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1 **Alien species and climate change drive shifts in a riverine fish community and trait**
2 **compositions over 35 years**

3

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25 **Abstract**

26 Alien fish substantially impact aquatic communities. However, their effects on trait
27 composition remain poorly understood, especially at large spatiotemporal scales. Here, we used
28 long-term biomonitoring data (1984-2018) from 31 fish communities of the Rhine river in
29 Germany to investigate compositional and functional changes over time. Average total
30 community richness increased by 49%: it was stable until 2004, then declined until 2010, before
31 increasing until 2018. Average abundance decreased by 9%. Starting from 198 individuals/m²
32 in 1984 abundance largely declined to 23 individuals/m² in 2010 (-88%), and then consequently
33 increased by 678% up to 180 individuals/m² until 2018. Increases in abundance and richness
34 starting around 2010 were mainly driven by the establishment of alien species: while alien
35 species represented 5% of all species and 0.1% of total individuals in 1993, it increased to 30%
36 (7 species) and 32% of individuals in 2018. Concomitant to the increase in alien species,
37 average native species richness and abundance declined by 26% and 50% respectively. We
38 identified increases in temperature, precipitation, abundance and richness of alien fish driving
39 compositional changes after 2010. To get more insights on the impacts of alien species on fish
40 communities, we used 12 biological and 13 ecological traits to compute four trait metrics each.
41 Ecological trait dispersion increased before 2010, probably due to diminishing ecologically
42 similar native species. No changes in trait metrics were measured after 2010, albeit relative
43 shares of expressed trait modalities significantly changing. The observed shift in trait
44 modalities suggested the introduction of new species carrying similar and novel trait
45 modalities. Our results revealed significant changes in taxonomic and trait compositions
46 following alien fish introductions and climatic change. To conclude, our analyses show
47 taxonomic and functional changes in the Rhine river over 35 years, likely indicative of future
48 changes in ecosystem services.

49

50 **Keywords:** *Rhine river, freshwater fish, long-term study, alien species, functional ecology,*
51 *traits*

52 **Introduction**

53 Extinction and speciation processes constitute the natural renewal of biodiversity (Johnson *et al.*, 2017). At global scales, the growing extent of human impacts on ecosystems worldwide, 54 including changes in climatic conditions and land-use, overexploitation of resources, or 55 introduction of alien species (Sala *et al.*, 2000; Cowx & Collares-Pereira, 2002), increases the 56 frequency of occurrence of these processes. At regional scales, the man-made alterations of 57 habitat quality greatly favour the increase of extinction rates, and cause a staggering decline in 58 biodiversity (Dirzo *et al.*, 2014; Johnson *et al.*, 2017; IPBES, 2019).

60 Among the above-mentioned drivers of biodiversity decline, biological invasions play 61 a central role, as growing numbers of alien species are introduced (deliberately or accidentally) 62 beyond their native range (Blackburn *et al.*, 2014; Turbelin *et al.*, 2017; Seebens *et al.*, 2018). 63 Once successfully established, some alien species can proliferate locally before spreading 64 geographically, and colonising new habitats, causing ecological and socio-economic impacts 65 on recipient ecosystems (Arim *et al.*, 2006). Invasive alien species can threaten biodiversity at 66 all organisational scales, in all regions, ecosystems, or habitats (Bellard *et al.*, 2016; Mollot *et al.*, 2017; Blackburn *et al.*, 2019; Pilotto *et al.*, 2020; Pyšek *et al.*, 2020). Biodiversity from 67 freshwater ecosystems is no exception: in addition to being critically affected by anthropogenic 68 activities (*e.g.* change in land use, climatic changes), environmental stressors can magnify the 69 deleterious impacts of alien species, potentially leading to species extinction and to the loss of 70 ecosystem functions (Sala *et al.*, 2000; Gherardi & Acquistapace, 2007; Villéger *et al.*, 2011; 71 Haubrock *et al.*, 2020).

73 Invasive alien fishes have been known to modify aquatic ecosystems (*e.g.* by altering 74 community structure and the trophic web) and displace native species through competition 75 and/or predation (Cambray, 2003; Gherardi *et al.*, 2011; Haubrock *et al.*, 2020, 2022). These 76 impacts have been recently monetised, and economic losses incurred by alien fishes were

77 reported as being mainly related to damages, resource losses, and impaired social welfare
78 (Haubrock *et al.*, 2022). Alien fish species can also negatively affect taxonomic diversity
79 (Cambray, 2003; Gallardo *et al.*, 2016), with potential consequences for the functional structure
80 of the community. In these circumstances, the loss of native species can result in the
81 concomitant losses of certain traits, whereas new species are prone to introduce “novel” traits
82 or even replace those lost (Nock *et al.*, 2016; Renault *et al.*, 2022).

83 Information on ecological preferences and biological (*i.e.* functional) traits (later on
84 referred to as simply ecological and biological traits) are characteristic to each individual or
85 species, determining its biology and functioning within a community, as well as shaping the
86 ecological space it occupies. Traits are often considered plastic, in several cases slowly
87 changing either by developmental acclimation or by on-time adjustments, allowing their
88 constant optimisation or the maintenance of biological performance, even when environmental
89 factors vary qualitatively or quantitatively (Hooper *et al.*, 2005; Mason *et al.*, 2005; Nock *et*
90 *al.*, 2016). In invaded habitats, the loss of individuals and species can be associated with the
91 loss of traits, and this can have profound impacts on community structure and functioning when
92 unique and/or specialised traits are lost (Clavel *et al.*, 2011; Dias *et al.*, 2021; Pilotto *et al.*,
93 2022). In several instances, taxonomic and functional changes induced by alien species, and
94 the consecutive loss of native species, have resulted in the homogenisation of freshwater
95 communities. Indeed, ~40% of European fish assemblages that experienced taxonomic
96 differentiation were also functionally homogenised (Olden & Rooney, 2006; Villéger *et al.*,
97 2011, 2014; Haubrock *et al.*, 2020). Functional homogenisation of all local communities within
98 a region (*e.g.* metacommunities) can increase vulnerability to large-scale environmental events
99 by synchronising local biological responses across individual communities (Olden *et al.*, 2004;
100 Clavel *et al.*, 2011; Villéger *et al.*, 2014). Although some taxonomic and functional changes –
101 commonly described with a series of trait metrics (see Baker *et al.*, 2021) – induced by alien

102 fish are known to occur in European lakes (Zhao *et al.*, 2019), not much is known about riverine
103 ecosystems. Furthermore, changing climatic or environmental conditions can induce shifts in
104 the establishment rates and concomitantly abundance of alien fish (Strayer *et al.*, 2017). This
105 also induces functional changes—an aspect pertaining to the compositional and functional
106 dimension of biological invasions that has not been studied in depth (but see Crozier &
107 Hutchings, 2014).

108 The Rhine river, one of the most heavily anthropogenically impacted freshwater rivers
109 in Europe with a total length of 1,250 km (Tittizer, 1997; Van der Velde *et al.*, 2000; Uehlinger
110 *et al.*, 2009), provides numerous services such as transportation, power generation and drinking
111 water (Cioc, 2002; Uehlinger *et al.*, 2009). The opening of the Rhine-Main-Danube canal in
112 1992 links the Rhine catchment via the Main tributary with the Danube river, and serves as a
113 major pathway facilitating the spread of alien species (Tittizer, 1997; Bij de Vaate *et al.*, 2002;
114 Balzani *et al.*, 2022). However, while information on the taxonomic identity of alien species
115 present in highly anthropogenically altered ecosystems like the Rhine river is available, not
116 much is known about the changes in the community and trait composition that have occurred
117 as a response to these introductions (Nehring & Klingenstein, 2008; Rabitsch *et al.*, 2013), nor
118 how environmental changes over time drove these.

119 In the current era of globalisation, ongoing climatic changes, and an increased interest
120 in the protection of natural resources, long-term studies have become increasingly necessary to
121 provide a better understanding of introduction processes, reconstruct introduction events, and
122 community dynamics (Franklin, 1989; Hooper *et al.*, 2005; Strayer *et al.*, 2006). Yet, long-
123 term data has rarely been used to disentangle the effects of alien species on the taxonomic and
124 trait diversity of fish communities, nor to investigate effects of climatic or environmental
125 changes (but see Guareschi *et al.*, 2021). Using long-term biomonitoring data from the Rhine
126 river in western Germany (1984–2018), we investigated changes in the taxonomic and trait

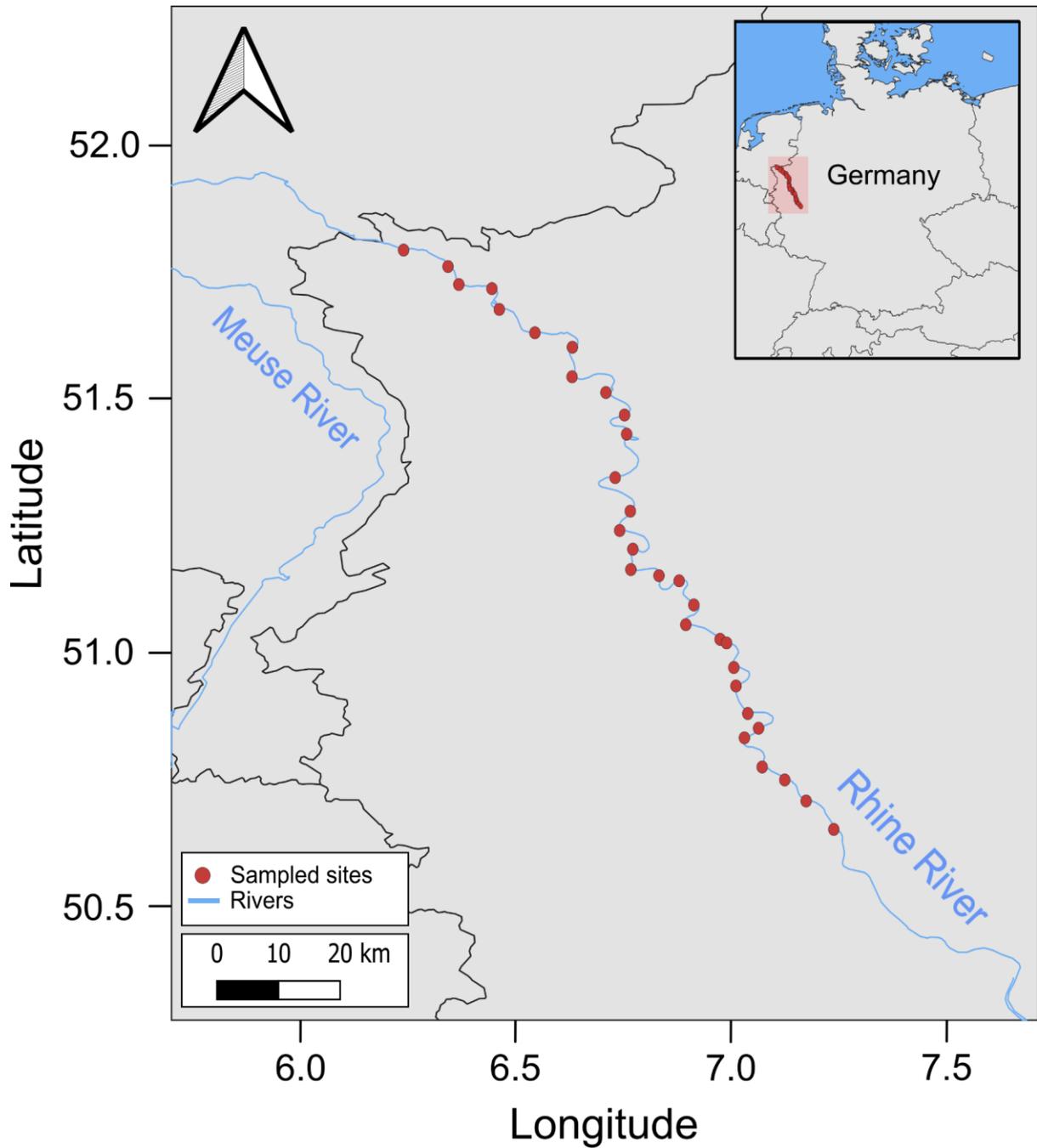
127 composition in invaded fish communities over time. To this end, we specifically investigated
128 if changes in the communities' taxonomic and trait composition were driven by disappearances
129 of native fishes or by replacements by alien fishes as well as climatic changes over time and
130 consequently, if identifiable changes were indicative of taxonomic and functional (*i.e.* trait)
131 homogenisation and subsequently, responding to environmental changes. We tested the
132 following hypotheses: (i) the increase in alien fish abundance will be at the expense of native
133 species, resulting in a decrease in the overall native communities' abundance, (ii) the arrival
134 and establishment of alien fishes will introduce novel functional traits, reshaping the ecological
135 space occupied by the community, (iii) changes in environmental parameters, and more
136 particularly climatic factors, will drive compositional changes.

137

138 **Methods**

139 *Data collection*

140 To investigate the changes in composition of freshwater fish communities over spatiotemporal
141 scales, we used a time series of fish abundance from the Rhine river in western Germany. Fish
142 samplings were conducted in the Rhine river between 1984–2018, although not continuously
143 (Table S1), over different seasons (*i.e.* spring and summer) and from 31 sites. In total, the
144 samplings covered ~170 km of the Rhine river from the border to the Netherlands upstream
145 (6°12'55''N–7°11'55''N; 50°39'49''E–51°49'46''E; Figure 1). Fish were collected at each
146 site following a standardised protocol by bank fishing from the right river bank through
147 continuous electrofishing. Over the study period, 403 samplings were conducted, capturing a
148 total of 43,888 individuals identified at the species level. For each fish species, abundance (*i.e.*
149 number of individuals per sampling) data were averaged across sites for each year.



150

151 **Figure 1.** Representation of 31 fish sampling sites along the Rhine river (Germany).

152

153 *Fish biological and ecological traits*

154 The sampled fish species were characterised with traits extracted from three resources:

155 *www.freshwaterecology.info* (Schmidt-Kloiber & Hering, 2015), *www.fishbase.org* (Froese &

156 Pauly, 2010) and *scholar.google.com* (Google Scholar sources). When trait data were missing

157 at the species level, information was retrieved from the genus. We retained a total of 25 traits

158 split into: (i) 13 ecological traits, sub-divided into 43 ‘modalities’ (*e.g.* if the considered
159 ecological trait is ‘habitat’, the different modalities correspond to the diversity of habitats which
160 the species can occupy; Table S2) describing the environmental tolerance of species, and (ii)
161 12 biological traits with 37 modalities reflecting characteristics of species (Table S2; Devin &
162 Beisel, 2007).

163

164 *Community metrics*

165 To investigate changes in the composition of the fish community over the investigated period
166 (1984–2018), five commonly used taxonomic metrics were computed for each site and year:
167 (i) total abundance, (ii) species richness, (iii) Shannon diversity, (iv) Pielou evenness and (v)
168 temporal species turnover (Table S3; Baranov *et al.*, 2020). Taxonomic metrics were computed
169 using the R package ‘vegan’ (Oksanen *et al.*, 2020). The turnover of overall communities,
170 native communities and alien communities were calculated using the *turnover* function
171 implemented in the R package ‘codyn’ (Hallett *et al.*, 2020). In addition, we calculated the
172 beta-diversity (*i.e.* as a measure of the ratio between regional and local species diversity) across
173 all sites sampled within each year as a proxy for taxonomic homogenisation using
174 *beta.div.comp* function implemented in the R package ‘adespatial’ (Dray *et al.*, 2022).

175

176 *Trait metrics*

177 To investigate trait changes in fish communities over time, all trait modalities (*i.e.* ecological
178 and biological) were ‘fuzzy coded’ with the *prep.fuzzy.var.function* following Chevenet *et al.*
179 (1994) using the R package ‘ade4’ (Dray & Dufour, 2007). The fuzzy coding procedure
180 indicates to which extent a taxon exhibits each trait category by proportionally scaling them
181 between 0 and 1 (Schmera *et al.*, 2015). This is essential as it simplifies the synthesis of trait
182 information and enables the synthesis of diverse kinds of biological information (Baker *et al.*,

183 2021). Then, four trait metrics: trait richness (TRic), trait divergence (TDiv), trait dispersion
184 (TDis) and trait evenness (TEve; Table S3; Baker *et al.*, 2021; Renault *et al.*, 2022) were
185 calculated using the *alpha.fd.multidim* function from the R package ‘mFD’ (Magneville *et al.*,
186 2022). Trait metrics were computed for ecological and biological traits separately.

187

188 *Abiotic and biotic predictors*

189 Site-specific characteristics were used to investigate the spatiotemporal changes in fish
190 communities. To assess the effects of climatic variables, we extracted the average daily
191 temperature and precipitation of the 12 months before the sampling event from a gridded
192 European-scale observation-based dataset for each year and site (spatial resolution: 0.1 °C;
193 www.ecad.eu/E-OBS/; Cornes *et al.*, 2018). Lastly, we estimated the richness and abundance
194 of alien species in each year of each time series as biotic predictors to investigate their effects
195 on the community.

196

197 *Statistical analyses*

198 To investigate changes in taxonomic and trait composition of communities over time, canonical
199 analysis of principal coordinates (CAPs) were performed based on the Bray-Curtis dissimilarity
200 index (Bray & Curtis, 1957). The CAPs were based on (i) species abundance and the
201 community weighted means (CWM, *i.e.* the mean value for each trait for the whole community
202 weighted by the abundance of the species carrying the trait) of (ii) ecological and (iii) biological
203 traits for each year. Further, permutational multivariate analysis of variance (PERMANOVA,
204 permutations = 9,999) based on the Bray-Curtis dissimilarity index were performed using
205 *adonis2* function of the R package ‘vegan’ (Oksanen *et al.*, 2020) on the species abundance
206 matrix to identify the significance of abiotic (*i.e.* latitude, temperature, precipitation) and biotic
207 predictors (*i.e.* alien species abundance and richness) and then the significance of ecological

208 and biological trait modalities. Then, two PERMANOVAs (Bray-Curtis dissimilarity index,
209 permutations = 9,999) were separately performed on the matrices of the ecological and
210 biological traits CWM to identify the significance of abiotic and biotic predictors.

211 Finally, taxonomic and trait metrics as well as climatic variables were modelled as the
212 response variables over time. In addition, we identified periods in which community and trait-
213 based metrics significantly changed over time by fitting an autoregressive AR (1) process
214 (Nathan *et al.*, 1999) for the residuals enabled the computation of derivatives of fitted splines
215 using the method of finite differences to estimate the rate of change (slope) in the fitted
216 smoother. This produced diagnostic plots of the time series with which periods of significant
217 changes were identified and superimposed within the respective time series upon the respective
218 trends over time (Simpson, 2018). Based on the results from the autoregressive models we
219 identified significant changes to have occurred in ~2010. Accordingly, we splitted the study
220 period into two (1984–2010: 25 years; 2010–2018: 8 years; Figure S1) to identify the effects
221 of alien fishes in the community. Taxonomic and trait metrics were modelled using the gam
222 function from the R package ‘mgcv’ (Wood, 2021). All metrics were modelled using a negative
223 binomial distribution except for species abundance and richness, modelled using a poisson and
224 gaussian distribution respectively. Hence, each model was composed of the specific response
225 variable (*i.e.* taxonomic, biological and ecological trait metrics) and the explanatory variables:
226 ‘year’, ‘latitude’, ‘temperature’ and ‘precipitation’. Considering non-linear trends over time,
227 our models included a cubic regression smooth spline for the predictor “year” (Wood, 2006)
228 and a smoothed spline for abiotic predictors.

229 All analyses were conducted in the R environment, version 4.1.1 (R Core Team, 2021).
230 The level of significance was set at $\alpha < 0.05$.

231 **Results**

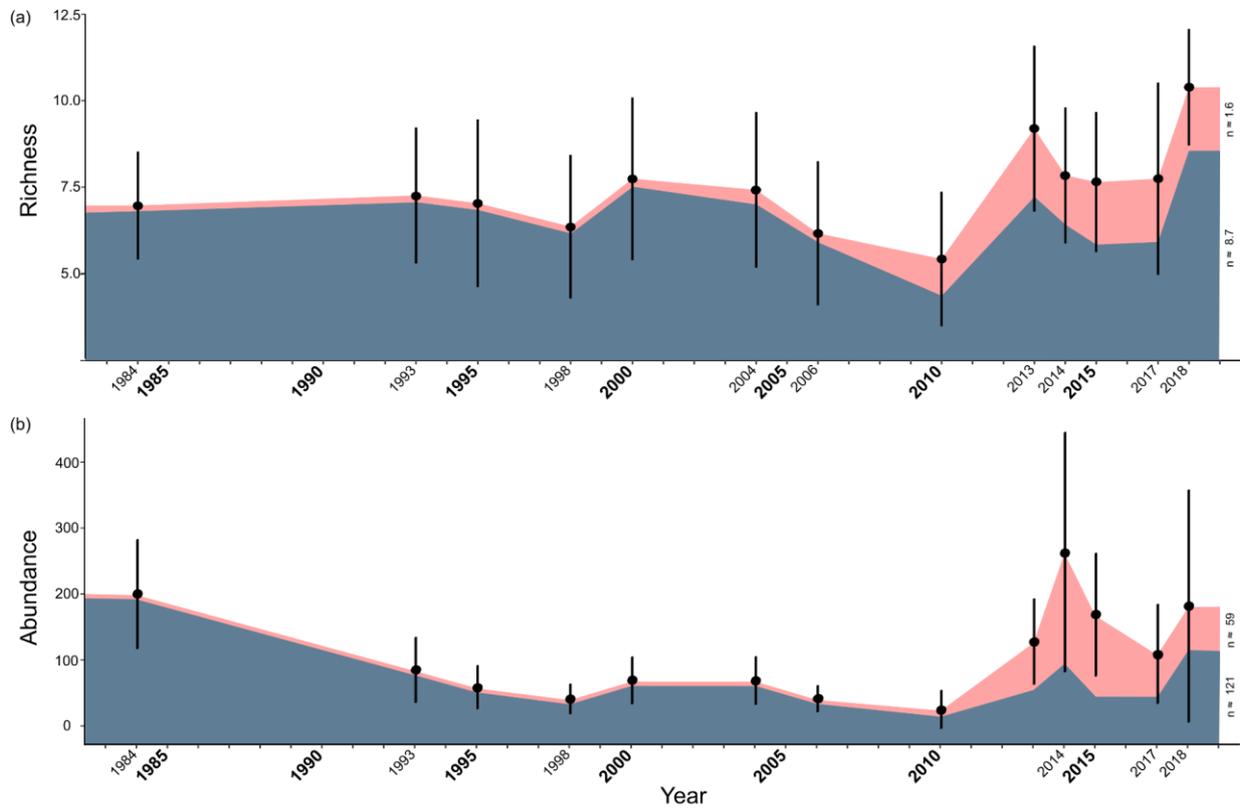
232 Over the 35 years of this study, 43 fish species (32 native and 11 alien species) were caught in
233 the lower part of the Rhine river (Table S4). The Cyprinidae was the most diverse family in
234 terms of species (20 out of the 43 sampled species), followed by Salmonidae (4 species),
235 Gobiidae (4 species) and Percidae (3 species). The 12 remaining families were represented by
236 a single species (Table S4; Figure S2). Over the studied period, the average annual temperature
237 and precipitation increased by ~ 0.94 °C (~ 0.03 °C year⁻¹) and ~ 0.26 mm (~ 0.008 mm year⁻¹),
238 respectively. While temperature fluctuated throughout the period, peaking in 2006,
239 precipitation steeply inclined after 2010 (Figure S3).

240

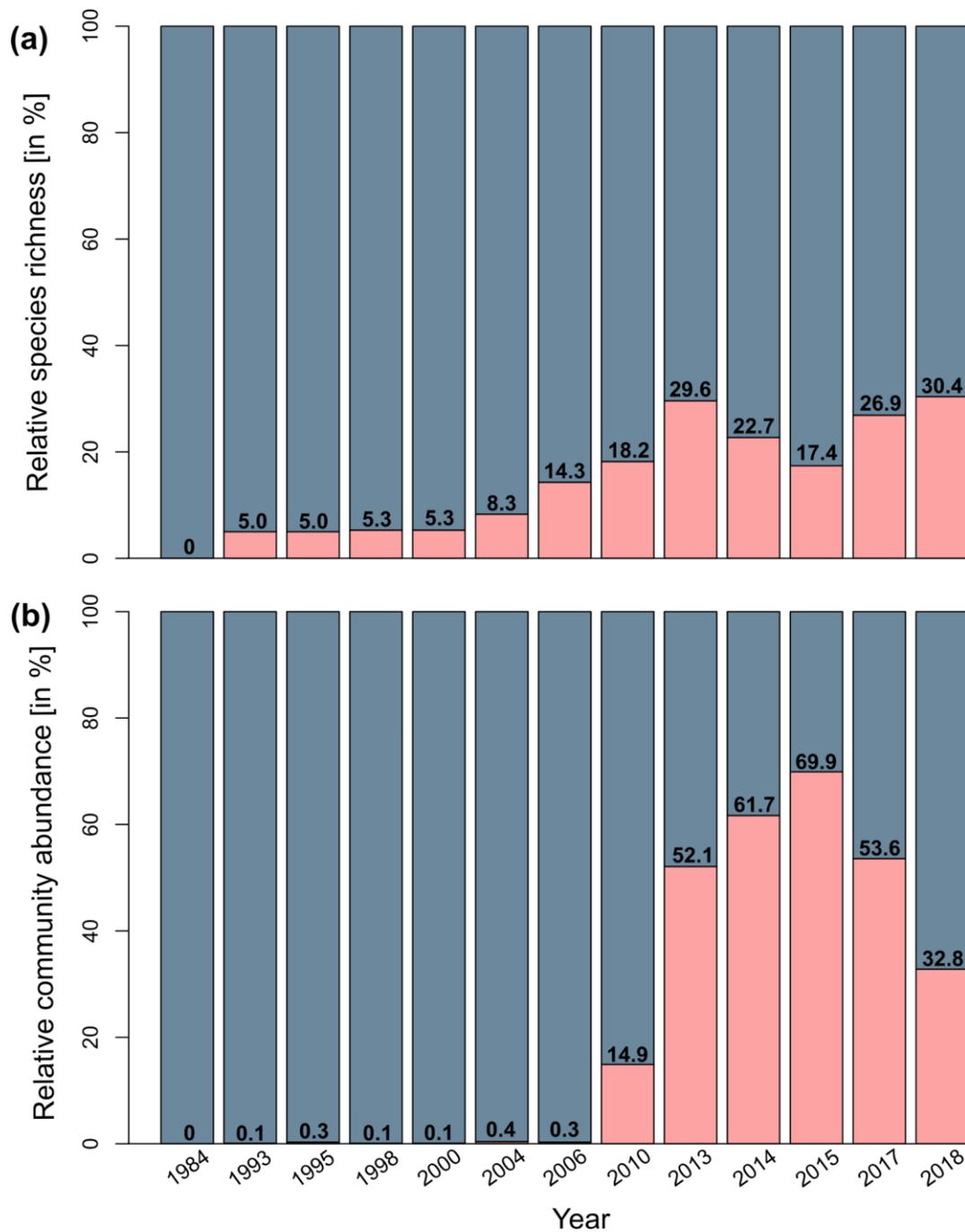
241 *Compositional changes in communities over time*

242 Between 1984 and 2018, total community richness increased by 49%, albeit declining between
243 2004 and 2010 before increasing again until 2018 (Figure 2a). Starting from 198 individuals/m²
244 in 1984, the overall community abundance largely declined to 23 individuals/m² in 2010 (-
245 88%), and then consequently increased by 678% up to 180 individuals/m² until 2018 (Figure
246 2b). Despite the slow increase at the beginning of the studied period, the richness and
247 abundance of alien species considerably increased after 2010 (Figure 2) and peaked in 2013
248 with a maximum of 8 alien species and again in 2018 with 7 alien species. The common carp
249 *Cyprinus carpio* (first recorded in 1993), the rainbow trout *Oncorhynchus mykiss* (first
250 recorded in 1998) and the European catfish *Silurus glanis* (first recorded in 2004) were the first
251 alien species that were detected and successfully established in the studied stretch of the Rhine
252 river (Table S4). While *C. carpio*, *O. mykiss*, and *S. glanis* represented 5.0–8.3% of the total
253 species richness before 2006 (Figure 3a), their abundance remained negligible (0.1–0.4%;
254 Figure 3b). In 2010, the abundance of five identified alien fish species increased to 14.9%. In
255 the period 2010-2013, six novel alien fish species established in the river: the pumpkinseed

256 *Lepomis gibbosus*, the monkey goby *Neogobius fluviatilis*, the bighead goby *Ponticola kessleri*,
 257 the round goby *N. melanostomus*, the topmouth gudgeon *Pseudorasbora parva*, and the
 258 European bitterling *Rhodeus amarus* (Table S4), constituting a total of 29.6% of the total
 259 species richness and 52.1% of the total communities abundance in 2013 (Figure 3).



260
 261 **Figure 2.** (a) Total species richness and (b) total community abundance of the Rhine river fish community from 1984 to 2018
 262 indicating the average values \pm the respective standard deviation for each sampled year and the respective share of native
 263 (blue) and alien species (red). Numbers on the right indicate the (a) total species richness and the (b) total community
 264 abundance of native and alien fish in 2018.



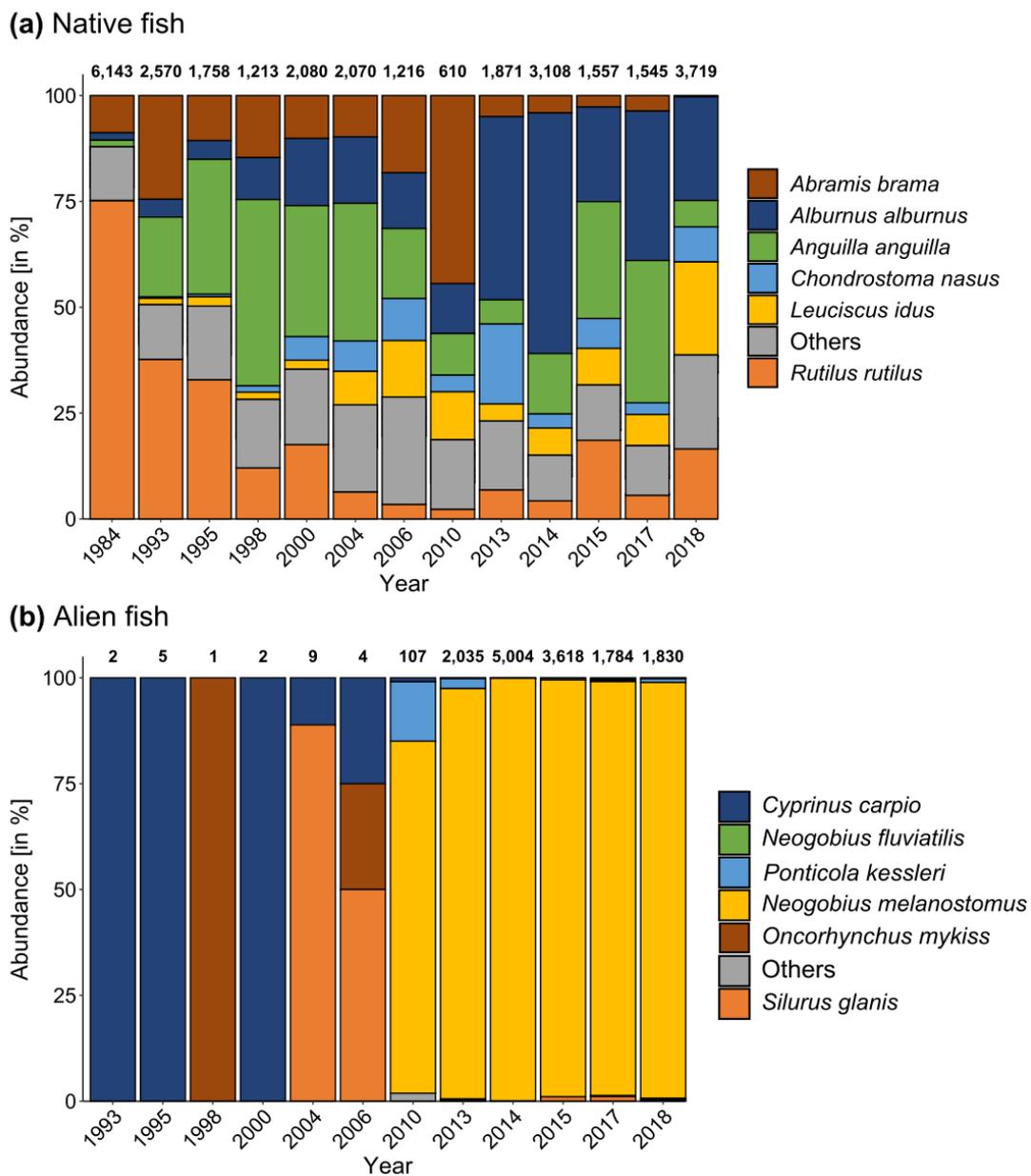
265

266 **Figure 3.** (a) Relative species richness and (b) relative community abundance of native (in blue) and alien (in red) fish species
 267 of the Rhine river from 1984 to 2018.

268

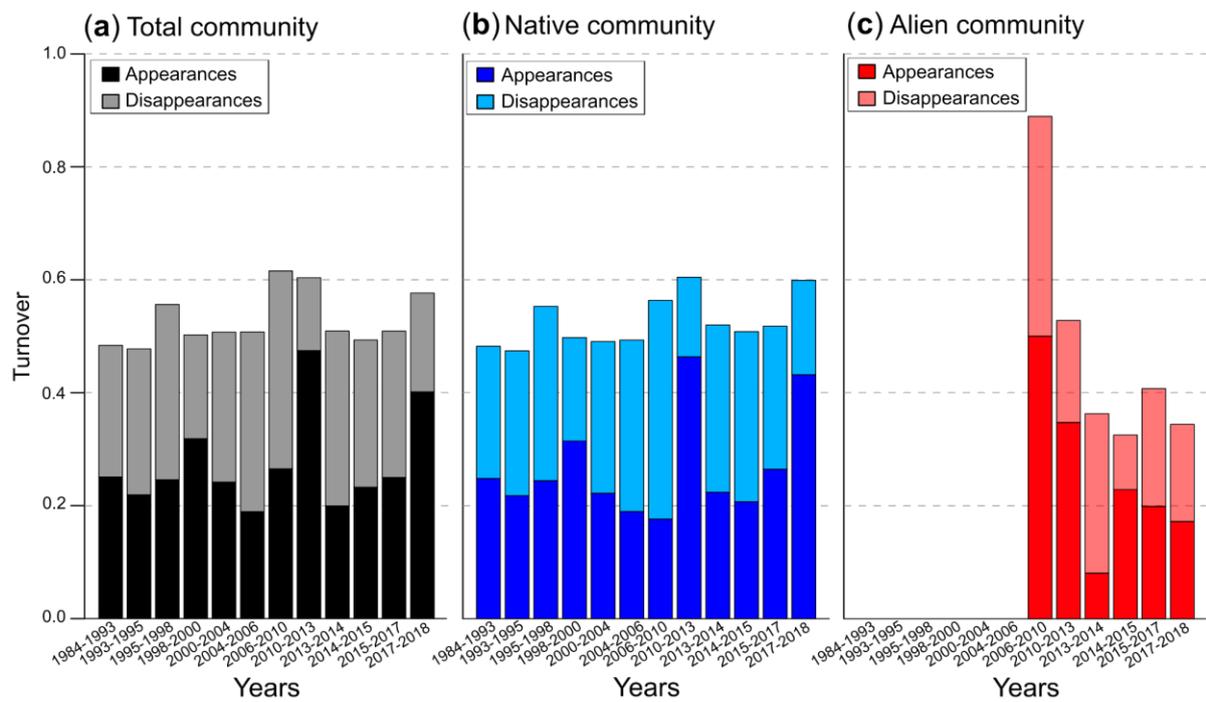
269 The establishment of the six novel species increased alien species richness, which
 270 peaked in 2018 (30.4%; Figure 3a), alongside with their abundance, totalling 69.9% in 2015
 271 (Figure 3b). Considering solely the alien community, species from the genus *Neogobius* sp.
 272 were first detected in 2010. In this genus, *N. melanostomus* dominated the alien fish community

273 in 2018 (98.25% of the total alien communities abundance, Figure 4b). For the native fish
 274 communities, the roach *Rutilus rutilus* was the most abundant species at the beginning of the
 275 study period (75.14%, Figure 4a) and declined sharply in 2010 (2.30%). The bleak *Alburnus*
 276 *alburnus*, which represented 1.76% of the total native community abundance in 1984, and the
 277 ide *Leuciscus idus*, 1.52% in 1993, became more abundant in 2018, respectively occupying
 278 24.47% and 22.00% (Figure 4a).



279
 280 **Figure 4.** Relative abundance of the six most abundant (a) native and (b) alien fishes during the study period. Number on top
 281 of each bar indicates the total abundance of all (a) native and (b) alien species collected in that respective year.

282 The communities' beta-diversity increased until 2010 before decreasing towards 2018
 283 (Figure S4). The total community turnover remained constant over time (Figure 5a), with the
 284 turnover of the native fish communities slightly increasing in recent years from 0.48 to 0.60,
 285 albeit being non-significant ($R^2 = 0.135$; $p = 0.238$; Figure 5b). The turnover of the alien fish
 286 community decreased significantly after 2006, from 0.89 to 0.34 ($R^2 = 0.210$; $p < 0.01$; Figure
 287 5c).

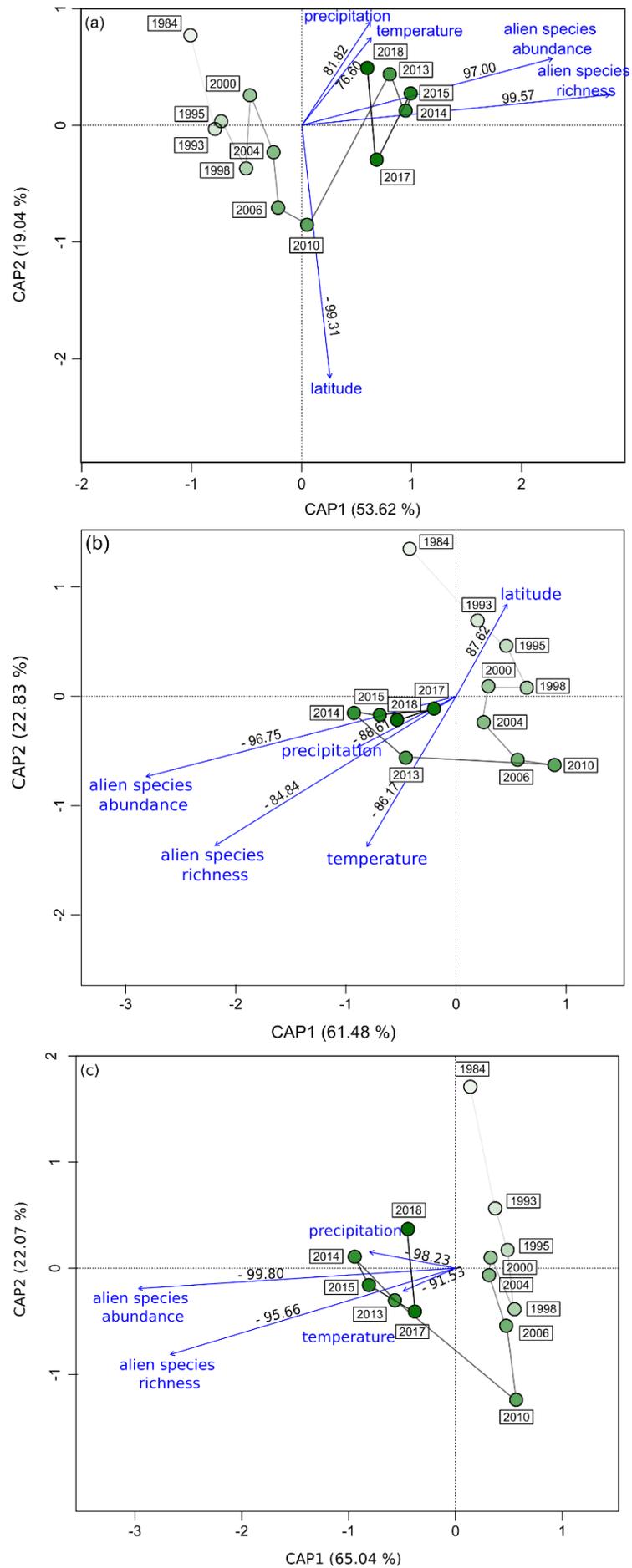


288
 289 **Figure 5.** Temporal turnover rate displaying appearances and disappearances of fish species in the Rhine river from 1984 to
 290 2018. (a) Overall turnover in the whole community. (b) Turnover for native fishes. (c) Turnover for alien fishes. Please note
 291 that there was no detectable turnover in the alien community before 2006 despite the presence of several, continuously present
 292 alien fish species.

293

294 The applied ordination suggested a clear segregation in the community's taxonomic
 295 composition, with years after 2010 characterised by a significant increase in alien species
 296 richness and abundance, as well as changes in temperature and precipitation, as confirmed by
 297 the applied PERMANOVA (Figure 6a; Table S5). Moreover, the applied PERMANOVA
 298 identified all community weighted means CWM (both ecological and biological) to

299 significantly vary over time (Table S6), as within each trait, modalities of both ecological
300 (Figure S5) and biological traits (Figure S6) changed in their relative prevalence, showing
301 notable differences in their respective proportion before and after 2010. After 2010, the
302 community was dominated by species characterised by smaller adult fishes and larvae, shorter
303 life span, moderate relative fecundity, bigger eggs, and provision of parental care (Figure S6;
304 Table S6). Regarding ecological traits, the post-2010 community was characterised by fishes
305 preferring rheophilic conditions in a demersal habitat, and feeding in benthic habitats (Figure
306 S5; Table S6). A comparable pattern was identified for the CWMs of ecological and biological
307 trait compositions (Figure 6b,c), as confirmed by the PERMANOVA (Table S7). Changes after
308 2010 were significantly related to an increase in alien species' richness and abundance as well
309 as increases in temperature and precipitation (Figure S3).



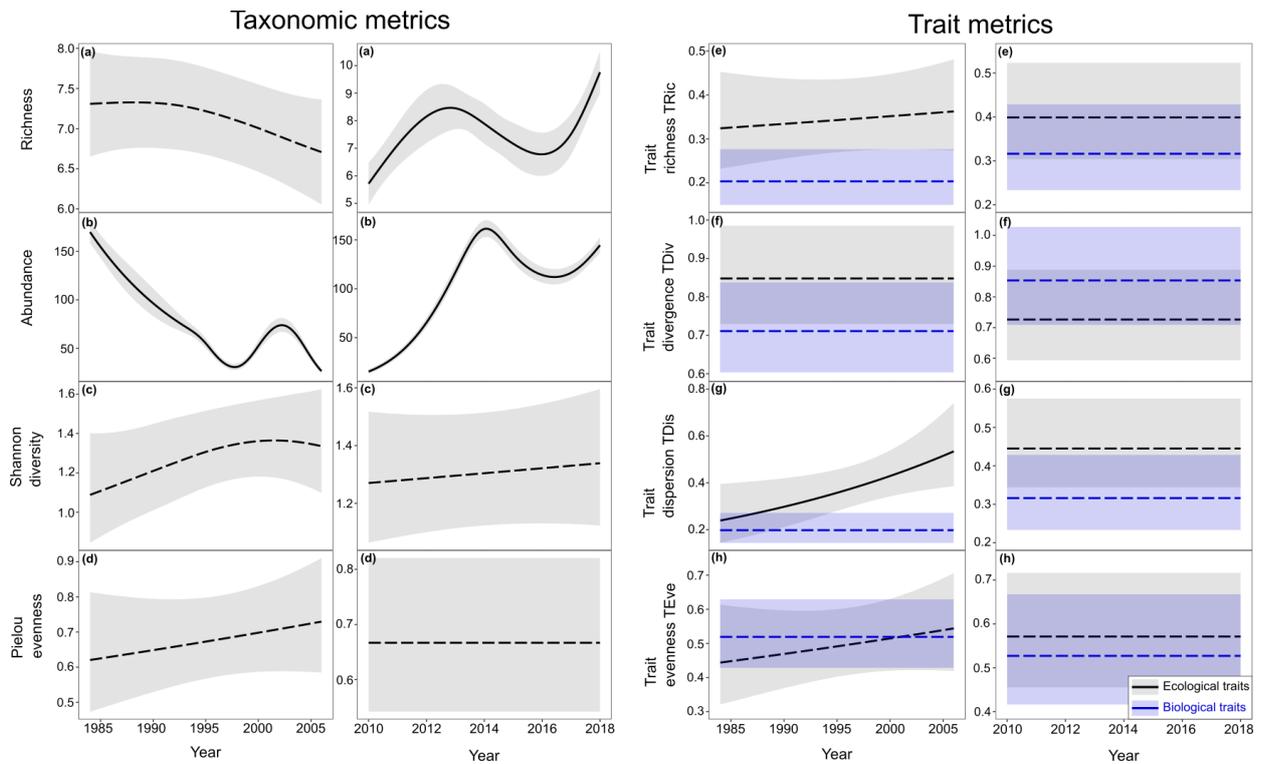
311 **Figure 6.** Canonical analysis of principal coordinates (CAP) on (a) the taxonomic community composition (based on species
312 abundances), the community weighted means (CWMs) of (b) ecological and (c) biological traits across years. Blue arrows
313 indicate significant abiotic and biotic predictors. Values along the arrows indicate the correlation with the dominant axis.

314

315 *Temporal patterns in the community taxonomic and trait metrics*

316 Without separating the time series into two distinct periods, we identified a fluctuation in
317 richness (*i.e.* a low around the year 2010 encompassed by two periods of high species richness),
318 a decline in abundance, and an incline in Shannon diversity as well as inclines in both
319 ecological and biological trait dispersion ($p < 0.05$; Figure S7). Between 1984 and 2010,
320 species richness did not change significantly (Figure 7a; Table S8), whereas species abundance
321 significantly decreased from on average 198 (± 82) to 23 (± 28) individuals/m² ($R^2 = 0.624$; p
322 = <0.01 ; Figure 7b). Species richness and abundance were significantly positively affected by
323 latitude, temperature and precipitation (Table S8). No significant changes in Shannon diversity
324 and Pielou's evenness were detected (Figure 7c,d). With regard to trait metrics, only an increase
325 in trait dispersion (TDis) of ecological traits was detected ($R^2 = 0.292$; $p = 0.01$; Figure 7g).

326 After 2010, species richness and abundance increased significantly from approximately
327 six to ten species, and from on average 23 (± 28) to 180 (± 176) individuals/m² (richness: $R^2 =$
328 0.425; $p = <0.01$; abundance: $R^2 = 0.731$; $p = <0.01$; Figure 7a,b). No further significant change
329 was detected (Figure 7; Table S8). While species richness after 2010 was positively related
330 with latitude, the identified increase in abundance was primarily driven by the increasing
331 richness (and abundance) of alien species as well as an increase in temperature, precipitation,
332 and latitude. Changes in Shannon diversity and Pielou's evenness were only significantly
333 negatively related to the abundance of alien species (Table S8).



334

335 **Figure 7.** Generalised additive models (GAMs) exploring the relationship between (a-d) taxonomic and (e-h) trait metrics of
 336 the studied communities. Trait metrics are divided into ecological (black lines) and biological (blue lines) traits. Solid lines
 337 indicate significant change over time, whereas dashed lines represent non-significant trends. See table S8 for other significant
 338 predictors.

339

340 Discussion

341 The direct and indirect impacts of alien fishes and meteorological conditions are major drivers
 342 of biodiversity loss in freshwater ecosystems worldwide (Villéger *et al.*, 2014; Gallardo *et al.*,
 343 2016; Bellard *et al.*, 2018; Haubrock *et al.*, 2020), often leading to taxonomic homogenisation
 344 (Olden *et al.*, 2004; Pilotto *et al.*, 2020; Haubrock *et al.*, 2022). Our data, collected with a
 345 standardised protocol over the period 1984-2018, revealed significant changes in taxonomic
 346 and trait composition following the introduction of alien fishes after 2010 and concomitant
 347 changes in climatic conditions. We further identified changes in community composition
 348 following declines in the richness and to some extent also the abundance of native communities
 349 until 2010, before indicating taxonomic homogenisation, while in the case of the abundance—
 350 after alien fish species were detected to dominate in 2015—reached levels comparable to those

351 measured in the first year, while richness reached a considerably higher level. While climatic
352 changes (*i.e.* in temperature and precipitation) likely benefited some alien species reflecting
353 characteristic traits, these however did not determine the transition to a community dominated
354 by aliens as both variables expressed no period of significant change during the study period
355 (Figure S1m, n).

356

357 *Patterns in community composition before 2010*

358 Trends in the abundance and richness of alien species can shift following external changes
359 (Rahel & Olden, 2008; Früh *et al.*, 2012), and have destabilising effects on invaded
360 communities (Cardoso & Free, 2008; Strayer *et al.*, 2011). The three alien fish species already
361 present before 2010 in the Rhine river (*C. carpio*, *O. mykiss*, and *S. glanis*) are well-known for
362 their substantial impacts on ecosystems and native species (*e.g.* through habitat degradation,
363 competition, predation and the spread of diseases; Cambray, 2003; Copp *et al.*, 2009; Gherardi
364 *et al.*, 2011; Guillerault *et al.*, 2015). Yet, their abundances remained at relatively low levels
365 (<0.5% of the overall community abundance), whereas these species can reach considerably
366 high biomasses with detectable impacts (Driver, 2005). The overall and significant decline in
367 native species abundances by -90% is however unlikely to have occurred due to their presence
368 (Copp *et al.*, 2009). Consistent with this assumption, several studies have reported that the
369 impacts of poorly abundant alien species on communities are often undetectable or absent
370 (Parker *et al.*, 1999; Ricciardi, 2003; Thiele *et al.*, 2010). Rather, there could be additional
371 stressors not encompassed by our data, *e.g.* anthropogenic alterations in the water quality of
372 the Rhine river (Tittizer, 1997; Van der Velde *et al.*, 2000), leading to a decline in native species
373 and increasing diversity across sites. Changes in climatic conditions or the nutrient load of the
374 Rhine following improvements in the sewage treatment plants in the early 1990s (Malle, 1996)
375 have limited the availability of prey (*e.g.* zoo- and phytoplankton), and this may partially

376 explain the community composition pattern found (pers. comm. Nikola Theissen). The opening
377 of the Rhine-Main-Danube canal in 1992 likely contributed further to the observed decline in
378 native fish communities, as their composition and numbers considerably changed from 1993
379 onwards. While the direct presence of Ponto-Caspian species is unlikely, increased shipping
380 and perturbation have likely contributed to the identified declines.

381

382 *Patterns in community composition after 2010*

383 In the Rhine river, the arrival of new alien species was likely linked to the opening of the Rhine-
384 Main-Danube canal in 1992 (Tittizer, 1997; Bij de Vaate *et al.*, 2002). Indeed, three out of the
385 six alien fish species (*N. fluviatilis*, *P. kessleri* and *N. melanostomus*) are native to the Ponto-
386 Caspian region, and it took 18 years before they arrived and established (Kottelat & Freyhof,
387 2007; Froese & Pauly, 2010). The introduction of other alien fishes (*L. gibbosus*, *P. parva* and
388 *R. amarus*) is most likely linked to intensified aquaculture and angling (Kottelat, 2006; Kottelat
389 & Freyhof, 2007; Froese & Pauly, 2010). It is, therefore, not surprising that our results suggest
390 compositional changes (driven by the spatiotemporal spread of highly abundant Ponto-Caspian
391 fish species) to have progressed along a latitudinal gradient. This finding however highlights
392 the effectiveness of riverine ecosystems to facilitate the spread of alien species through the
393 increase of propagule pressure (Lockwood *et al.*, 2005; Cassey *et al.*, 2018). Furthermore, the
394 introduction of these alien species after 2010 led to profound changes in the taxonomic
395 composition and homogenisation of invaded communities. While this is in line with previous
396 studies (Van der Veer & Nentwig, 2015; Olden & Rooney, 2006), the overall communities'
397 turnover remained stable due to a slight increase in the native species' turnover, and a decrease
398 in alien species' turnover after 2010, depicting the process of alien species reshaping invaded
399 communities.

400 After 2010, the richness of alien fish increased to ~30% of the overall community
401 richness, constituting up to 70% of the overall abundance (an increase in the overall
402 communities abundance from 2010 by +1,031% in 2014 or +678% in 2018) before declining
403 by ~33% and possibly stabilising at lower abundance levels in 2018, resembling typical invader
404 boom-bust dynamics (Strayer *et al.*, 2017; Soto *et al.*, *in press*). Compositional changes have
405 often been observed in the invasion of freshwater ecosystems (Clavero & García-Berthou,
406 2006; Gallardo *et al.*, 2016; Haubrock *et al.*, 2020). In our study, the observed changes can be
407 ascribed to the establishment of six additional alien species between 2010 and 2013, with *L.*
408 *gibbosus*, *N. fluviatilis*, *P. kessleri*, *N. melanostomus*, *P. parva*, and *R. amarus* contributing
409 69.9% of the total community abundance in 2015. Although all of these are comparably small
410 species (≤ 20 cm), they can reach high densities and thus, biomass, accompanied by notable
411 impacts to invaded ecosystems (see e.g. Britton *et al.*, 2010; Števove & Kováč, 2016). These
412 alien species probably benefited from previous declines of native species and environmental
413 changes (Marvier *et al.*, 2004; Früh *et al.*, 2012; Heringer *et al.*, 2020), while their added
414 abundance has resulted in the overall community reaching comparable abundances to those
415 measured in 1984 – underlining the turnover from native to alien species communities
416 (Haubrock *et al.*, 2020) – suggesting that total community abundances are somewhat limited
417 due to the productivity of the ecosystem (Storch *et al.*, 2018). Indeed, as ectothermic animals,
418 fish species are sensitive to environmental changes (Magnuson *et al.*, 1979). Several studies
419 have highlighted the negative impacts of temperature increases and precipitation shifts on
420 native fish communities (Magnuson *et al.*, 1979; Marchetti & Moyle, 2001; Lamouroux &
421 Cattaneo, 2006) but also on aquatic communities in general (Durance & Ormerod, 2007;
422 Chessman, 2009; Heino *et al.*, 2009). Yet, their effects remain difficult to predict (Haase *et al.*,
423 2019; Baranov *et al.*, 2020). Concomitantly, we found temperature and precipitation to incline
424 over the studied period, consequently driving compositional changes in the investigated

425 community. Although observed increases in temperature (~ 0.94 °C) and precipitation (~ 0.26
426 mm) seem low, even minor changes – as well as fluctuations – can lead to substantial (*i.e.*
427 seasonal) changes in native communities (Magnuson *et al.*, 1979; Lamouroux & Cattaneo,
428 2006) and benefit alien species (Rahel & Olden, 2008; Radinger & García- Berthou, 2020).
429 As such, the increase in water temperature has likely permitted invasive fish species to cross
430 physiological thresholds that were formerly limiting or slowing down their biology.

431 The introduction of alien species after 2010 induced further changes in the community's
432 traits composition. These were mainly driven by the alien species *N. melanostomus*, which
433 dominated the communities' abundance since the first year it was observed by contributing up
434 to ~98% of the total abundance of alien fish in 2018. The dominance of *N. melanostomus* which
435 can severely impact invaded ecosystems (Kipp & Ricciardi, 2012), may be linked with the
436 demonstrated presence of Asian clam *Corbicula fluminea* in Rhine river (Merschel & Bau,
437 2015), used as its main food resource (Nagelkerke *et al.*, 2018; Coughlan *et al.*, 2022). Other
438 alien fish species not belonging to the *Neogobius* genus were observed with less than 100
439 individuals throughout the entire period. This dominance of *N. melanostomus* suggests that
440 comparably less abundant species occupied a less dominant role (Grabowska *et al.*, 2019).
441 However, it is possible that the sampling method applied here (*i.e.* electrofishing) was not
442 adequate to capture the total abundance, *e.g.* *C. carpio* or *S. glanis*, which tend to occupy deep
443 benthic zones. For this reason, it is possible that the abundances of these species were
444 underestimated (compared to the abundances observed by Singh *et al.*, 2010 or Šmejkal *et al.*,
445 2015).

446

447 *Patterns in trait composition*

448 Taxonomic changes in communities over time are often accompanied by trait changes (Baker
449 *et al.*, 2021; Haubrock *et al.*, 2020; Liu *et al.*, 2022). Here, we found trait changes to follow a

450 similar pattern to the shift in taxonomic composition, with the communities' trait structuring
451 driven by alien species richness and abundance, as well as changes in temperature and
452 precipitation. On the other hand, no significant changes in trait metrics were observed in
453 parallel to compositional changes. This result is peculiar, as it contrasts with other studies
454 showing alien species simultaneously leading to both taxonomic changes and functional
455 homogenisation (Pool & Olden, 2012; Villéger *et al.*, 2014; Pilotto *et al.*, 2022). However,
456 functional homogenisation may be hard to detect in the early stages of an invasion, in particular
457 during the establishment and possible lag phase, which may explain the discrepancy of our
458 results with the available literature. This additionally stresses the importance of computing
459 different diversity indices to properly prove impacts of alien invasions on recipient
460 communities.

461 In the present work, the only trait metric changing over time was trait dispersion (TDis)
462 of ecological traits before 2010. Often, TDis is expected to increase at the onset of the invasion
463 process, in particular when alien species introduce novel traits which extend trait space
464 (Renault *et al.*, 2022). Yet, this metric rose before alien species started to invade the
465 investigated communities, and rather accompanied the increase in beta-diversity. Beta-
466 diversity is widely known to increase when the studied habitats become more heterogeneous,
467 augmenting dissimilarity, and likely increasing the dispersion of species traits that cope with a
468 large diversity of environmental conditions. The demise (at least in abundance, also evidenced
469 by the temporal species turnover analysis) of ecologically similar species in the native
470 community is consistent with this assumption. This has increased trait divergence (TDiv)
471 among species from the different habitats, in addition to removing the less adapted individuals
472 (species). After 2010, TDis remained stable, even if beta-diversity declined, and confirmed the
473 maintenance of species' TDiv among habitats.

474 Other ecological and biological trait metrics were stable over time, providing
475 unexpected, yet interesting insights. The non-significant changes in trait richness indicate that
476 the demise of species carrying a certain set of traits is accompanied by acquiring new (alien)
477 species carrying new sets of trait modalities not expressed by the native community (McGeoch
478 & Jetz, 2019). Furthermore, it should be considered that due to its zoogeographic history, the
479 natural fish communities of western Europe are relatively species-poor (Kottelat & Freyhof,
480 2007). As such, it is possible that introductions of alien species, which are often functionally
481 and especially morphologically different (*e.g.* the asp *Leuciscus aspius*) as compared with other
482 fish species, lead to an initial increase in trait diversity and richness. This does not mean that
483 the demised species were functionally replaced, but rather that the losses in traits were
484 counterbalanced by the invader carrying the same or similar traits, as well as bringing new
485 traits into the community, resulting in a similarly occupied trait space. In other words, invaders
486 may have a high degree of functional identity as compared with native species, suggesting,
487 here again, that environmental filtering may have been strong and contributed to the selection
488 of particular traits.

489 The shifts in ecological and biological traits observed after 2010 were mainly driven by
490 one abundant alien species, namely *N. melanostomus*. This can explain trait metrics not
491 indicating significant changes, albeit the high abundance (and thus also biomass and density)
492 of this species likely impacting the studied sites. This lack of an identifiable trait change,
493 however, could also be caused by a high redundancy between the traits of native and alien
494 species (Loreau, 2004; Baiser & Lockwood, 2011; Villéger *et al.*, 2011) or by alien species
495 compensating for declining alien species by invading empty niches (Herbold & Moyle, 1986;
496 Liew *et al.*, 2016; Nagelkerke *et al.*, 2018). High functional redundancy has been shown to
497 increase both community resilience and ecological stability (Pillar *et al.*, 2013; Biggs *et al.*,

498 2020), and likely arises when environmental conditions are restricting (reduced phenotypic
499 variation in trait expression and characteristics). The possibility of a high functional
500 redundancy between alien and native species in the Rhine river could indicate that ecosystem
501 functions (Vilà *et al.*, 2010, 2011) were maintained despite the detected taxonomic turnover
502 and alterations in the ratio of several expressed biological and ecological traits. This further
503 suggests that alien species can, while simultaneously altering invaded habitats and recipient
504 biodiversity when becoming abundant, maintain functional roles or fill previously arisen voids.
505 By contributing to stabilise community resilience in perturbed habitats, alien species may
506 seemingly help to preserve an ecosystem's functioning in the short-term, although the long-
507 term effects remain unknown raising the controversy of the effects of alien species (Schlaepfer
508 *et al.*, 2011; Russell & Blackburn, 2017). Finally, our study advocates the need for the inclusion
509 of additional or new traits introduced with the establishment of alien fish species which are not
510 considered in *www.freshwaterecology.info* (Schmidt-Kloiber & Hering, 2015), further
511 increasing the resolution of functional changes (D'Andrea & Ostling, 2016).

512

513 **Conclusion**

514 Our results underline the importance of analysing long-term trends in invasion dynamics and
515 facilitating drivers, which has often been limited by a lack of adequate long-term data or the
516 non-continuity of annual sampling over time (Daufresne *et al.*, 2004; Durance & Ormerod,
517 2007; Comte & Grenouillet, 2013; Jourdan *et al.*, 2018; Haubrock *et al.*, 2020, 2022). This has
518 concomitantly limited insights into changing community functioning or ecological changes due
519 to changes in alien species' trait composition. This was also the case in this work, as highlighted
520 by the lack of information on the fish community between 1984 and 1993. Yet, having
521 identified a disconnect between visible responses in taxonomic composition, and no detectable
522 changes in functional metrics due to the invasion of alien species, suggests that both aspects

523 pertaining to fish invasions must be considered in conjunction and separated at the same time.
524 More specifically, taxonomic changes directly reflect biodiversity alterations, often at the
525 expense of native species, which possibly cause changes in the occurrence of trait modalities
526 and hence, potentially also functional changes, which can, but may not necessarily impact
527 ecosystem functioning.

528 Our results further lead to the question, why native species declined pre-2010 and communities
529 became more diverse, before alien species became abundant and increased taxonomic
530 homogenisation. This finding highlights the importance of considering further variables to
531 investigate the temporal effects of species introductions. These variables could reflect habitat
532 fragmentation (Cowx & Collares-Pereira, 2002), the regulation of water flow (Poff *et al.*, 1997;
533 Albert *et al.*, 2021), changes in the land use (Albert *et al.*, 2021), pollution (Matthews *et al.*,
534 1992; Taylor *et al.*, 1993; Lappalainen & Soininen, 2006), the pressure of fishing (Cowx &
535 Collares-Pereira, 2002; Butchart *et al.*, 2010) – predictors which were not adequately available
536 over the entire period to be considered in our models.

537

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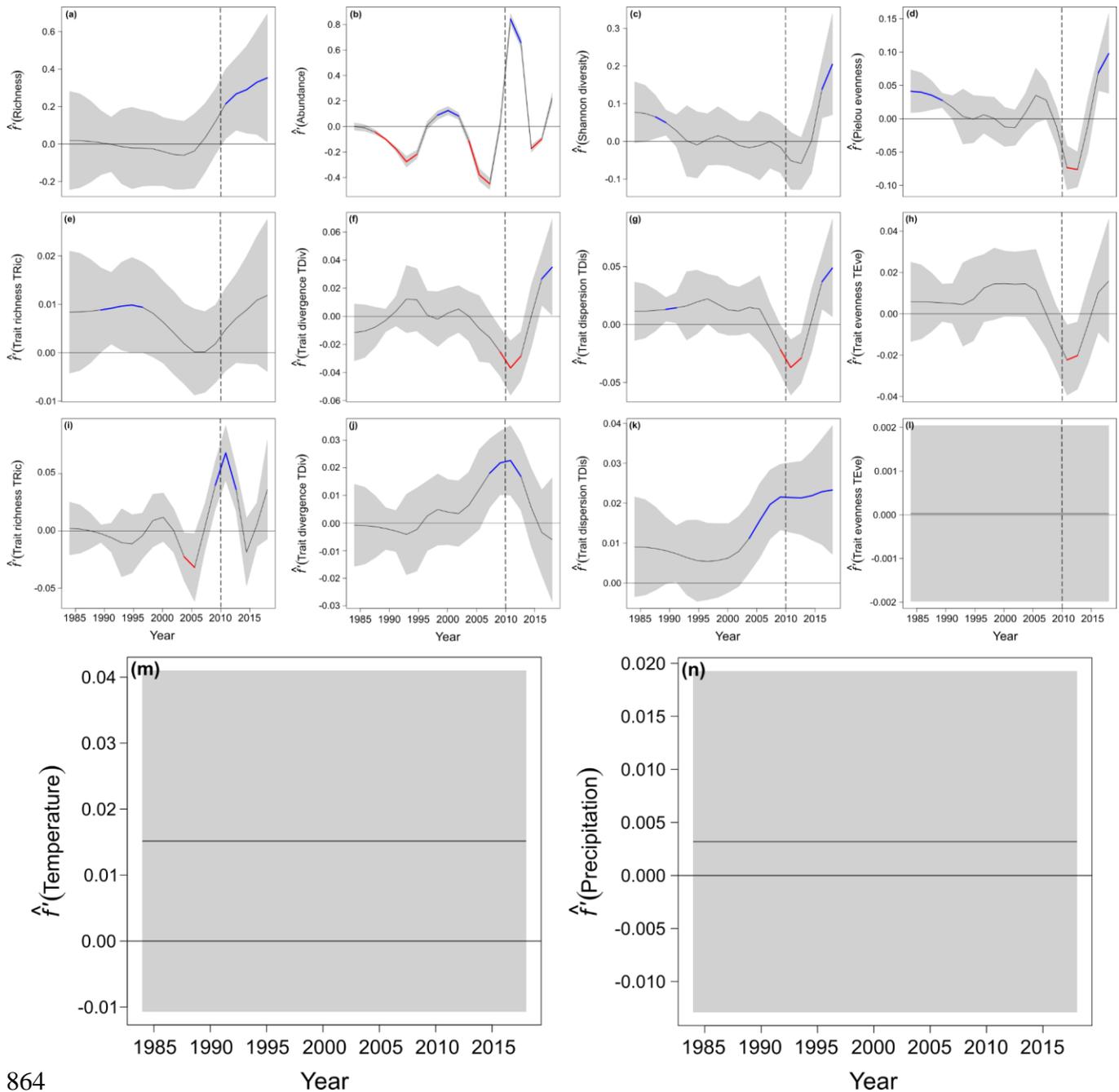
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861 **Supplements**

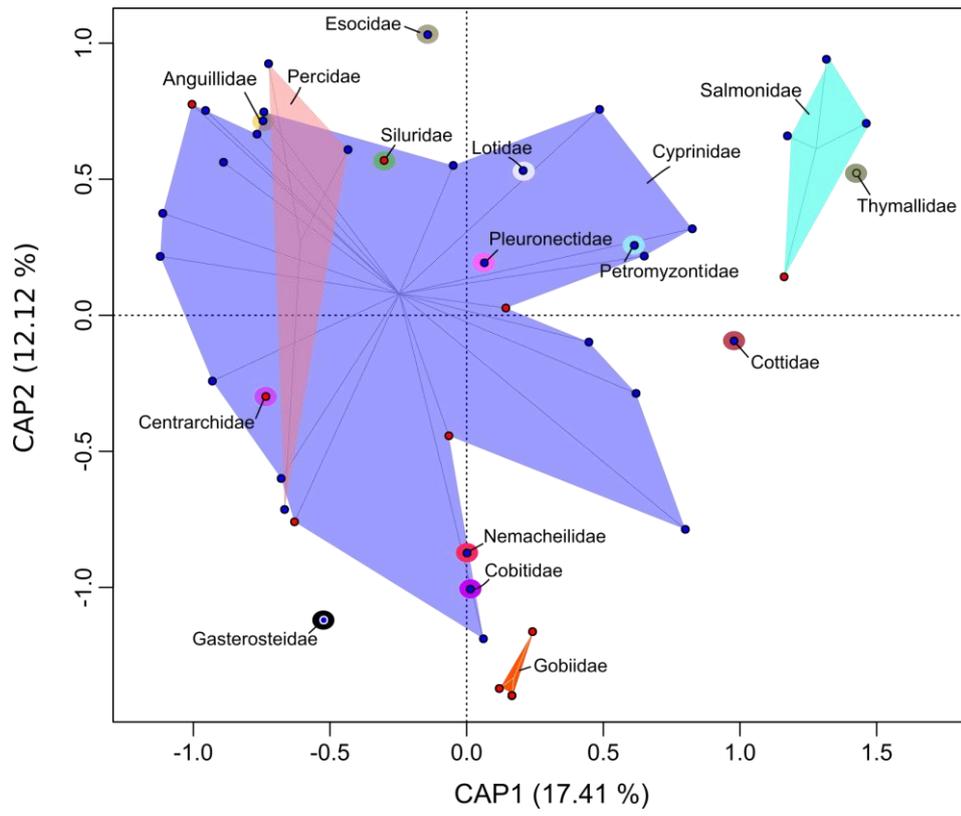
862 **Figure S1.** Diagnostic plots of the time series models concerning changes in (a-d) taxonomic metrics, (e-h) ecological traits

863 metrics, (i-l) biological trait metrics and (m-n) temperature and precipitation.



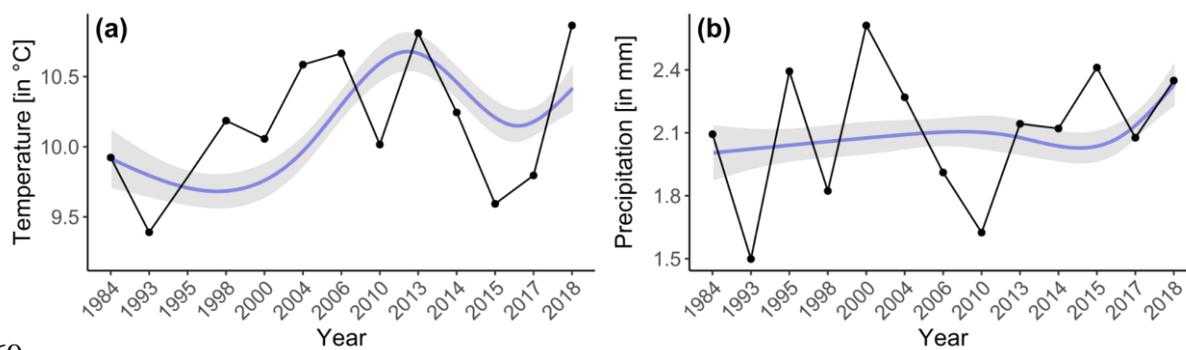
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865 **Figure S2.** Distribution of species in trait space based on both biological and ecological traits.



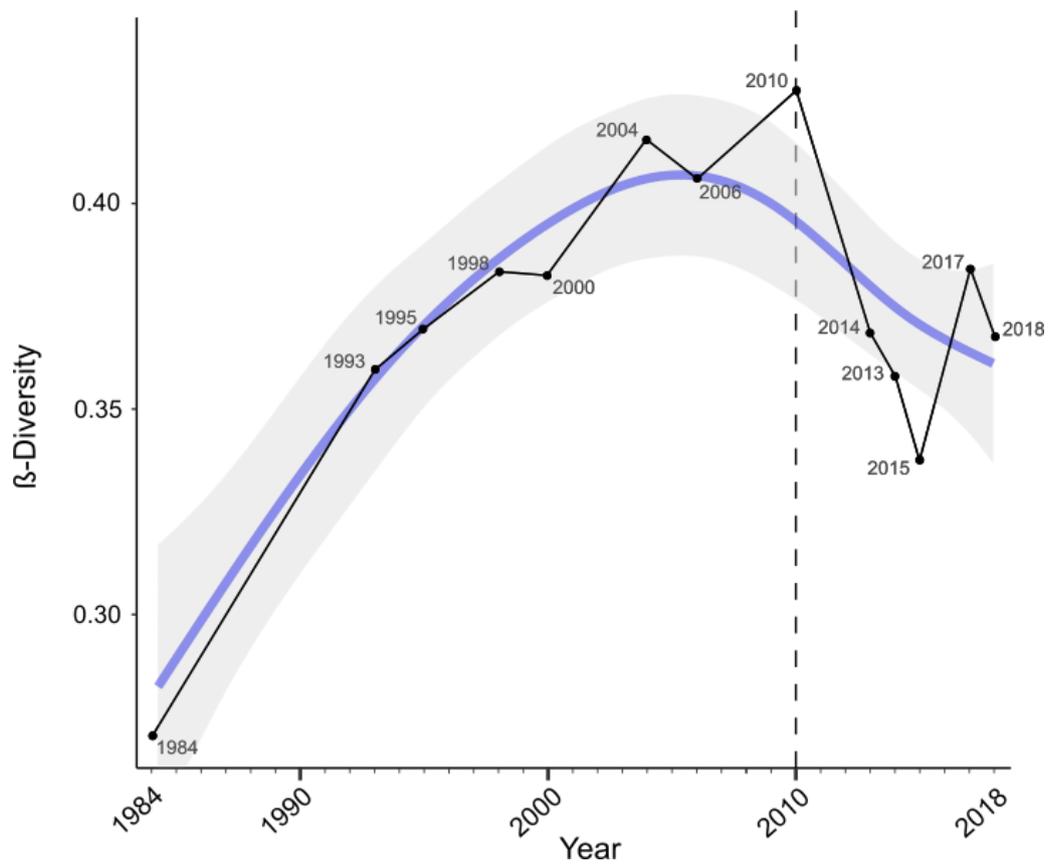
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867 **Figure S3.** Generalised additive models (GAMs) exploring the relationship between (a) temperature and (b) precipitation
868 during the study period. Solid lines indicate significant change over time.



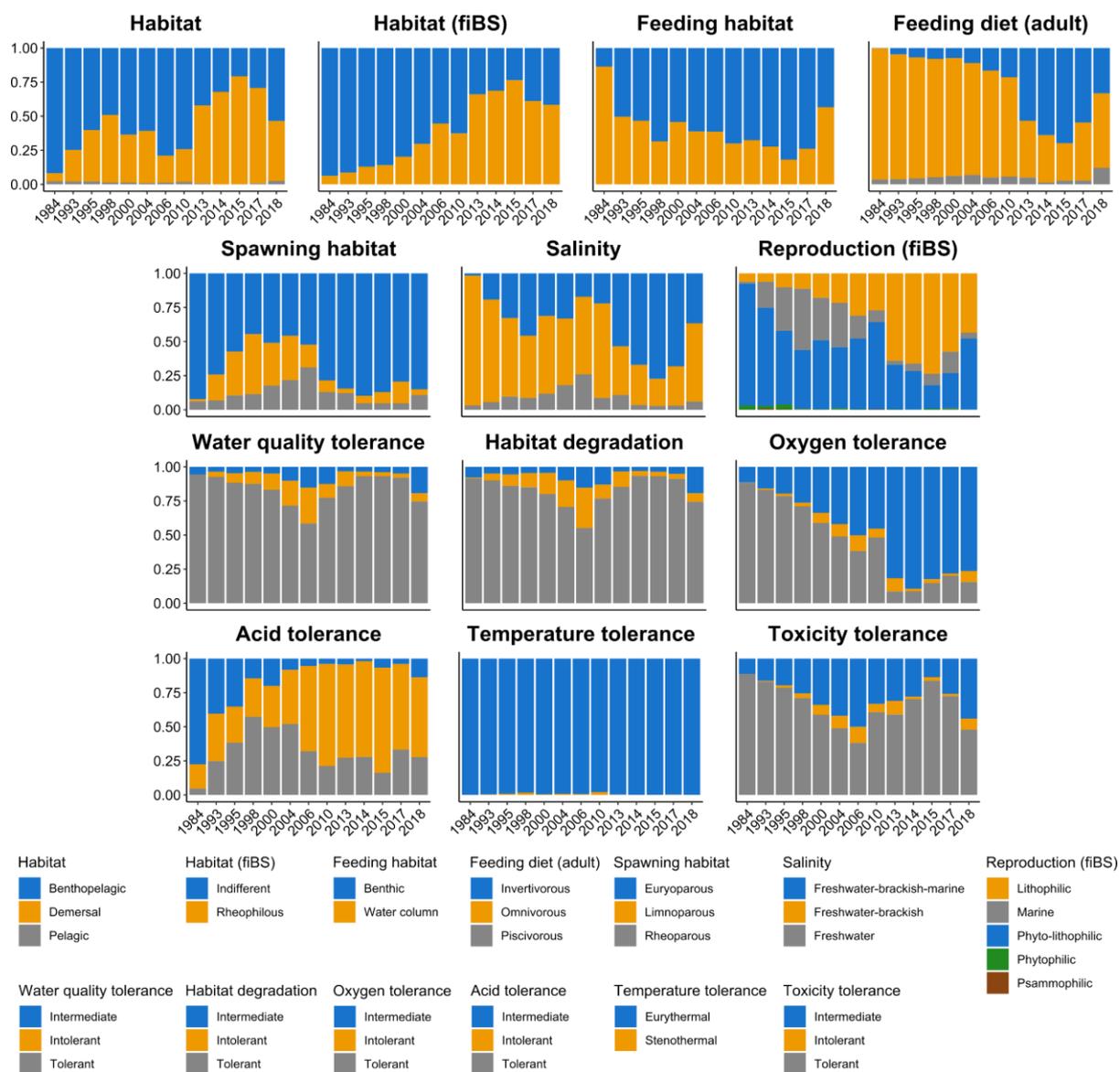
869

870 **Figure S4.** Beta-diversity over time using the Jaccard similarity Index (solid black lines) and the response of GAM model
871 (blue line) using “year” as a unique predictor. Dashed line split the period into before the arrival of alien species (< 2010) and
872 after (> 2010).



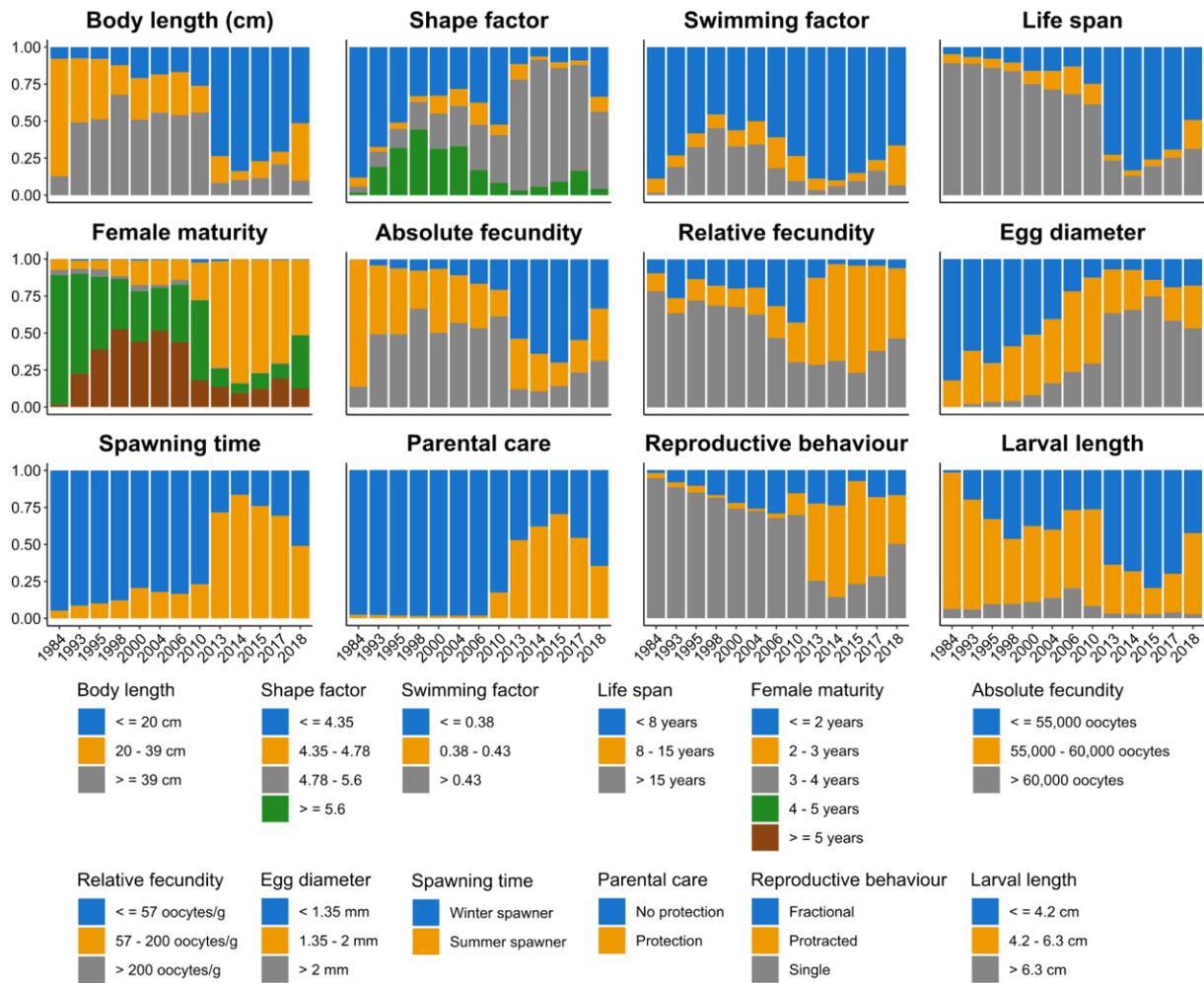
873

874 **Figure S5.** Relative abundance of each ecological trait modalities over time.



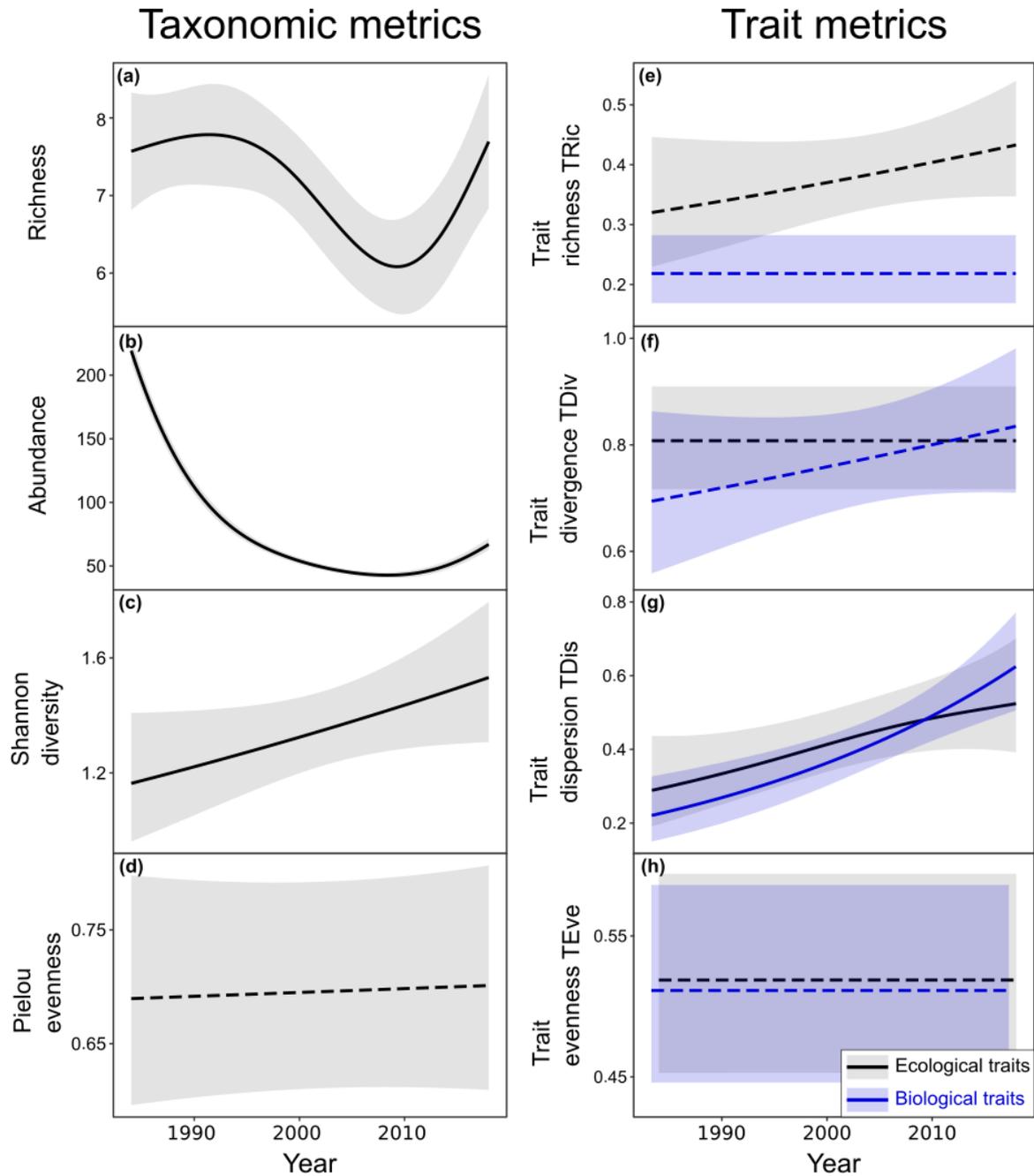
875

876 **Figure S6.** Relative abundance of each biological trait modalities over time.



877

878 **Figure S7.** Generalised additive models (GAMs) exploring the relationship between (a-d) taxonomic and (e-h) trait metrics of
879 the studies communities. Trait metrics are divided into ecological (black lines) and biological (blue lines) traits. Solid lines
880 indicate significant change over time, whereas dashed lines represent non-significant trends.



881

Time series	Sampling years	Total sampled years	Period
1	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
2	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
3	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
4	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
5	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
6	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
7	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
8	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
9	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
10	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
11	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
12	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
13	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
14	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
15	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
16	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013,	13	1984-2018

	2014, 2015, 2017, 2018		
17	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
18	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
19	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
20	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
21	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
22	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
23	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
24	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
25	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
26	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
27	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
28	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
29	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
30	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
31	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018

884 **Table S2.** Definitions of all ecological and biological traits used in this study; extracted from www.freshwaterecology.info
 885 (Schmidt-Kloiber & Hering, 2015).

Biological		Ecological	
Body length	Body length in cm	Habitat	Where species living and feeding
Shape factor	Ratio between the length and the width	Habitat (fiBS)	Flow conditions of the habitat
Swimming factor	Ratio of the minimum depth of the caudal peduncle to the maximum caudal fin depth	Feeding habitat	Feeding in the bottom or in the water column
Life span	Life span in years	Reproduction (fiBS)	Habitat reproduction
Female maturity	Age of females mature for the first time	Spawning habitat	Flow conditions of the spawning habitat
Absolute fecundity	Number of oocytes produces per female	Habitat degradation tolerance	Tolerance or not to habitat degradation
Relative fecundity	Number of oocytes produces per gram	Water quality tolerance	Tolerance to usual water quality parameters
Egg diameter	Egg diameter in mm	Temperature tolerance	Eury- or stenothermal
Spawning time	Season of fish spawning	Oxygen tolerance	Tolerance to low oxygen concentration
Reproductive behaviour	One or several spawning events in the potential season	Toxicity tolerance	Tolerance to toxic contamination
Parental care	Protection of eggs and/or larvae or not	Acid tolerance	Tolerance to acidification
Larval length	Length of larval fish in cm	Salinity	Salinity habitat
		Feeding diet (adult)	Type of feeding diet

886

887 **Table S3.** Definitions of all metrics and measurements used in this study.

Metric	Definition
Species richness	Number of species (Baker <i>et al.</i> , 2021)
Abundance	Number of individuals per species
Shannon diversity	A function of both richness and evenness (Maurer & McGill, 2011)
Pielou evenness	Distribution of abundances across all species within a community (Baker <i>et al.</i> , 2021)
Trait richness (TRic)	The amount of niche space occupied by all species within a given community (Mason <i>et al.</i> , 2005)
Trait divergence (TDiv)	The degree to which the abundance distribution utilises differences in traits within the community (a measure of how spread or clumped species are within the niche space, weighted by the relative abundance) (Mason <i>et al.</i> , 2005; Villéger <i>et al.</i> , 2008)
Trait dispersion (TDis)	The average distance of individual species to the group centroids of all species (Villéger <i>et al.</i> , 2008)
Trait evenness (TEve)	The regularity in the distribution of the abundance in trait space of the organisms composing an ecological unit (Villéger <i>et al.</i> , 2008; Schleuter <i>et al.</i> , 2010)

888

889 **Table S4.** Species sampled in the Rhine river between 1984 and 2018, their family, status (native/alien, the latter highlighted
 890 in red) and sampled occurrences.

Species	Family	Status	Period
<i>Abramis brama</i>	Cyprinidae	Native	1984-2018
<i>Alburnoides bipunctatus</i>	Cyprinidae	Native	2017
<i>Alburnus alburnus</i>	Cyprinidae	Native	1984-2018
<i>Anguilla anguilla</i>	Anguillidae	Native	1984-2018
<i>Barbatula barbatula</i>	Nemacheilidae	Native	2004-2017
<i>Barbus barbus</i>	Cyprinidae	Native	1984-2018
<i>Blicca bjoerkna</i>	Cyprinidae	Native	1984-2004
<i>Carassius carassius</i>	Cyprinidae	Native	1984
<i>Carassius gibelio</i>	Cyprinidae	Native	2013-2015
<i>Chondrostoma nasus</i>	Cyprinidae	Native	1984-2018
<i>Cobitis taenia</i>	Cobitidae	Native	2013
<i>Cottus gobio</i>	Cottidae	Native	2004
<i>Cyprinus carpio</i>	Cyprinidae	Alien	1993-2018
<i>Esox lucius</i>	Esocidae	Native	1984-2017
<i>Gasterosteus aculeatus</i>	Gasterosteidae	Native	2015-2017
<i>Gobio gobio</i>	Cyprinidae	Native	1984-2010
<i>Gymnocephalus cernua</i>	Percidae	Native	1993-2015
<i>Lampetra fluviatilis</i>	Petromyzontidae	Native	2004
<i>Lepomis gibbosus</i>	Centrarchidae	Alien	2013-2017
<i>Leuciscus aspius</i>	Cyprinidae	Native	1993-2018
<i>Leuciscus idus</i>	Cyprinidae	Native	1993-2018
<i>Leuciscus leuciscus</i>	Cyprinidae	Native	1984-2018
<i>Lota lota</i>	Lotidae	Native	2000-2018
<i>Neogobius fluviatilis</i>	Gobiidae	Alien	2013-2018
<i>Ponticola kessleri</i>	Gobiidae	Alien	2010-2018
<i>Neogobius melanostomus</i>	Gobiidae	Alien	2010-2018
<i>Oncorhynchus mykiss</i>	Salmonidae	Alien	1998-2010
<i>Perca fluviatilis</i>	Percidae	Native	1984-2018
<i>Platichthys flesus</i>	Pleuronectidae	Native	1993-2018
<i>Proterorhinus marmoratus</i>	Gobiidae	Alien	2006

<i>Pseudorasbora parva</i>	Cyprinidae	Alien	2013-2018
<i>Rhodeus amarus</i>	Cyprinidae	Alien	2013
<i>Rutilus rutilus</i>	Cyprinidae	Native	1984-2018
<i>Salmo salar</i>	Salmonidae	Native	2000-2017
<i>Salmo trutta fario</i>	Salmonidae	Native	1984-2018
<i>Salmo trutta trutta</i>	Salmonidae	Native	1984-2015
<i>Sander lucioperca</i>	Percidae	Native	1984-2018
<i>Scardinius erythrophthalmus</i>	Cyprinidae	Native	2006-2018
<i>Silurus glanis</i>	Siluridae	Alien	2004-2018
<i>Squalius cephalus</i>	Cyprinidae	Native	1984-2018
<i>Thymallus thymallus</i>	Thymallidae	Native	2010-2017
<i>Tinca tinca</i>	Cyprinidae	Native	1984
<i>Vimba vimba</i>	Cyprinidae	Alien	2017-2018

891

892 **Table S5.** Results of the PERMANOVA on community species composition using abiotic and biotic predictors.

	Df	SumOfSqs	R2	F	Pr(>F)
Latitude	1	5.670	0.063	35.044	0.0001
Alien species abundance	1	10.454	0.115	64.617	0.0001
Alien species richness	1	7.222	0.080	44.640	0.0001
Precipitation	1	1.283	0.014	7.929	0.0001
Temperature	1	1.854	0.020	11.460	0.0001
Residual	397	64.229	0.708		
Total	402	90.711	1.000		

893

894 **Table S6.** Results of the PERMANOVA on community species composition using abundance-weighted ecological and
 895 biological traits as predictors.

Ecological traits					
	Df	SumOfSqs	R2	F	Pr(>F)
habitat_ben	1	16.739	0.185	384.976	0.0001
habitat_dem	1	2.324	0.026	53.450	0.0001
habitat_fibs_indifferent	1	11.853	0.131	272.589	0.0001
habitat_fibs_rheophil	1	0.213	0.002	4.908	0.0001
feeding_habitat_ben	1	7.496	0.083	172.392	0.0001
reproduction_lithopelagophilic	1	0.196	0.002	4.508	0.0001
reproduction_lithophilic	1	6.982	0.077	160.575	0.0001
reproduction_marine	1	5.686	0.063	130.772	0.0001
reproduction_ostracophilic	1	0.076	0.001	1.742	0.048
reproduction_phytolithophilic	1	1.149	0.013	26.431	0.0001
reproduction_phytophilic	1	0.632	0.007	14.534	0.0001
reproduction_psammophilic	1	0.154	0.002	3.535	0.0001
spawning_habitat_eury	1	2.360	0.026	54.278	0.0001
spawning_habitat_limno	1	0.624	0.007	14.339	0.0001
hab_degr_tol_im	1	1.273	0.014	29.281	0.0001
hab_degr_tol_intol	1	1.323	0.015	30.431	0.0001
wat_qual_tol_im	1	0.366	0.004	8.409	0.0001
wat_qual_tol_intol	1	1.521	0.017	34.980	0.0001

temp_tol_eury	1	0.949	0.010	21.831	0.0001
oxy_tol_im	1	5.006	0.055	115.140	0.0001
oxy_tol_intol	1	3.665	0.040	84.299	0.0001
tox_tol_im	1	0.917	0.010	21.100	0.0001
tox_tol_intol	1	0.841	0.009	19.352	0.0001
acid_tol_im	1	0.281	0.003	6.453	0.0001
acid_tol_intol	1	0.457	0.005	10.507	0.0001
salinity_fbm	1	0.367	0.004	8.446	0.0001
salinity_fbr	1	0.461	0.005	10.595	0.0001
diet_invert	1	0.165	0.002	3.790	0.0001
diet_omni	1	0.368	0.004	8.470	0.0001
diet_parasit	1	0.091	0.001	2.099	0.0388
Residual	372	16.175	0.178		
Total	402	90.711	1.000		

Biological traits

body_length_bl1	1	16.563	0.183	372.089	0.0001
body_length_bl2	1	8.241	0.091	185.126	0.0001
shape_factor_sh1	1	6.093	0.067	136.878	0.0001
shape_factor_sh2	1	7.764	0.086	174.419	0.0001
shape_factor_sh3	1	6.112	0.067	137.295	0.0001
swimming_factor_sw1	1	4.451	0.049	99.983	0.0001
swimming_factor_sw2	1	1.416	0.016	31.805	0.0001

life_span_ls1	1	3.136	0.035	70.445	0.0001
life_span_ls2	1	2.527	0.028	56.758	0.0001
maturity_ma1	1	0.712	0.008	15.988	0.0001
maturity_ma2	1	1.195	0.013	26.843	0.0001
maturity_ma3	1	2.069	0.023	46.474	0.0001
maturity_ma4	1	1.310	0.014	29.420	0.0001
abs_fecundity_fe1	1	6.164	0.068	138.471	0.0001
abs_fecundity_fe2	1	0.942	0.010	21.168	0.0001
rel_fecundity_fr1	1	0.925	0.010	20.771	0.0001
rel_fecundity_fr2	1	0.451	0.005	10.127	0.0001
egg_ed1	1	1.082	0.012	24.304	0.0001
egg_ed2	1	0.681	0.008	15.296	0.0001
spawning_time_st1	1	0.429	0.005	9.638	0.0001
repr_behav_fra	1	0.379	0.004	8.508	0.0001
repr_behav_pro	1	0.563	0.006	12.653	0.0001
parental_care_no	1	0.266	0.003	5.971	0.0001
larval_length_ll1	1	0.271	0.003	6.089	0.0001
larval_length_ll2	1	0.190	0.002	4.260	0.0001
Residual	377	16.782	0.185		
Total	402	90.711	1.000		

897 **Table S7.** Results of the PERMANOVA on abundance-weighted ecological and biological traits using abiotic and biotic
898 predictors.

Ecological traits					
	Df	SumOfSqs	R2	F	Pr(>F)
Latitude	1	0.622	0.044	25.237	0.0001
Alien species abundance	1	2.282	0.162	92.612	0.0001
Alien species richness	1	0.813	0.058	33.006	0.0001
Precipitation	1	0.080	0.006	3.234	0.0193
Temperature	1	0.490	0.035	19.869	0.0001
Residual	397	9.782	0.695		
Total	402	14.068	1.000		

Biological traits					
	Df	SumOfSqs	R2	F	Pr(>F)
Latitude	1	0.661	0.032	22.641	0.0001
Alien species abundance	1	5.882	0.286	201.484	0.0001
Alien species richness	1	2.164	0.105	74.129	0.0001
Precipitation	1	0.091	0.004	3.128	0.0341
Temperature	1	0.209	0.010	7.172	0.001
Residual	397	11.590	0.563		
Total	402	20.598	1.000		

899

900 **Table S8.** Coefficients of significant predictors in generalised additive models (gam) exploring the relationship between
 901 taxonomic and trait metrics over study time. Abbreviations “NS” indicate non significant predictors and “AS” refer to alien
 902 species.

Period	Metrics	R ²	Year	Latitude	Temperature	Precipitation	AS abundance	AS richness
1984 to 2006	Richness	0.110	NS	0.81	1.54	3.60	NS	NS
	Abundance	0.624	5.92	8.56	7.86	8.33	NS	NS
	FDis ecological	0.292	0.84	NS	NS	NS	NS	NS
2010 to 2018	Richness	0.425	4.06	1.65	NS	NS	NS	1.00
	Abundance	0.731	4.93	7.64	8.21	7.70	2.97e-03	6.83e-02
	Shannon diversity	0.353	NS	NS	NS	NS	-0.003	NS
	Pielou evenness	0.477	NS	NS	NS	NS	-0.003	NS

903

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Table S1. Description of each time series

Time series	Sampling years	Total sampled years	Period
1	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
2	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
3	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
4	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
5	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
6	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
7	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
8	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
9	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
10	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
11	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
12	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
13	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
14	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018

15	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
16	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
17	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
18	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
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25	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
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28	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
29	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018

30	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018
31	1984, 1993, 1995, 1998, 2000, 2004, 2006, 2013, 2014, 2015, 2017, 2018	13	1984-2018

Table S2. Definitions of all ecological and biological traits used in this study; extracted from *www.freshwaterecology.info* (Schmidt-Kloiber & Hering, 2015).

Biological		Ecological	
Body length	Body length in cm	Habitat	Where species living and feeding
Shape factor	Ratio between the length and the width	Habitat (fiBS)	Flow conditions of the habitat
Swimming factor	Ratio of the minimum depth of the caudal peduncle to the maximum caudal fin depth	Feeding habitat	Feeding in the bottom or in the water column
Life span	Life span in years	Reproduction (fiBS)	Habitat reproduction
Female maturity	Age of females mature for the first time	Spawning habitat	Flow conditions of the spawning habitat
Absolute fecundity	Number of oocytes produces per female	Habitat degradation tolerance	Tolerance or not to habitat degradation
Relative fecundity	Number of oocytes produces per gram	Water quality tolerance	Tolerance to usual water quality parameters
Egg diameter	Egg diameter in mm	Temperature tolerance	Eury- or stenothermal
Spawning time	Season of fish spawning	Oxygen tolerance	Tolerance to low oxygen concentration
Reproductive behaviour	One or several spawning events in the potential season	Toxicity tolerance	Tolerance to toxic contamination
Parental care	Protection of eggs and/or larvae or not	Acid tolerance	Tolerance to acidification
Larval length	Length of larval fish in cm	Salinity	Salinity habitat
		Feeding diet (adult)	Type of feeding diet

Table S3. Definitions of all metrics and measurements used in this study.

Metric	Definition
Species richness	Number of species (Baker <i>et al.</i> , 2021)
Abundance	Number of individuals per species
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Trait divergence (TDiv)	The degree to which the abundance distribution utilises differences in traits within the community (a measure of how spread or clumped species are within the niche space, weighted by the relative abundance) (Mason <i>et al.</i> 2005; Villéger <i>et al.</i> , 2008)
Trait dispersion (TDis)	The average distance of individual species to the group centroids of all species (Villéger <i>et al.</i> , 2008)
Trait evenness (TEve)	The regularity in the distribution of the abundance in trait space of the organisms composing an ecological unit (Villéger <i>et al.</i> , 2008; Schleuter <i>et al.</i> , 2010)

Table S4. Species sampled in the Rhine River between 1984 and 2018, their family, status (native/alien, the latter highlighted in red) and sampled occurrences.

Species	Family	Status	Period
<i>Abramis brama</i>	Cyprinidae	Native	1984-2018
<i>Alburnoides bipunctatus</i>	Cyprinidae	Native	2017
<i>Alburnus alburnus</i>	Cyprinidae	Native	1984-2018
<i>Anguilla anguilla</i>	Anguillidae	Native	1984-2018
<i>Barbatula barbatula</i>	Nemacheilidae	Native	2004-2017
<i>Barbus barbus</i>	Cyprinidae	Native	1984-2018
<i>Blicca bjoerkna</i>	Cyprinidae	Native	1984-2004
<i>Carassius carassius</i>	Cyprinidae	Native	1984
<i>Carassius gibelio</i>	Cyprinidae	Native	2013-2015
<i>Chondrostoma nasus</i>	Cyprinidae	Native	1984-2018
<i>Cobitis taenia</i>	Cobitidae	Native	2013
<i>Cottus gobio</i>	Cottidae	Native	2004
<i>Cyprinus carpio</i>	Cyprinidae	Alien	1993-2018
<i>Esox lucius</i>	Esocidae	Native	1984-2017
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<i>Neogobius kessleri</i>	Gobiidae	Alien	2010-2018
<i>Neogobius melanostomus</i>	Gobiidae	Alien	2010-2018
<i>Oncorhynchus mykiss</i>	Salmonidae	Alien	1998-2010
<i>Perca fluviatilis</i>	Percidae	Native	1984-2018
<i>Platichthys flesus</i>	Pleuronectidae	Native	1993-2018
<i>Proterorhinus marmoratus</i>	Gobiidae	Alien	2006
<i>Pseudorasbora parva</i>	Cyprinidae	Alien	2013-2018
<i>Rhodeus amarus</i>	Cyprinidae	Alien	2013
<i>Rutilus rutilus</i>	Cyprinidae	Native	1984-2018
<i>Salmo salar</i>	Salmonidae	Native	2000-2017
<i>Salmo trutta fario</i>	Salmonidae	Native	1984-2018
<i>Salmo trutta trutta</i>	Salmonidae	Native	1984-2015
<i>Sander lucioperca</i>	Percidae	Native	1984-2018
<i>Scardinius erythrophthalmus</i>	Cyprinidae	Native	2006-2018
<i>Silurus glanis</i>	Siluridae	Alien	2004-2018
<i>Squalius cephalus</i>	Cyprinidae	Native	1984-2018
<i>Thymallus thymallus</i>	Thymallidae	Native	2010-2017
<i>Tinca tinca</i>	Cyprinidae	Native	1984

Vimba vimba

Cyprinidae

Alien

2017-2018

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Temperature	1	1.854	0.02	11.46	0.0001
Residual	397	64.229	0.708		
Total	402	90.711	1		

Table S6. Results of the PERMANOVA on community species composition using abundance-weighted ecological and biological traits as predictors.

Ecological traits						
	Df	SumOfSqs	R2	F	Pr(>F)	
habitat_ben	1	16.739	0.185	384.976	0.0001	
habitat_dem	1	2.324	0.026	53.45	0.0001	
habitat_fibs_indifferent	1	11.853	0.131	272.589	0.0001	
habitat_fibs_rheophil	1	0.213	0.002	4.908	0.0001	
feeding_habitat_ben	1	7.496	0.083	172.392	0.0001	
reproduction_lithopelagophilic	1	0.196	0.002	4.508	0.0001	
reproduction_lithophilic	1	6.982	0.077	160.575	0.0001	
reproduction_marine	1	5.686	0.063	130.772	0.0001	
reproduction_ostracophilic	1	0.076	0.001	1.742	0.048	
reproduction_phytolithophilic	1	1.149	0.013	26.431	0.0001	
reproduction_phytophilic	1	0.632	0.007	14.534	0.0001	
reproduction_psammophilic	1	0.154	0.002	3.535	0.0001	
spawning_habitat_eury	1	2.36	0.026	54.278	0.0001	
spawning_habitat_limno	1	0.624	0.007	14.339	0.0001	
hab_degr_tol_im	1	1.273	0.014	29.281	0.0001	
hab_degr_tol_intol	1	1.323	0.015	30.431	0.0001	
wat_qual_tol_im	1	0.366	0.004	8.409	0.0001	
wat_qual_tol_intol	1	1.521	0.017	34.98	0.0001	
temp_tol_eury	1	0.949	0.01	21.831	0.0001	
oxy_tol_im	1	5.006	0.055	115.14	0.0001	
oxy_tol_intol	1	3.665	0.04	84.299	0.0001	
tox_tol_im	1	0.917	0.01	21.1	0.0001	
tox_tol_intol	1	0.841	0.009	19.352	0.0001	
acid_tol_im	1	0.281	0.003	6.453	0.0001	
acid_tol_intol	1	0.457	0.005	10.507	0.0001	
salinity_fbm	1	0.367	0.004	8.446	0.0001	
salinity_fbr	1	0.461	0.005	10.595	0.0001	
diet_invert	1	0.165	0.002	3.79	0.0001	
diet_omni	1	0.368	0.004	8.47	0.0001	
diet_parasit	1	0.091	0.001	2.099	0.0388	
Residual	372	16.175	0.178			
Total	402	90.711	1			
Biological traits						
body_length_bl1	1	16.563	0.183	372.089	0.0001	
body_length_bl2	1	8.241	0.091	185.126	0.0001	
shape_factor_sh1	1	6.093	0.067	136.878	0.0001	
shape_factor_sh2	1	7.764	0.086	174.419	0.0001	
shape_factor_sh3	1	6.112	0.067	137.295	0.0001	
swimming_factor_sw1	1	4.451	0.049	99.983	0.0001	
swimming_factor_sw2	1	1.416	0.016	31.805	0.0001	
life_span_ls1	1	3.136	0.035	70.445	0.0001	
life_span_ls2	1	2.527	0.028	56.758	0.0001	

maturity_ma1	1	0.712	0.008	15.988	0.0001
maturity_ma2	1	1.195	0.013	26.843	0.0001
maturity_ma3	1	2.069	0.023	46.474	0.0001
maturity_ma4	1	1.31	0.014	29.42	0.0001
abs_fecundity_fe1	1	6.164	0.068	138.471	0.0001
abs_fecundity_fe2	1	0.942	0.01	21.168	0.0001
rel_fecundity_fr1	1	0.925	0.01	20.771	0.0001
rel_fecundity_fr2	1	0.451	0.005	10.127	0.0001
egg_ed1	1	1.082	0.012	24.304	0.0001
egg_ed2	1	0.681	0.008	15.296	0.0001
spawning_time_st1	1	0.429	0.005	9.638	0.0001
repr_behav_fra	1	0.379	0.004	8.508	0.0001
repr_behav_pro	1	0.563	0.006	12.653	0.0001
parental_care_no	1	0.266	0.003	5.971	0.0001
larval_length_ll1	1	0.271	0.003	6.089	0.0001
larval_length_ll2	1	0.19	0.002	4.26	0.0001
Residual	377	16.782	0.185		
Total	402	90.711	1		

Table S7. Results of the PERMANOVA on abundance-weighted ecological and biological traits using abiotic and biotic predictors.

Ecological traits

	Df	SumOfSqs	R2	F	Pr(>F)
Latitude	1	0.622	0.044	25.237	0.0001
Alien species abundance	1	2.282	0.162	92.612	0.0001
Alien species richness	1	0.813	0.058	33.006	0.0001
Precipitation	1	0.08	0.006	3.234	0.0193
Temperature	1	0.49	0.035	19.869	0.0001
Residual	397	9.782	0.695		
Total	402	14.068	1		

Biological traits

Latitude	1	0.661	0.032	22.641	0.0001
Alien species abundance	1	5.882	0.286	201.484	0.0001
Alien species richness	1	2.164	0.105	74.129	0.0001
Precipitation	1	0.091	0.004	3.128	0.0341
Temperature	1	0.209	0.01	7.172	0.001
Residual	397	11.59	0.563		
Total	402	20.598	1		

Table S8. Coefficients of significant predictors in generalised additive models (gam) exploring the relationship between taxonomic and trait metrics over study time. Abbreviations “NS” indicate non significant predictors and “AS” refer to alien species.

Period	Metrics	R ²	Year	Latitude	Temperature	Precipitation	AS abundance	AS richness
1984 to 2006	Richness	0.11	NS	0.81	1.54	3.6	NS	NS
	Abundance	0.624	5.92	8.56	7.86	8.33	NS	NS
	FDis ecological	0.292	0.84	NS	NS	NS	NS	NS
2010 to 2018	Richness	0.425	4.06	1.65	NS	NS	NS	1
	Abundance	0.731	4.93	7.64	8.21	7.7	2.97E-03	6.83E-02
	Shannon diversity	0.353	NS	NS	NS	NS	-0.003	NS
	Pielou evenness	0.477	NS	NS	NS	NS	-0.003	NS

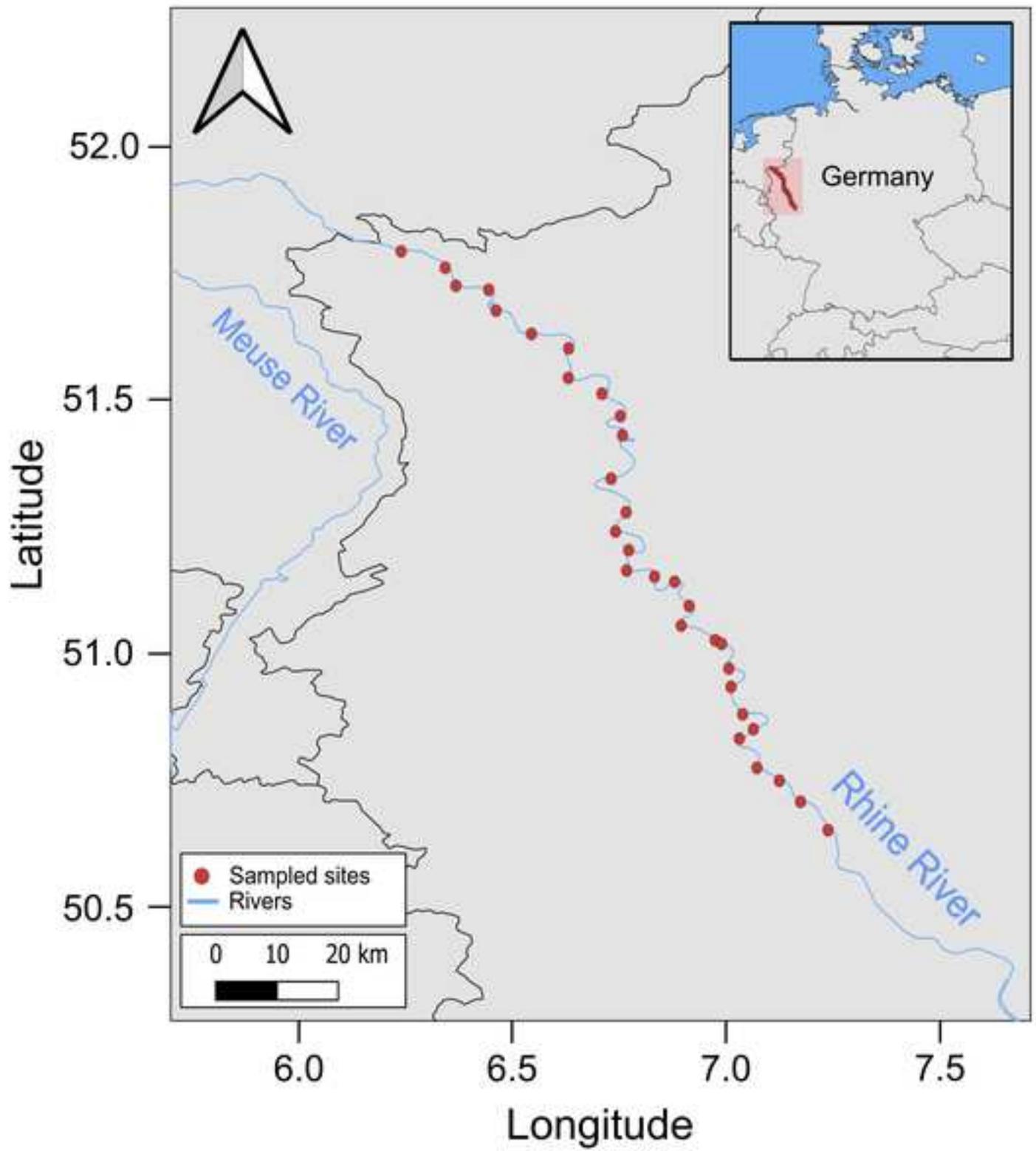
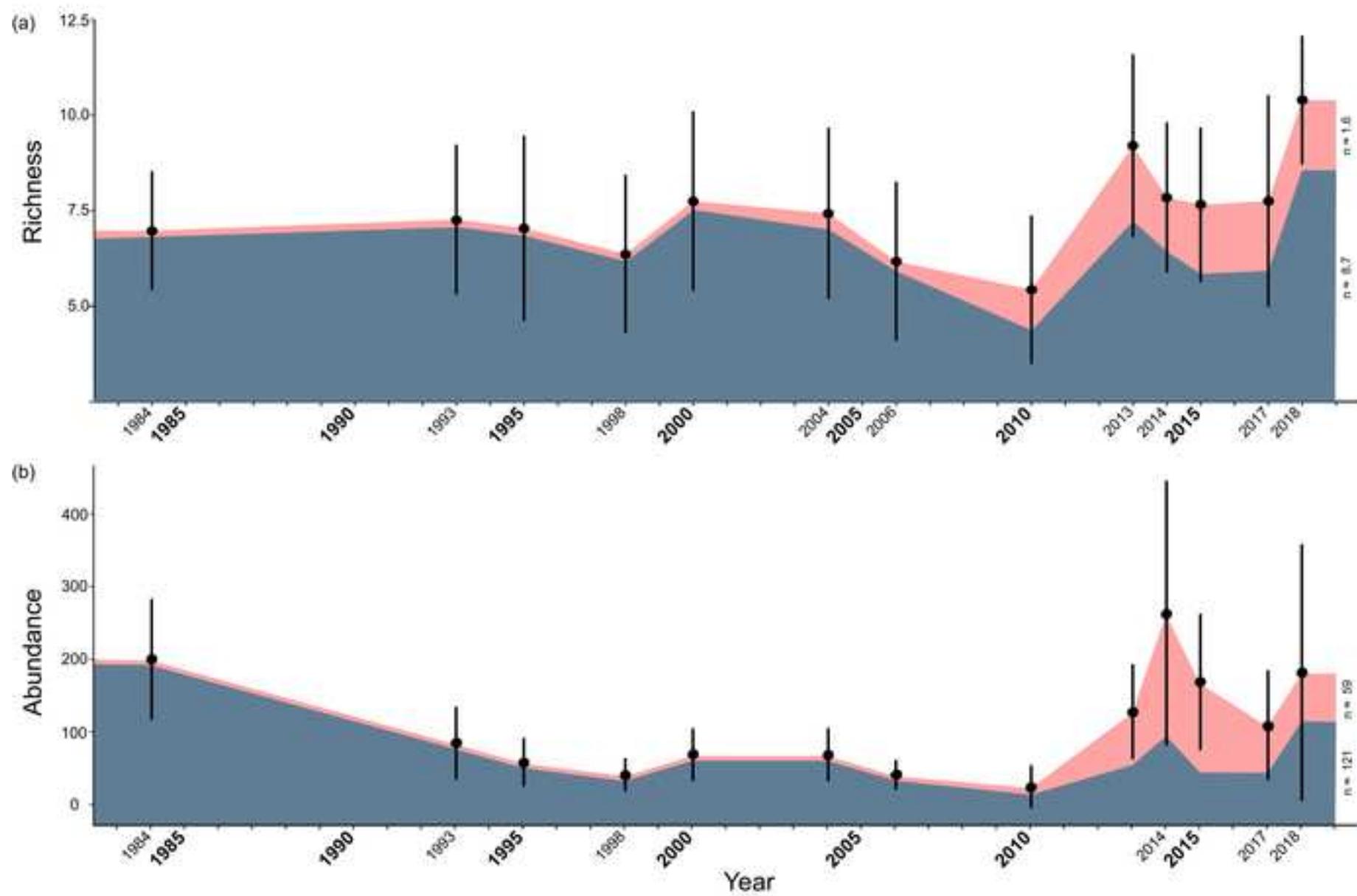
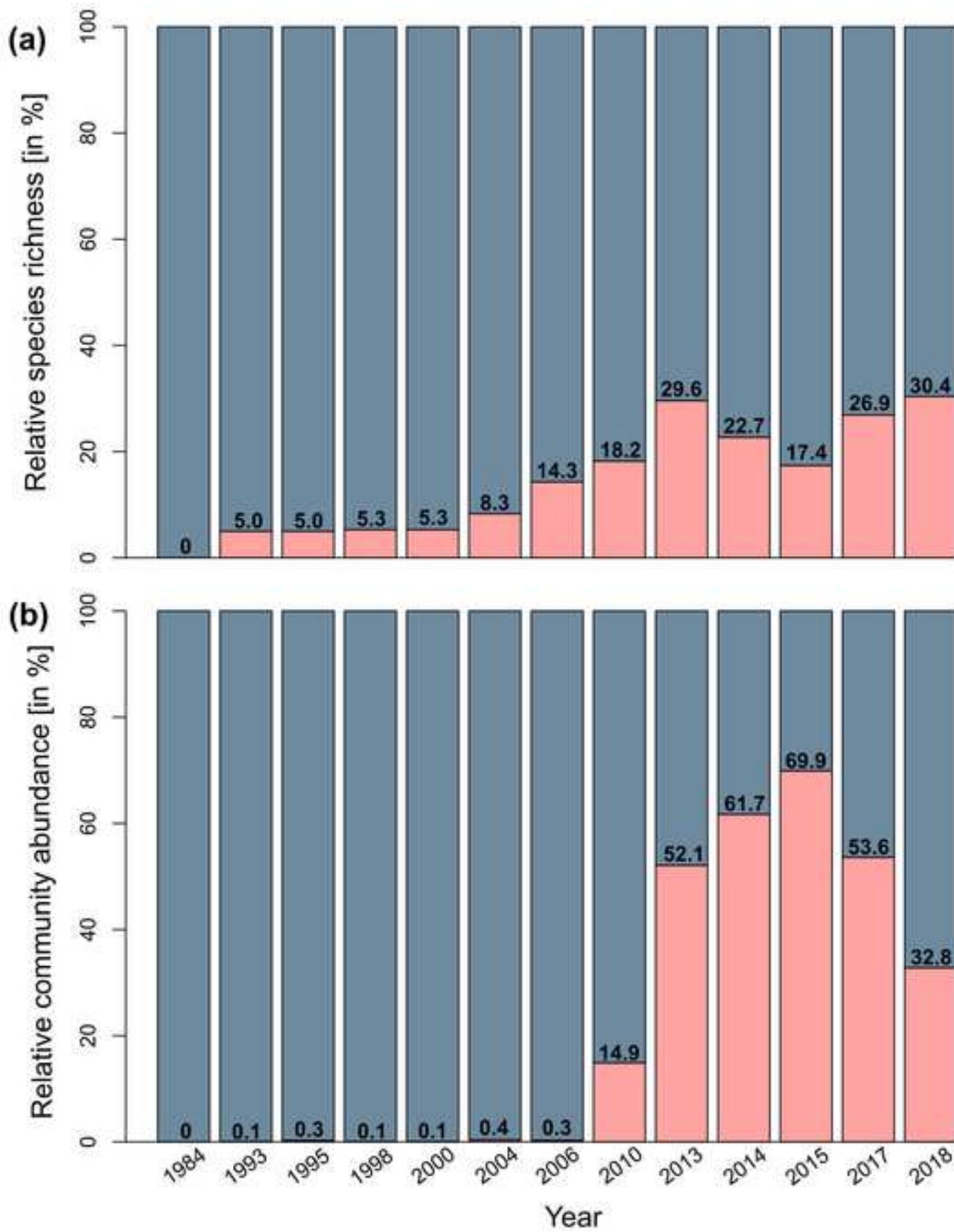
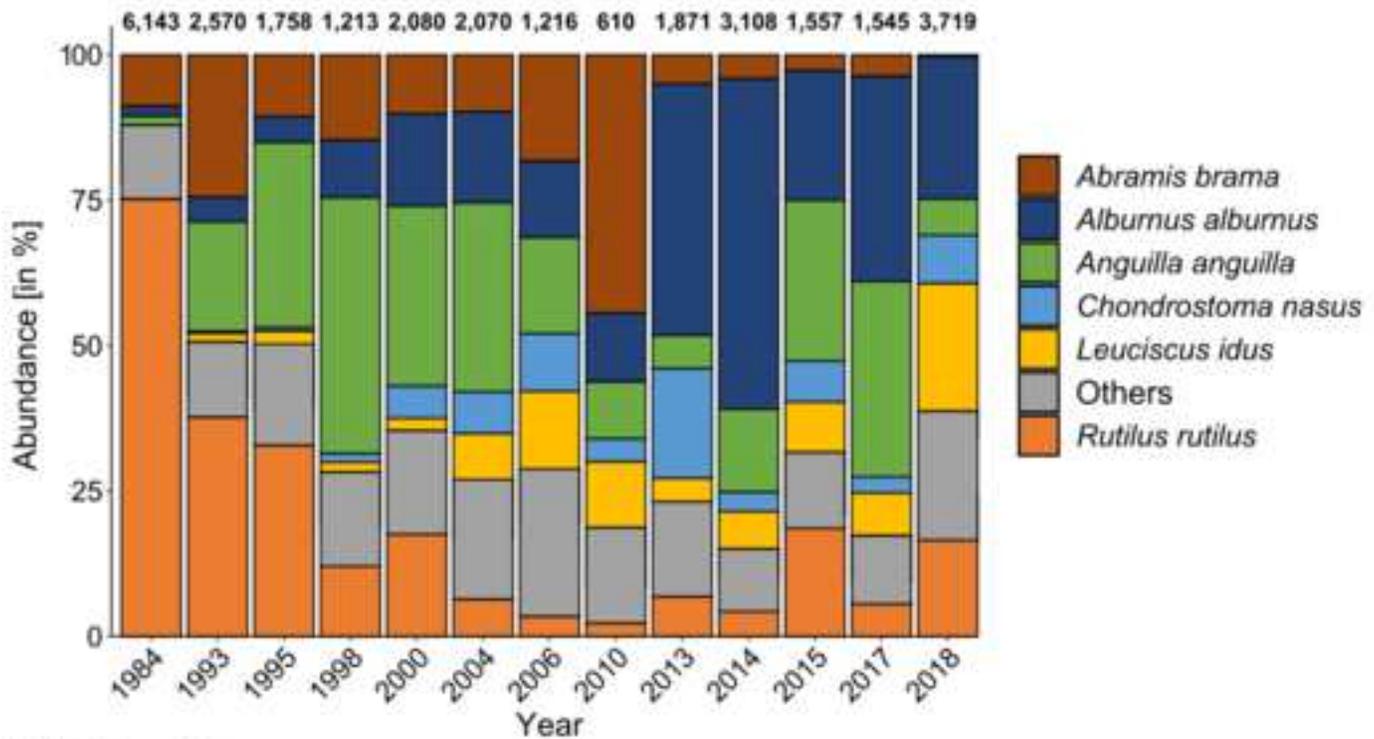
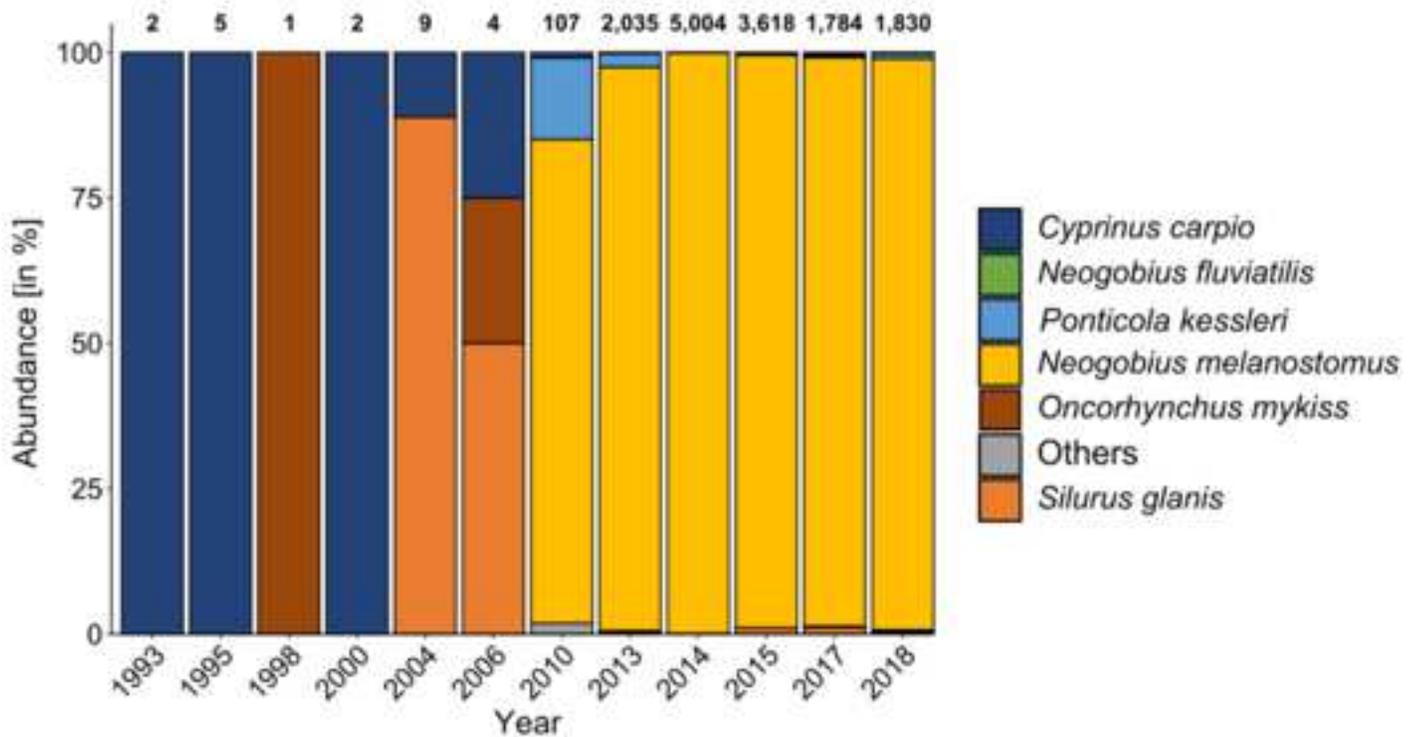
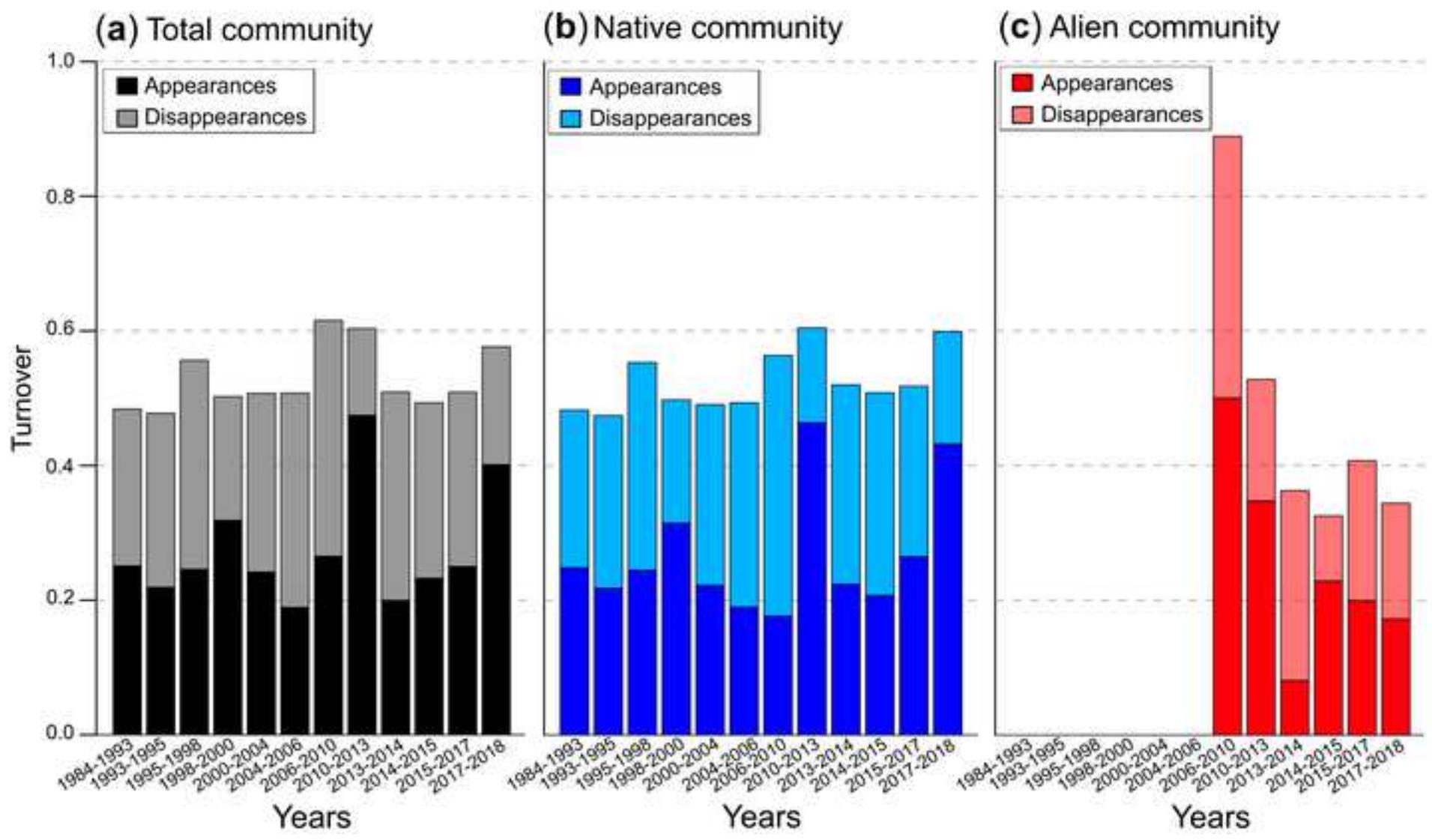


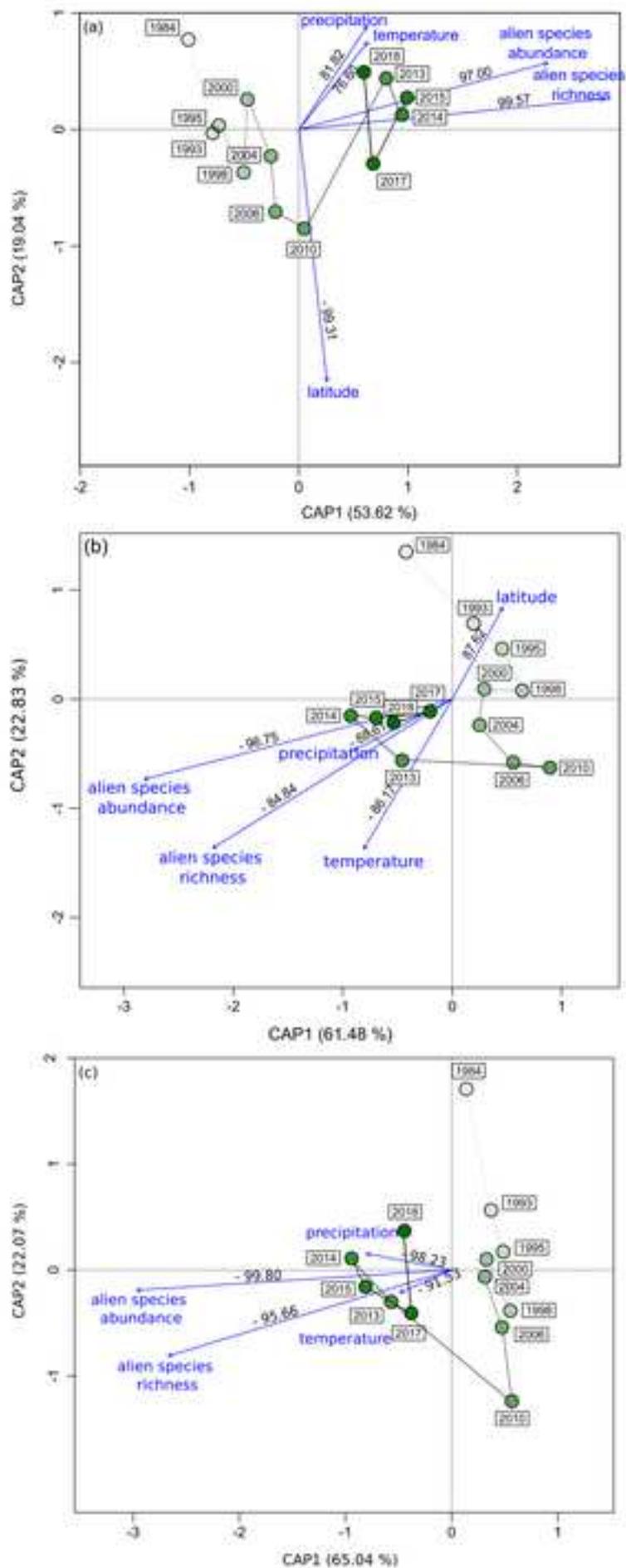
Figure 2

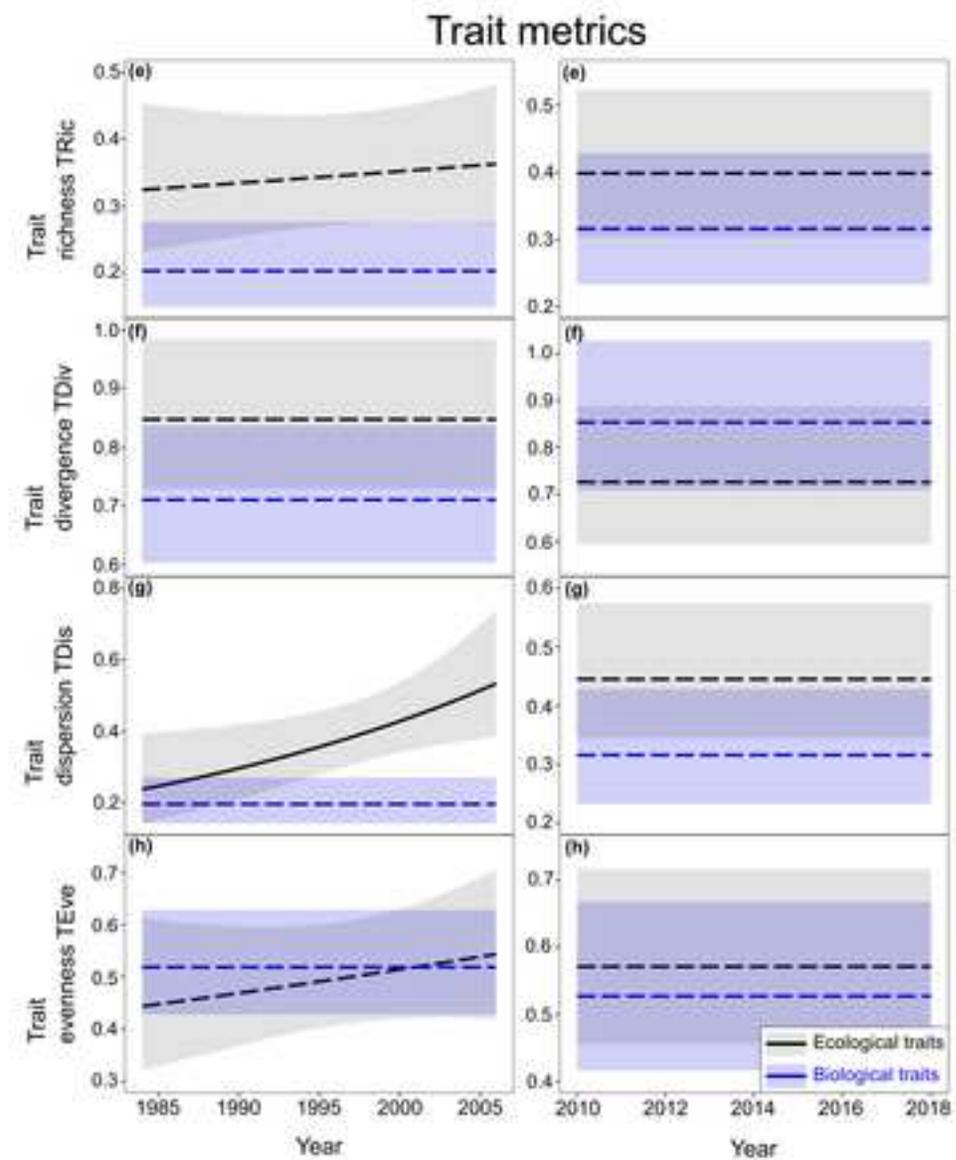
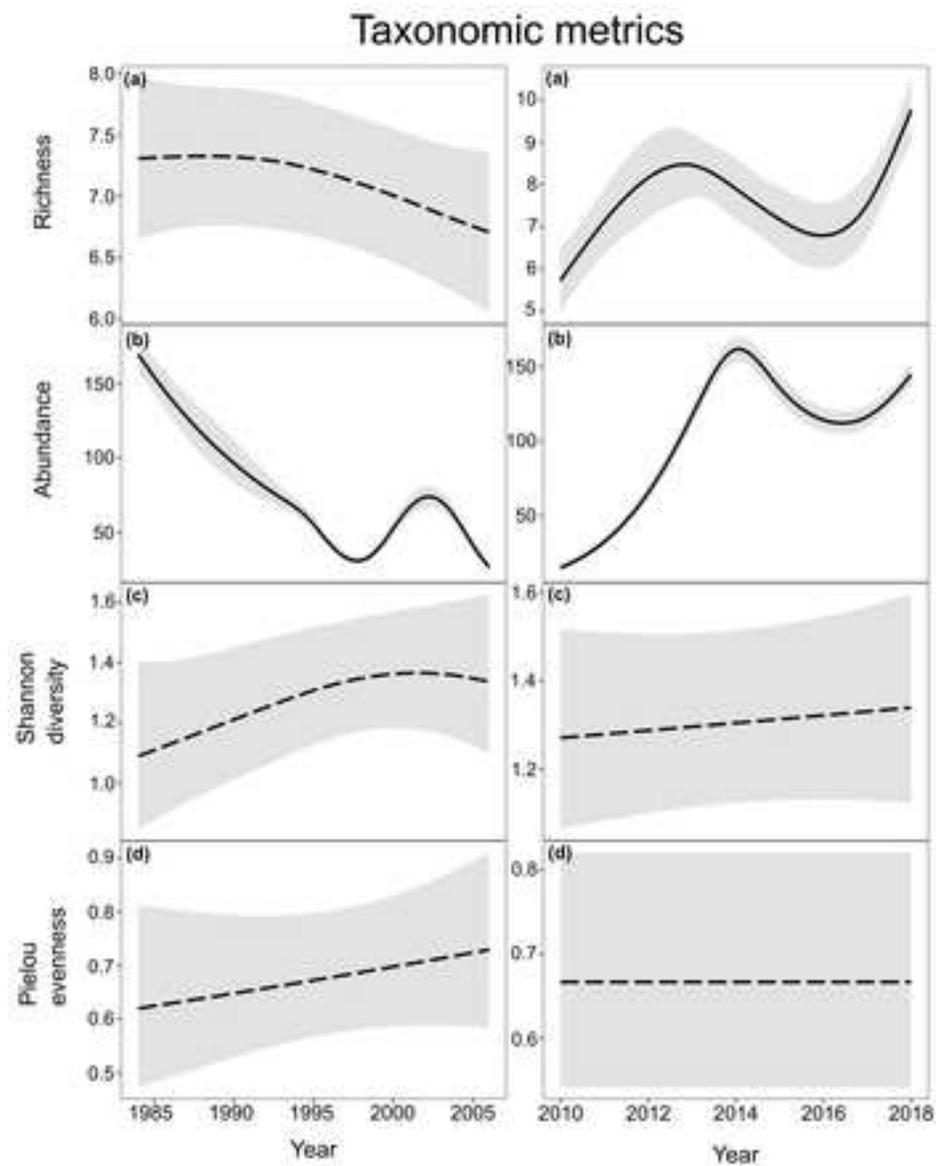


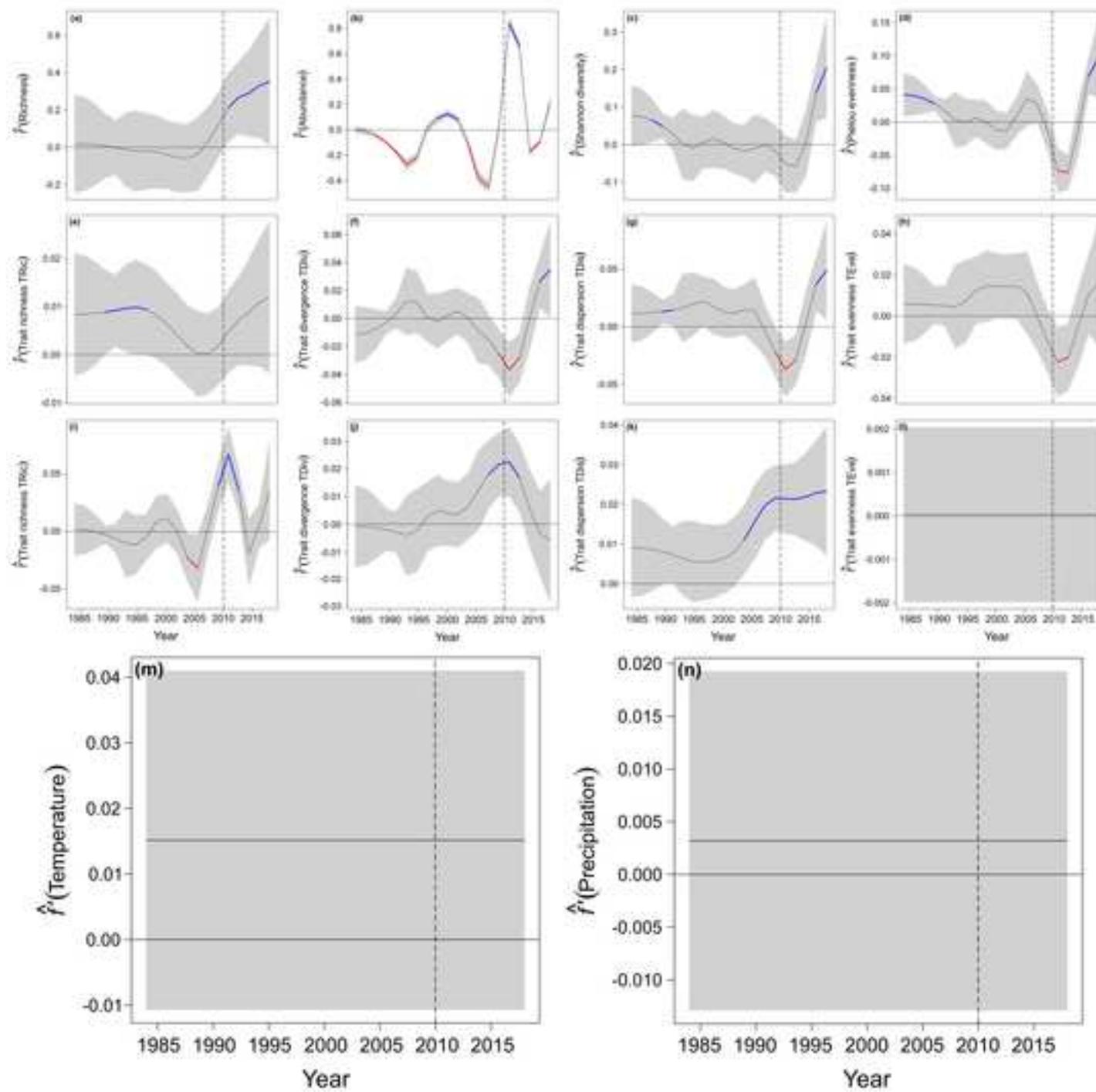


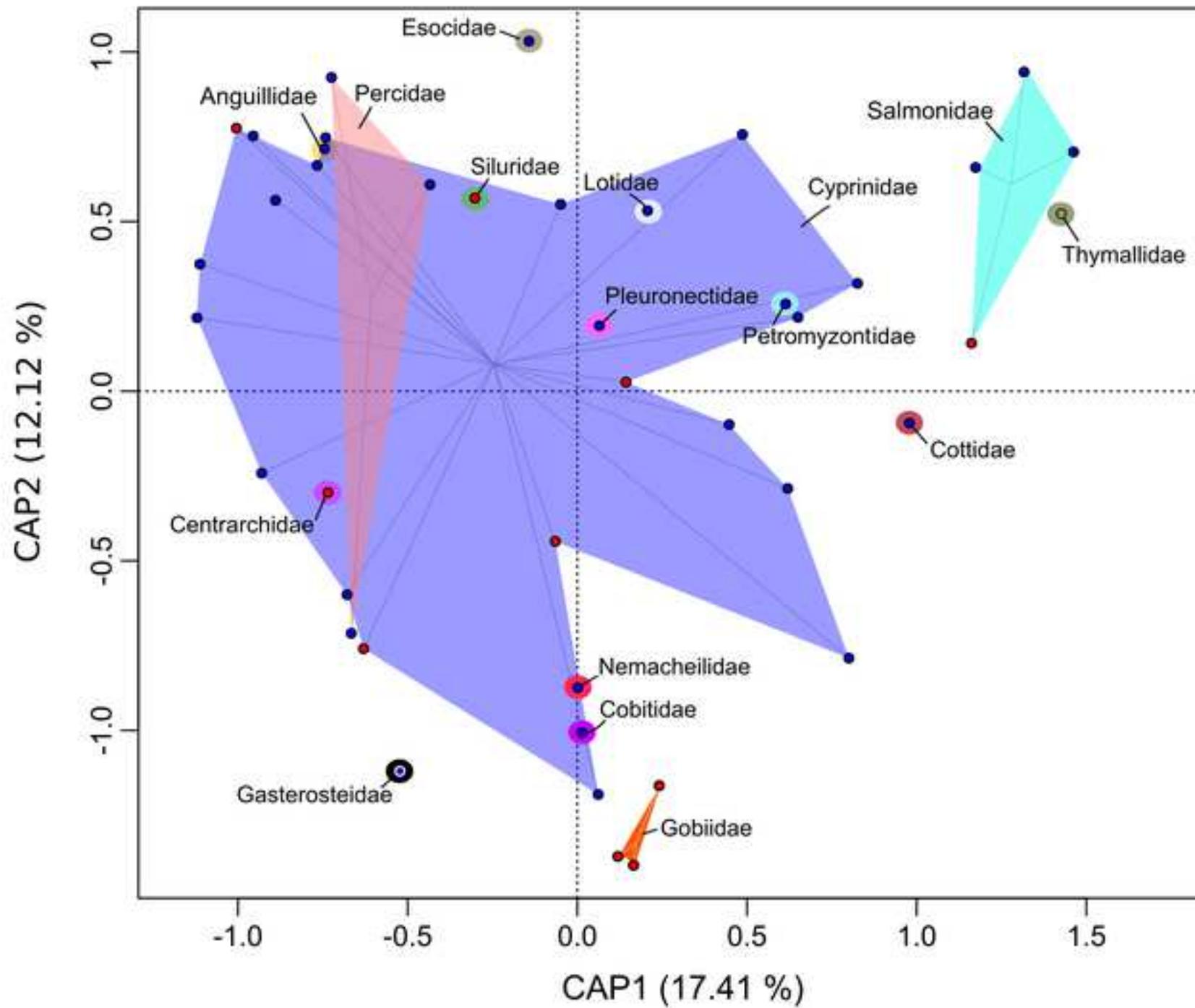
(a) Native fish**(b) Alien fish**

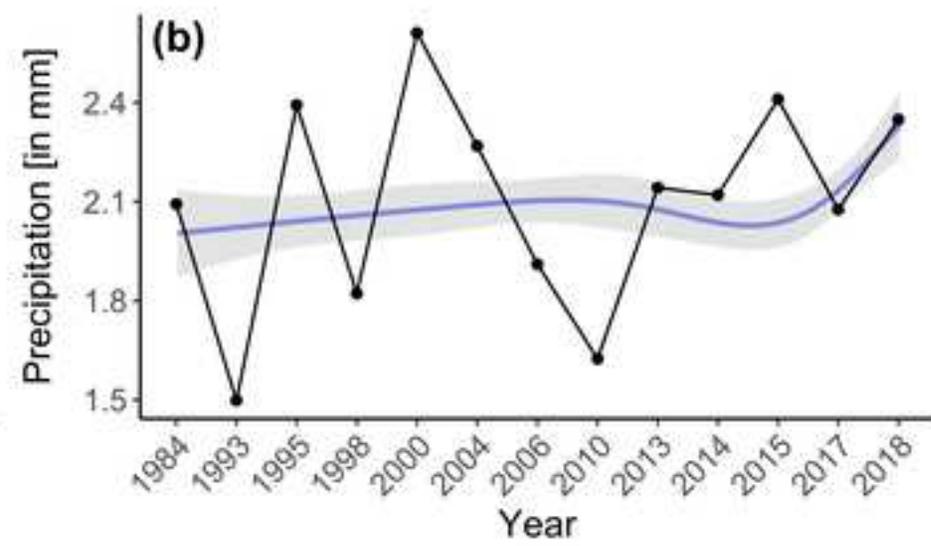
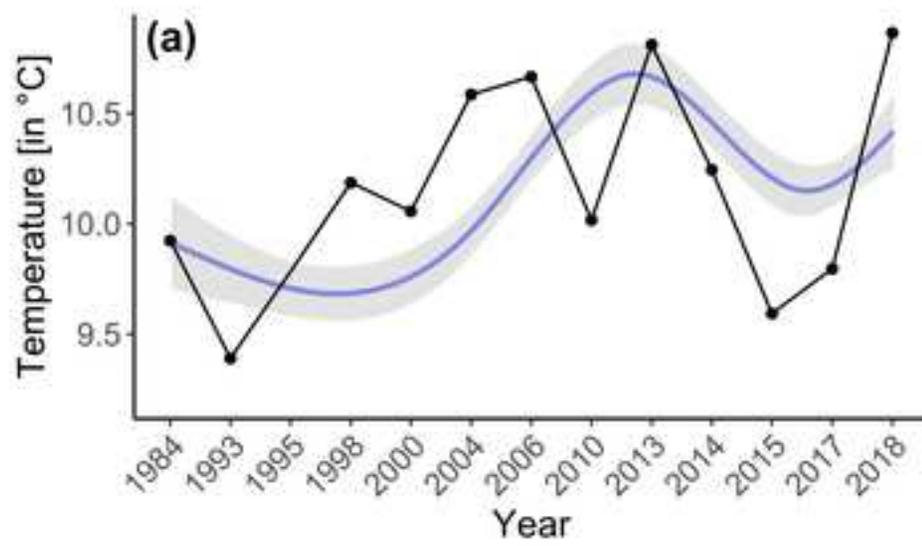


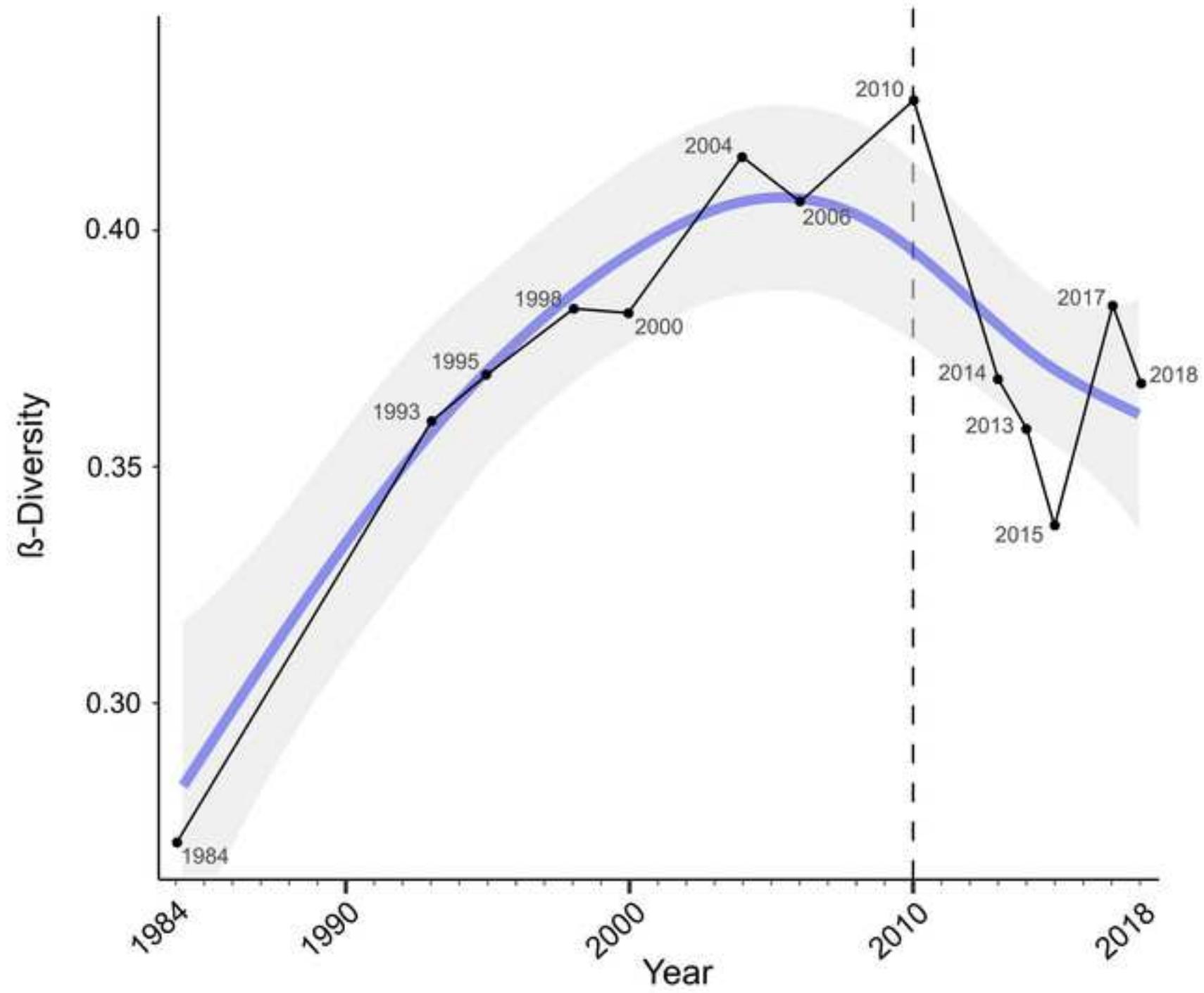


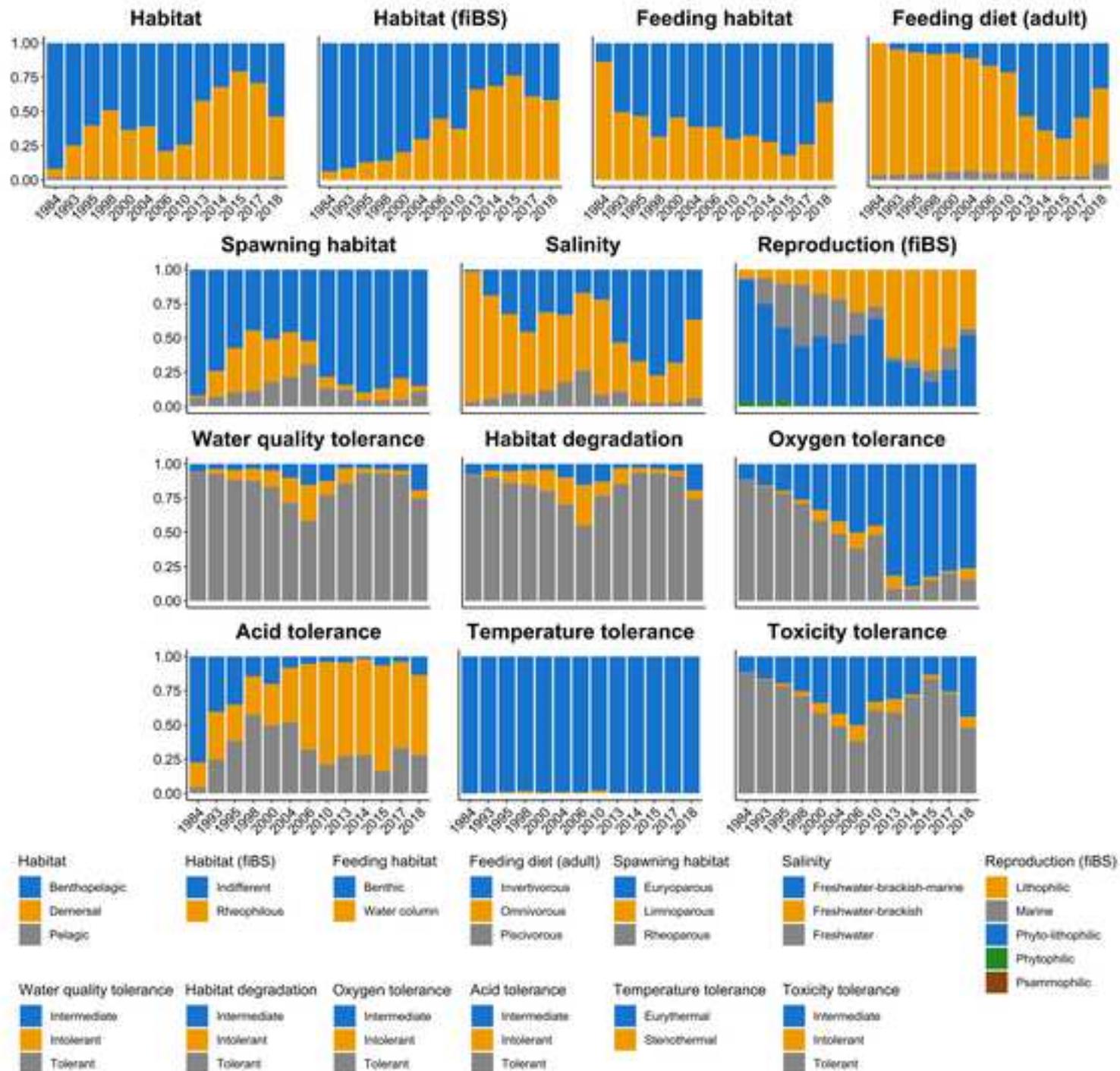


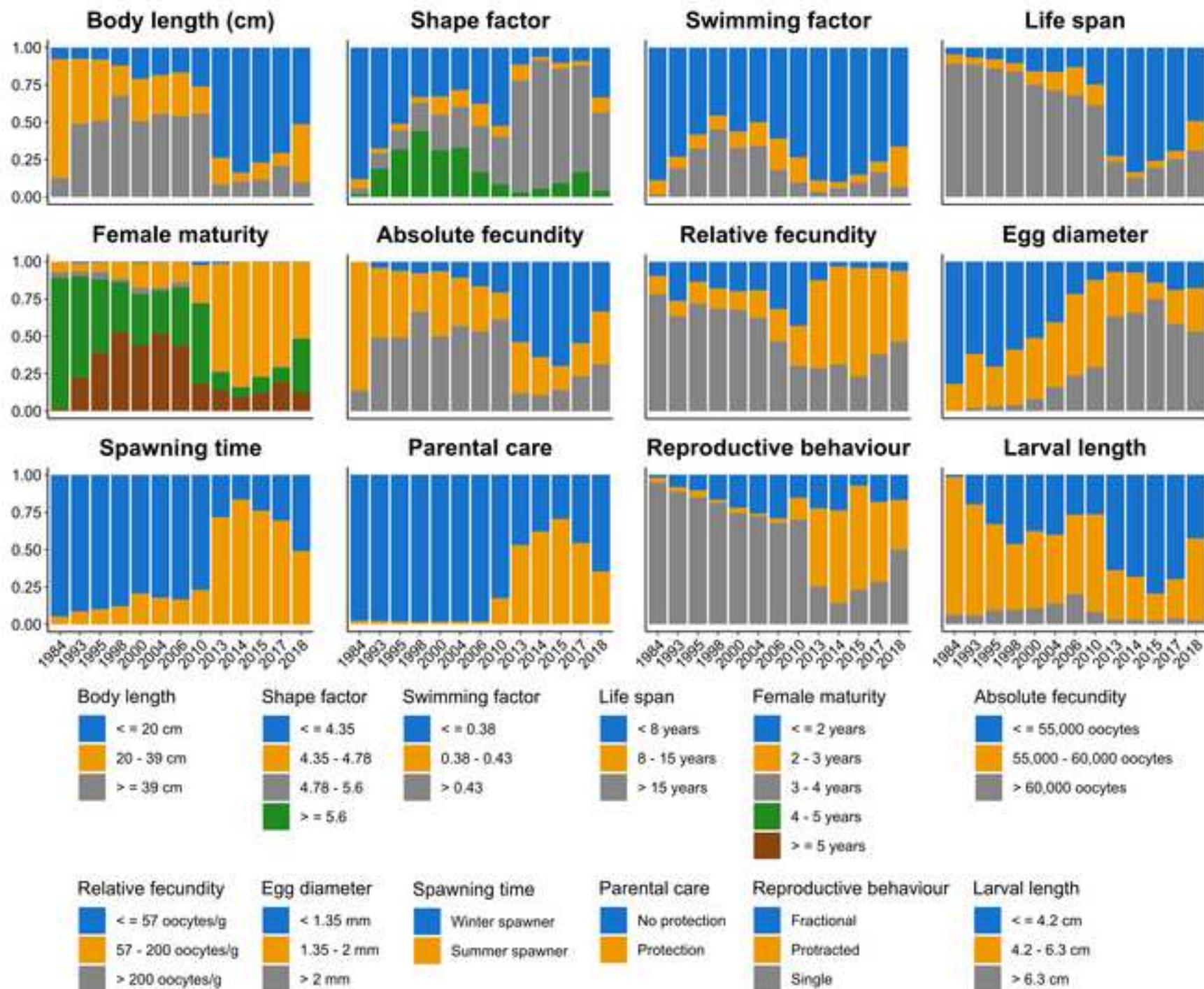


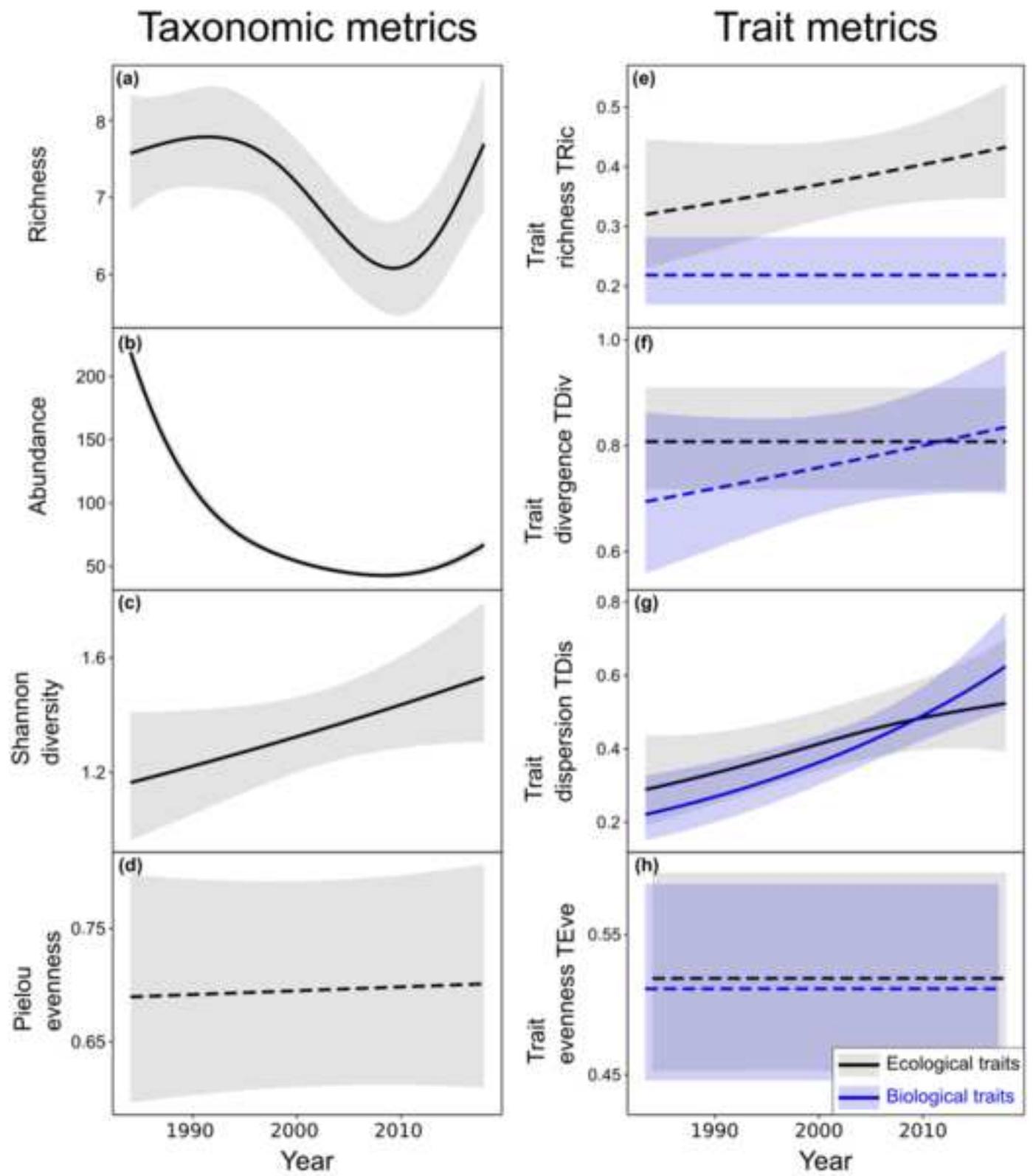












Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRedit authorship contribution statement

GLH: Methodology, Formal analysis, Data Curation, Writing - Original Draft, Writing - review & editing.

IS, PJH, PB: Conceptualization, Supervision, Formal analysis, Writing - Original Draft, Writing - review & editing

PH, AK, CL, LN, NT, DR: Resources, Writing - Review & Editing.