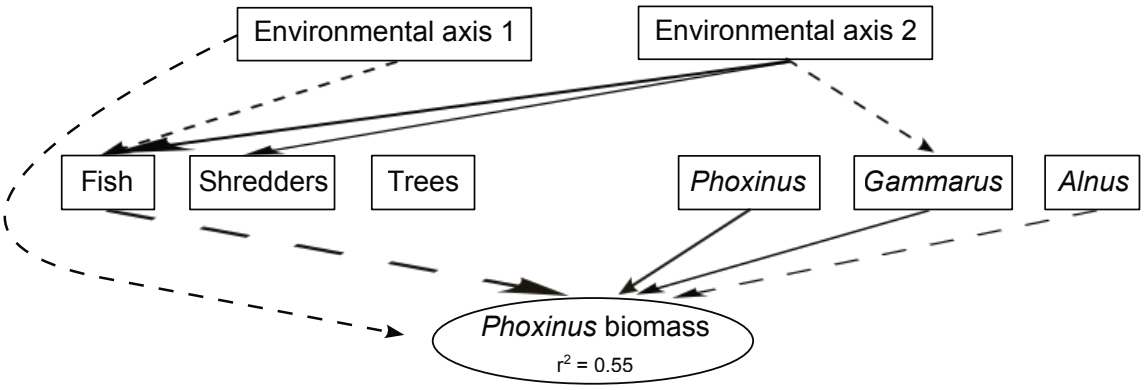
861 **Figure 3 (continued)**

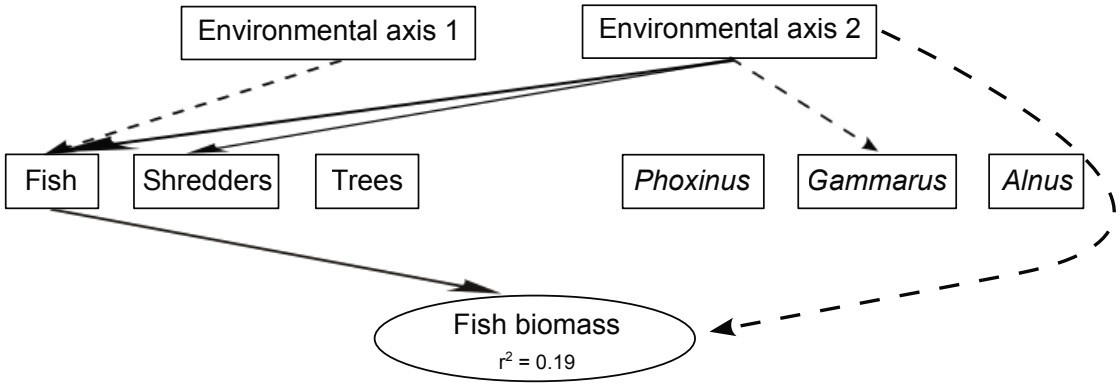
(e) Invertebrate biomass



(f) Phoxinus biomass



(g) Fish biomass



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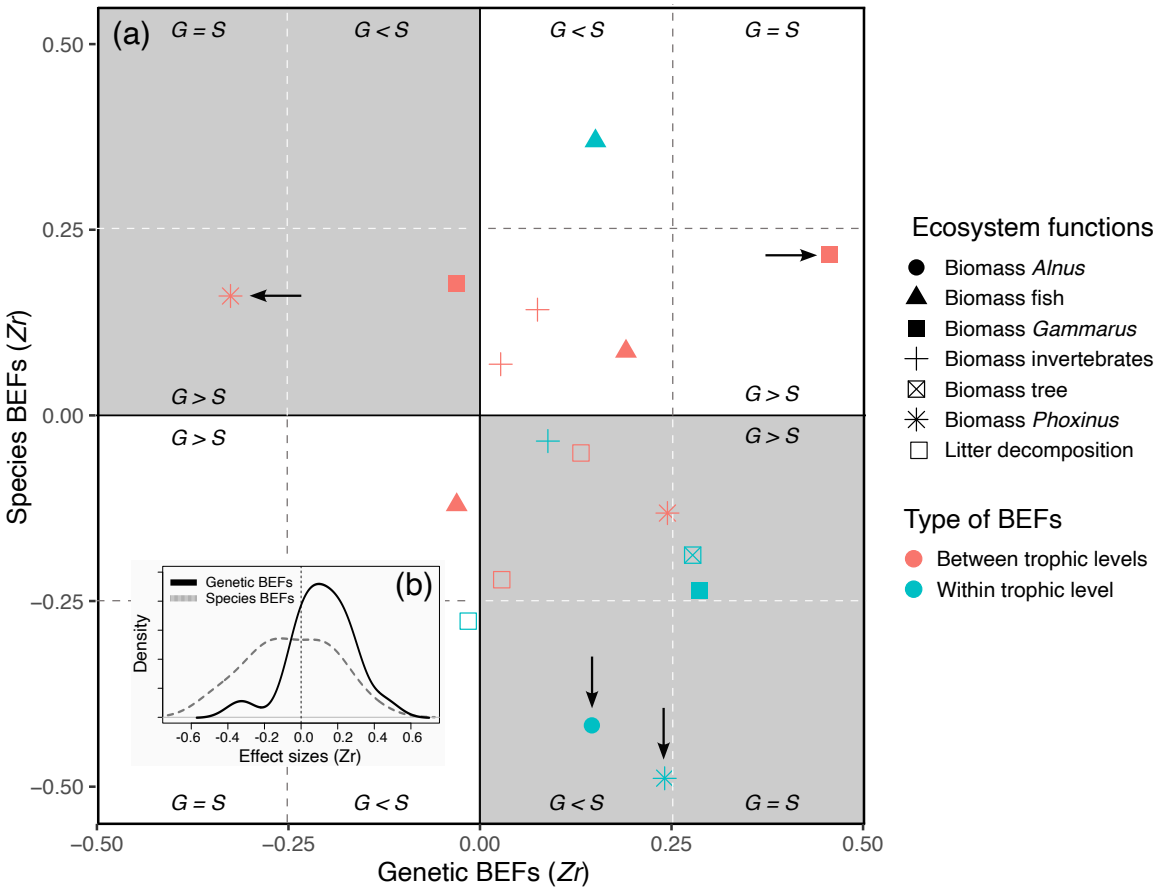
863 **Figure 4**



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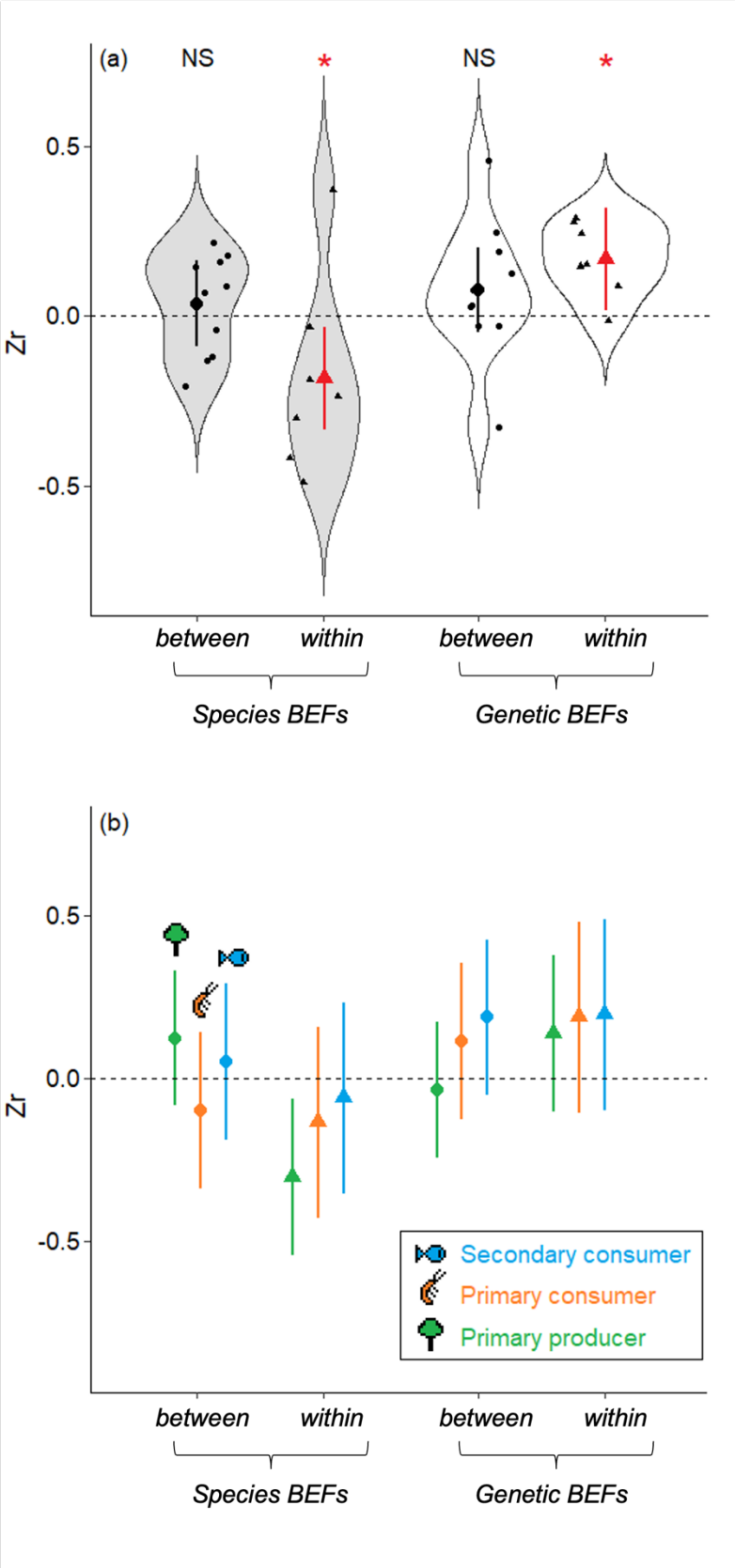
865 **Figure 5**



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868 **SUPPLEMENTARY MATERIALS**

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870

871 **Table S1.** ANOVA table for the linear mixed model testing whether the relationships between
872 biodiversity and ecosystem functions measured in a riverine trophic chain differ between the
873 biodiversity facets (species or genetic diversity), the types of BEF (*within*- or *between-trophic*
874 *levels*) and the trophic levels at which BEFs are estimated (primary producers, primary
875 consumers or secondary consumers). A Wald chi-quare test is used to test the significance of
876 each fixed effect.

877

	Degree of freedom	Chisq-value	P-value
(Intercept)	1	1.453	0.228
Biodiversity facet	1	1.293	0.255
Type of BEF	1	7.498	**0.006**
Trophic level	2	1.976	0.372
Biodiversity facet*Type of BEF	1	7.884	**0.005**
Biodiversity facet*Trophic level	2	3.520	0.172
Trophic level*Type of BEF	2	2.901	0.234
Biodiversity facet*Type of BEF*Trophic level	1	2.994	0.224

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Table S2 – Estimates of individual effect sizes of BEFs (Zr, n=34) for each ecosystem function, each biodiversity facet (genetic or species diversity) and each type of BEF (within- or between-trophic levels). 95% confidence intervals are provided together with the estimate of each BEF. BEFs are considered as significant when the 95%CI does not overlap 0. P-values estimated from t-test are also provided.

Ecosystem function	Predictor	Biodiversity facet	Type of BEF	*Zr*	negative 95% CI	positive 95% CI	P values
***Phoxinus* biomass**	**Fish species diversity**	**Species diversity**	**Whithin-trophic levels**	**-0.529**	**-0.803**	**-0.166**	**0.006**
***Alnus* biomass**	**Tree species diversity**	**Species diversity**	**Whithin-trophic levels**	**-0.447**	**-0.695**	**-0.143**	**0.006**
***Phoxinus* biomass**	***Alnus* genetic diversity**	**Genetic diversity**	**Between-trophic levels**	**-0.321**	**-0.602**	**-0.020**	**0.045**
Litter decomposition	Tree species diversity	Species diversity	Whithin-trophic levels	-0.293	-0.630	0.060	0.115
Gammarus biomass	Invertebrate species diversity	Species diversity	Whithin-trophic levels	-0.273	-0.661	0.128	0.194
Litter decomposition	Fish species diversity	Species diversity	Between-trophic levels	-0.210	-0.625	0.210	0.337
Tree biomass	Tree species diversity	Species diversity	Whithin-trophic levels	-0.183	-0.485	0.122	0.250
Fish biomass	Invertebrate species diversity	Species diversity	Between-trophic levels	-0.138	-0.428	0.153	0.506
Phoxinus biomass	Invertebrate species diversity	Species diversity	Between-trophic levels	-0.129	-0.471	0.215	0.362
Invertebrate biomass	Invertebrate species diversity	Species diversity	Whithin-trophic levels	-0.051	-0.381	0.279	0.766
Fish biomass	*Gammarus* genetic diversity	Genetic diversity	Between-trophic levels	-0.047	-0.457	0.364	0.825
Gammarus biomass	*Alnus* genetic diversity	Genetic diversity	Between-trophic levels	-0.043	-0.438	0.352	0.833
Litter decomposition	Invertebrate species diversity	Species diversity	Between-trophic levels	-0.020	-0.401	0.361	0.918
Litter decomposition	*Alnus* genetic diversity	Genetic diversity	Whithin-trophic levels	-0.012	-0.394	0.370	0.950
Litter decomposition	*Phoxinus* genetic diversity	Genetic diversity	Between-trophic levels	0.018	-0.379	0.416	0.930
Invertebrate biomass	*Alnus* genetic diversity	Genetic diversity	Between-trophic levels	0.025	-0.306	0.356	0.885
Invertebrate biomass	Tree species diversity	Species diversity	Between-trophic levels	0.063	-0.236	0.362	0.682
Invertebrate biomass	*Phoxinus* genetic diversity	Genetic diversity	Between-trophic levels	0.085	-0.260	0.429	0.634
Fish biomass	Tree species diversity	Species diversity	Between-trophic levels	0.087	-0.276	0.449	0.644
Invertebrate biomass	*Gammarus* genetic diversity	Genetic diversity	Whithin-trophic levels	0.097	-0.242	0.435	0.580
Litter decomposition	*Gammarus* genetic diversity	Genetic diversity	Between-trophic levels	0.110	-0.281	0.500	0.586
Alnus biomass	*Alnus* genetic diversity	Genetic diversity	Whithin-trophic levels	0.128	-0.179	0.433	0.422
Invertebrate biomass	Fish species diversity	Species diversity	Between-trophic levels	0.144	-0.219	0.504	0.444
Fish biomass	*Phoxinus* genetic diversity	Genetic diversity	Whithin-trophic levels	0.159	-0.260	0.575	0.464
Gammarus biomass	Tree species diversity	Species diversity	Between-trophic levels	0.161	-0.198	0.516	0.388
Phoxinus biomass	Tree species diversity	Species diversity	Between-trophic levels	0.165	-0.100	0.426	0.233
Gammarus biomass	Fish species diversity	Species diversity	Between-trophic levels	0.185	-0.249	0.614	0.413
Fish biomass	*Alnus* genetic diversity	Genetic diversity	Between-trophic levels	0.193	-0.211	0.592	0.360
Phoxinus biomass	*Gammarus* genetic diversity	Genetic diversity	Between-trophic levels	0.228	-0.074	0.522	0.151
Phoxinus biomass	*Phoxinus* genetic diversity	Genetic diversity	Whithin-trophic levels	0.244	-0.064	0.542	0.133
Tree biomass	*Alnus* genetic diversity	Genetic diversity	Whithin-trophic levels	0.280	-0.062	0.609	0.121
Gammarus biomass	*Gammarus* genetic diversity	Genetic diversity	Whithin-trophic levels	0.292	-0.120	0.688	0.178
Fish biomass	Fish species diversity	Species diversity	Whithin-trophic levels	0.326	-0.123	0.753	0.169

***Gammarus* biomass**	***Phoxinus* genetic diversity**	**Genetic diversity**	**Between-trophic levels**	**0.446**	**0.007**	**0.830**	**0.047**

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