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3 **Trophic consequences of introduced species: comparative impacts of increased**
4 **inter-specific versus intra-specific competitive interactions**

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15 **Running title:** Trophic niche variation

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20 **Abstract**

21

22 1. Invasive species can cause substantial ecological impacts on native
23 biodiversity. Whilst ecological theory attempts to explain the processes
24 involved in the trophic integration of invaders into native food webs and their
25 competitive impacts on resident species, results are equivocal. In addition,
26 quantifying the relative strength of impacts from non-native species (inter-
27 specific competition) versus the release of native conspecifics (intra-specific
28 competition) is important but rarely completed.

29 2. Two model non-native fishes, the globally invasive *Cyprinus carpio* and
30 *Carassius auratus*, and the model native fish *Tinca tinca*, were used in a pond
31 experiment to test how increased intra- and inter-specific competition
32 influenced trophic niches and somatic growth rates. This was complemented
33 by samples collected from three natural fish communities where the model
34 fishes were present. The isotopic niche, calculated using stable isotope data,
35 represented the trophic niche.

36 3. The pond experiment used additive and substitutive treatments to quantify the
37 trophic niche variation that resulted from intra- and inter-specific competitive
38 interactions. Although the trophic niche sizes of the model species were not
39 significantly altered by any competitive treatment, they all resulted in patterns
40 of inter-specific niche divergence. Increased inter-specific competition caused
41 the trophic niche of *T. tinca* to shift to a significantly higher trophic position,
42 whereas intra-specific competition caused its position to shift towards elevated
43 $\delta^{13}\text{C}$. These patterns were independent of impacts on fish growth rates, which
44 were only significantly altered when inter-specific competition was elevated.

- 45 4. In the natural fish communities, patterns of trophic niche partitioning between
46 the model fishes was evident, with no niche sharing. Comparison of these
47 results with those of the experiment revealed the most similar results between
48 the two approaches were for the niche partitioning between sympatric *T. tinca*
49 and *C. carpio*.
- 50 5. These results indicate that trophic niche divergence facilitates the integration
51 of introduced species into food webs, but there are differences in how this
52 manifests between introductions that increase inter- and intra-specific
53 competition. In entirety, these results suggest that the initial ecological
54 response to an introduction appears to be a trophic re-organisation of the food
55 web that minimises the trophic interactions between competing species.

56

57 **Keywords:** Biological invasions; global change; isotopic niche; niche divergence.

58

59 **Introduction**

60

61 Biological invasions are a substantial driver of global environmental change that have
62 major implications for native biodiversity (Simerloff et al. 2013). When an introduced
63 species establishes a population then ecological impacts on native species can be
64 incurred through competitive interactions (Gozlan et al. 2010). These impacts can be
65 particularly strong where the invader and native species are closely related (Ricciardi
66 & Atkinson 2004; Li et al. 2015) or functionally similar (Dick et al. 2016, 2017), as
67 the species are more likely to be foraging on the same food resources (Buoro et al.
68 2016).

69

70 A number of niche-based hypotheses have been suggested to explain the processes
71 that facilitate the development of successful invasions and enable native species to
72 coexist trophically with the invader (Ricciardi et al. 2013). For example, the
73 utilisation of unexploited resources by an invader would avoid competitive
74 interactions with native species and so facilitate their integration into the food web
75 (Okabe & Agetsuma 2007; Mason et al. 2008; Juncos et al. 2015). In situations where
76 resources are fully exploited then niche theory predicts that competitive interactions
77 between invasive and native species will result in both their niches being smaller than
78 in allopatry (Bolnick et al. 2010; Tran et al. 2015; Jackson et al. 2016a). Conversely,
79 increased inter-specific competition might result in species increasing their niche
80 breadths to maintain their energy requirements (Svanbäck & Bolnick 2007).
81 Moreover, where the non-native species is a superior competitor, they can compete
82 for the same resources as used by native species and result in the competitive
83 exclusion of that native species (Tran et al. 2015). This can cause niche shift or

84 replacement in the native species, leading to reduced food intake, slower growth rates
85 and/ or reduced population density (Bøhn, Amundsen & Sparrow 2008).

86

87 Ecological impacts resulting from increased competitive interactions due to
88 introductions are, however, not limited to non-native species. Impacts can also
89 develop when the population of a native species increases in abundance. Whilst this
90 can occur naturally through strong recruitment, it also occurs when conspecifics are
91 released to increase population size ('stocking') (Bašić & Britton 2016). In fishes of
92 the Salmonidae family, ecological impacts from stocking with conspecifics are often
93 stronger than those resulting from non-native fishes (Buoro et al. 2016). This is due to
94 the 'pre-adaptation hypothesis', where the released fishes have almost identical
95 functional traits as resident species and so have a similar ability to acquire resources
96 (Ricciardi & Mottiar 2006). The subsequent increase in intra-specific competition
97 then potentially results in similar ecological consequences to those resulting from
98 inter-specific competition (Carey et al. 2012), and can be tested using the similar
99 hypotheses (Svanbäck & Bolnick 2007; Bøhn, Amundsen & Sparrow 2008; Ricciardi
100 et al. 2013). However, differences in the processes by which ecological impacts result
101 from intra- versus inter-specific competitive interactions remain poorly understood for
102 many taxa.

103

104 The aim of this study was to therefore test the trophic consequences for populations of
105 a model native species from increased intra- and inter-specific competitive
106 interactions that result from introductions. The model species were freshwater fishes,
107 as they are adaptable and tractable experimental animals that provide excellent model
108 systems for competitive studies (Ward, Webster & Hart 2006). For example, their

109 indeterminate nature of growth enables correlation with competitive success (Ward,
110 Webster & Hart 2006). The model native species was tench *Tinca tinca*, a fish of the
111 Cyprinidae family with a native range across most of Eurasia (Fishbase 2017). Its
112 rationale for use as a model was that its omnivory and broad environmental tolerances
113 potentially provide highly plastic responses to increased competition (Guo et al.
114 2016). As the drivers of invasion success of non-native fishes include their functional
115 similarities to many native fishes, then the model species used to increase inter-
116 specific competition were the global cyprinid invaders carp *Cyprinus carpio* and
117 goldfish *Carassius auratus*. Both fishes are primarily benthic foragers that exploit a
118 wide range of trophic resources and so they are trophic analogues of *T. tinca* (Weber
119 & Brown 2011; Guo et al. 2016). All of the model fishes are exploitative competitors
120 and thus were assumed to overlap in their diet in situations where food resources are
121 limited.

122

123 Understanding the trophic interactions of invasive and native fishes is enhanced when
124 experimental approaches are coupled with studies of invaded natural communities as,
125 in combination, they enable ecological patterns and processes to be understood over a
126 range of temporal and spatial scales (Tran et al. 2015). Consequently, using stable
127 isotope analysis (SIA; $\delta^{13}\text{C}$, $\delta^{15}\text{N}$) to determine trophic relationships, the model
128 species were initially used in a pond experiment based on additive and substitutive
129 treatments. This experiment tested the following predictions: (1) competitive
130 interactions between the model fishes significantly alters the size of their trophic
131 niches and reduces their somatic growth rates; and (2) impacts from inter- and intra-
132 specific competition are similar on the size and position of the trophic niche of the
133 native species. Then, samples of the model fishes collected from invaded natural

134 communities tested the prediction that: (3) the trophic relationships of the model
135 species are similar in the experimental simulations and their populations in the wild.

136

137 **Materials and Methods**

138

139 *Experimental design*

140 The experimental design (hereafter referred to as the ‘experiment’) used 10 additive
141 and substitutive treatments across a combination of allopatric and sympatric contexts,
142 with each treatment replicated three times (Table 1). Three control treatments used
143 each species in allopatry (‘Allopatry’; N = 8; Table 1). Three substitutive treatments
144 then paired the native and non-native species in their different sympatric
145 combinations (‘Sympatry’; n = 4+4, N = 8; Table 1). It was these three treatments
146 whose isotopic niches of the sympatric fishes were compared to those from the
147 invaded natural communities (Table 1, 2). Three additive treatments then used all
148 three species in sympatry across three different abundances (‘Inter-specific
149 competition’; n = 4+4+4, 8+8+8, 12+12+12; N = 12, 24, 36; Table 1). Finally, a
150 single treatment used the native species in higher abundance (‘Intra-specific
151 competition’; N = 12). All the fish used in the treatments were juveniles and had been
152 hatchery reared. As their starting lengths were 45 to 60 mm and starting weights < 10
153 g, the predicted stable isotope half-life for their dorsal muscle was 36 and 38 days for
154 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively (Thomas & Crowther 2015).

155

156 The experiment was completed using the treatments within enclosures that sat within
157 a larger, man-made pond (30 x 30 m; 1 m consistent depth) that was located in
158 Southern England. Following Bašić & Britton (2016), the enclosures comprised of an

159 aluminium frame (length 1.66 m; width: 1.05 m; height: 1.2 m) within a net (mesh: 7
160 mm²) that prevented fish in- and egress, but allowed movements of invertebrates. The
161 enclosures were placed randomly across the pond, with at least 0.5 m between them;
162 they were sufficiently heavy that they remained *in-situ* throughout the experimental
163 period without movement and they sat on the substrate, with macrophytes able to
164 grow within each of them (primarily *Elodea* spp.). Bird predation was prevented via
165 netting over the enclosures (15 mm mesh). The experiment ran for 150 days from
166 April 2016. This duration enabled fish dorsal muscle to undergo approximately 4 half-
167 lives and so by its conclusion, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data of the experimental fish would
168 represent their diet in the ponds (Thomas & Crowther 2015). All fish were weighed
169 (nearest 0.1g) prior to release into the enclosures. Temperature loggers (TinyTag
170 TGP-4017) in the larger pond revealed the mean water temperature was 18.1 ± 0.6 °C
171 during the experiment. On day 150, all the fish were recovered from the enclosures,
172 euthanized (anaesthetic overdose, MS-222) and taken to the laboratory. For the
173 purpose of stable isotope analysis, macroinvertebrate and macrophyte samples were
174 taken from the larger pond, sorted into samples (one sample = 3 to 9 individuals per
175 species), with triplicate samples taken.

176

177 *Invaded wild fish communities*

178 Three wild pond fish communities (hereafter referred to as the ‘invaded
179 communities’) were used, with each having two of the model species present within a
180 mixed community of other fishes (Table 2). At each site, the model fishes had been
181 present for at least five years prior to sampling. Although data from each of these fish
182 communities have been reported previously, the data used in this paper have not
183 previously been compared (Table 2). Note that replicates of each combination of

184 species were not used due to inherent logistical difficulties of locating sites where each
185 species was present and sufficiently abundant to provide adequate sample sizes. Also,
186 at Site 2, whilst *C. carpio* was present in sympatry with a *Carassius* species, this was
187 identified in the field as *Carassius gibelio*. However, Busst & Britton (2017)
188 indicated that *Carassius* species generally have high trophic similarity due to their
189 similar functional traits (Busst & Britton 2017) and thus *C. gibelio* was used as a
190 surrogate of *C. auratus* at Site 2. Site 1 and 2 was sampled in spring 2013, whereas
191 Site 3 was sampled in March 2008. At each site, fish sampling incorporated electric
192 fishing, seine nets, fish traps and fyke nets. Following their capture, the fish were
193 euthanized and returned to the laboratory for processing. The sample size for stable
194 isotope analysis was a minimum of 10 individuals per species, with individuals
195 randomly selected across the length range sampled (Table 2). As these were fish
196 sampled from the wild then this random selection resulted in a wider length range of
197 fish being used than was the case in the experiment (Table 2).

198

199 *Stable isotope analysis*

200 In the laboratory, fish from the experiment and the invaded communities were
201 measured and weighed, and a dorsal muscle sample taken for stable isotope analysis
202 (SIA). SIA sample sizes were 10 fish per species for Sites 1 and 2, and 15 per species
203 for Site 3. Along with the macro-invertebrate samples, all samples were dried at 60°C
204 to constant mass before stable isotope analysis (SIA) ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) at the Cornell
205 University Stable Isotope Laboratory, New York, USA, where they were ground to
206 powder and weighed precisely to ~1000 μg in tin capsules and analysed on a Thermo
207 Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a
208 NC2500 elemental analyser (CE Elantach Inc., USA). Analytical precision associated

209 with the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ sample runs was estimated at 0.42 and 0.15 ‰ respectively.
210 Data outputs were in the format of delta (δ) isotope ratios expressed per mille (‰).
211 There was no lipid correction applied to the data as C:N ratios indicated very low
212 lipid content (Post et al. 2007).

213

214 *Data analysis*

215 The SIA data from the experiment and invaded communities were used to calculate
216 the trophic niche size of each fish species using the isotopic niche (Jackson et al.
217 2011). Whilst closely related to the trophic niche, the isotopic niche is also influenced
218 by factors including growth rate and metabolism, and thus represents a close
219 approximation of the trophic niche (Jackson et al. 2011). It was calculated using
220 standard ellipse areas (SEA) in SIBER (Jackson et al. 2012), a bivariate measure of
221 the distribution of individuals in isotopic space; as each ellipse encloses $\approx 40\%$ of
222 data, they reveal the population's typical resource use (Jackson et al. 2011). The
223 generally small sample sizes used in both study components (i.e. <30) meant a
224 Bayesian estimate of SEA (SEA_B) was used to test differences in niche sizes between
225 species, calculated using a Markov chain Monte Carlo simulation (10^4 iterations per
226 group) (Jackson et al. 2012). Where 95% confidence intervals of SEA_B overlapped
227 between comparator species, the isotopic niches were interpreted as not being
228 significantly different in size. The stable isotope data were then used to calculate
229 isotopic niche overlap (%) between the species in each treatment and across
230 treatments using SEA_c calculated in SIBER, where subscript 'c' indicates a small
231 sample size correction was used (Jackson et al. 2012). Use of SEA_c was only to get a
232 representation of the extent of niche overlap between species, as it is more strongly

233 affected by small sample sizes <30 than SEA_B (Jackson et al. 2012; Syväranta et al.
234 2013).

235

236 For the invaded communities, SEA_B and SEA_c was calculated for each model species
237 and compared between the species within each site, but not between sites due to the
238 multiple context dependencies that can influence niche sizes between wild
239 populations (Tran et al. 2015). For the experiment, as the treatments were completed
240 within the same larger pond, all the fish had the same isotopic baseline and thus their
241 SI data and niche data were able to be compared between species and treatments
242 without any correction. Data per species were combined from replicates for each
243 treatment to provide representative sample sizes sufficient for analyses of SEA_B and
244 SEA_c . A minimum of four randomly chosen individuals was used from each replicate
245 to provide both a balanced dataset across the experiment and a minimum sample size
246 per treatment of 12 fish per species (Table 1; Appendix S1 in Supporting information,
247 Fig. S1 to S3).

248

249 In the experiment, to then test differences in the SI data between species and
250 treatments, $\delta^{15}N$ was converted to trophic position (TP) from $TP_i = [(\delta^{15}N_i -$
251 $\delta^{15}N_{base})/3.4] + 2$, where TP_i = trophic position of the individual fish, $\delta^{15}N_i$ = fish
252 isotopic ratio, $\delta^{15}N_{base}$ = macro-invertebrate isotopic ratio, 3.4 = discrimination
253 between trophic levels, and 2 = trophic position of baseline macro-invertebrates
254 (Jackson and Britton 2014). TP and $\delta^{13}C$ data were used in linear mixed effects
255 models (LMEM) to test differences between treatments per species, with enclosure
256 used as a random effect on the intercept to avoid inflating the degrees of freedom that
257 would occur if individual fish were used as true replicates (Tran et al. 2015). The

258 starting mass of fish in the enclosure was also initially used as a covariate, but was
259 removed from all final models due to its effects not being significant ($P > 0.05$ in all
260 cases). For each model, differences between species and treatment were assessed
261 using estimated marginal means and linearly independent pairwise comparisons with
262 Bonferroni adjustment for multiple comparisons.

263

264 To determine fish growth rates in the experiment, the mean specific growth rate
265 (SGR) per model species and replicate was determined from: $[(\ln W_{t+1}) - (\ln W_t)]/n/t$,
266 where W_t = total starting weight, W_{t+1} = total end weight, n = the number of fish used
267 to determine W , and t = the duration of the experiment (days). A generalized linear
268 model (GLM) tested the differences in SGR between treatments for each species. In
269 each GLM, SGR was the dependent variable and treatment was the independent
270 variable; total starting mass of fish per replicate initially used as a covariate and was
271 retained in the final model when its effect on SGR was significant.

272

273 **Results**

274

275 *Trophic impacts of inter-specific competition from non-native species*

276 Across all of the experimental treatments, the isotopic niche sizes (as SEA_B) of each
277 species varied, but their 95% confidence intervals always overlapped between
278 allopatry and sympatry, indicating no significant differences in isotopic niche size
279 caused by the competition scenarios (Table 3). The LMEMs testing differences in TP
280 and $\delta^{13}C$ between treatments for each species were significant ($P < 0.01$), except TP
281 in *C. carpio* ($P = 0.47$) (Table S1 to S3). Pairwise comparisons revealed the
282 significant shifts TP and $\delta^{13}C$ were mainly between the allopatric treatments and the

283 inter-specific competition (8) and (12) treatments ($P < 0.05$; Fig. 1; Tables S1 to S3).
284 In the inter-specific competition treatments, the pattern for *T. tinca* was a shift to
285 significantly higher TP and higher $\delta^{13}\text{C}$, for *C. carpio*, the only shift was to
286 significantly higher $\delta^{13}\text{C}$, and for *C. auratus*, the significant shifts were to lower TP
287 and higher $\delta^{13}\text{C}$ (Fig. 1). Where shifts were to elevated values of $\delta^{13}\text{C}$, the fish were
288 moving towards using macrophyte as an energy source (mean $\delta^{13}\text{C}$: $-24.37 \pm 0.88 \text{ ‰}$),
289 away from macro-invertebrate prey resources (Chironomid larvae, Corixidae,
290 Odonata and Ephemeroptera: mean $\delta^{13}\text{C}$: $-30.57 \pm 1.28 \text{ ‰}$).

291

292 These shifts in isotopic positions resulted in some divergence in the isotopic niches of
293 each species between allopatry and sympatry. In allopatry, *T. tinca* shared 39 % of
294 their isotopic niche with *C. auratus*, but this reduced to 14 % in sympatry; *T. tinca*
295 only shared 2 % of their isotopic niche with *C. carpio* in allopatry, but this reduced to
296 0.3 % in sympatry (Fig. S4). In contrast, *C. carpio* shared 74 % of their niche with *C.*
297 *auratus*, and only reduced to 52 % in sympatry (Fig. S4). In the three inter-specific
298 competition treatments (Table 1), *T. tinca* no longer shared any of their isotopic niche
299 with either non-native species, whereas the extent of shared *C. carpio* niche with *C.*
300 *auratus* was reduced to between 15 and 26 % (Fig. S5).

301

302 *Trophic impacts of intra- versus inter-specific competition*

303 The LMEMs testing differences in *T. tinca* $\delta^{13}\text{C}$ and TP between allopatry and the
304 intra- and inter-specific competition experimental treatments were significant ($P <$
305 0.01 ; Table S4). For $\delta^{13}\text{C}$, there was a significant difference between allopatry and the
306 intra-specific competition treatment (allopatry: $-27.72 \pm 0.51 \text{ ‰}$; intra-specific
307 competition: $-26.25 \pm 0.54 \text{ ‰}$; $P < 0.01$), and between allopatry and the inter-specific

308 competition (12) treatment (-25.82 ± 0.38 ‰, $P < 0.01$) (Table S4; Fig. 1). Their TP
309 was significantly higher in allopatry than the intra-specific competition treatment
310 (allopatry: 3.21 ± 0.06 ‰; intra-specific competition: 3.10 ± 0.05 ; $P = 0.05$; Table S4;
311 Fig. 2). In the inter-specific competition treatments, the *T. tinca* niche shift was to
312 higher trophic positions compared to allopatry, with these differences significant in
313 the (8) and (12) treatments ($P \leq 0.05$; Table S4; Fig. 2). In the treatments when
314 numbers of fish were equal ($N = 12$), allopatric TP (3.10 ± 0.05) was significantly
315 lower than when in competition with the two non-native fishes (3.33 ± 0.07) ($P <$
316 0.01 ; Fig. 2). Regarding overlaps in isotopic niches (as SEA_c), the intra-specific
317 competition treatment shared 15 % of their niche with the allopatric treatment and 3
318 % with the inter-specific competition (4) treatment (Fig. 2); this reduced to 0 % for
319 inter-specific competition (12) treatment.

320

321 *Impacts of intra- and inter-specific competition on specific growth rates*

322 In each experimental treatment, there was an increase in total fish mass in each
323 species across the 150 days and thus all mean SGRs were positive (Fig. 3). The GLM
324 testing the effect of treatment on SGR was significant for each species (*T. tinca*: Wald
325 $\chi^2 = 139.39$, d.f. = 6, $P < 0.01$; *C. carpio*: Wald $\chi^2 = 35.50$, d.f. = 5, $P < 0.01$; *C.*
326 *carassius*: Wald $\chi^2 = 13.73$, d.f. = 5, $P = 0.02$). The effect of starting mass as a
327 covariate on SGR was significant for *T. tinca* ($P < 0.01$) and *C. carpio* ($P = 0.02$) and
328 so it was retained in their final models. It was not significant for *C. auratus* ($P = 0.48$)
329 and so it was removed from their final model. Pairwise comparisons of differences in
330 mean SGR between allopatry and the other treatments revealed that for each species,
331 significantly decreased SGR was only apparent in the inter-specific competition (8)
332 and (12) treatments ($P < 0.01$) (Fig. 3).

333 *Trophic relationships in the invaded communities versus the experiment*

334 In the invaded communities of Sites 1 and 3, there were no significant differences in
335 the isotopic niche sizes of the sympatric model fishes (Site 1: *T. tinca*: 1.92 to 3.01
336 ‰², *C. auratus*: 1.64 to 2.41 ‰²; Site 3: *T. tinca*: 3.04 to 4.27 ‰², *C. carpio*: 2.97 to
337 4.03 ‰²) (Fig. 5). In Site 2, however, the isotopic niche of *C. carpio* (3.12 to 4.70)
338 was significantly higher than the *Carassius* spp. (0.94 to 1.89 ‰²) (Fig. 4). In the
339 invaded communities, the isotopic niches of fishes were highly divergent with no
340 overlap. Whilst they showed some consistency with the patterns identified within the
341 experiment, their niches were also more divergent (Fig. 4). There were also
342 similarities in the relative positions of their niches in isotopic space between the
343 experiment and invaded communities (Fig. 4). For example, in both the invaded
344 communities and the experiment, the isotopic niche of *T. tinca* was at a higher trophic
345 position than *C. carpio*, but had similar values of $\delta^{13}\text{C}$ (Fig. 4). The sympatric
346 treatment of *T. tinca* and *C. auratus* was the least similar to their pattern in the
347 invaded communities, although the *T. tinca* niche was at the higher trophic position in
348 both contexts (Fig. 4).

349

350 **Discussion**

351

352 The results of the experiment revealed that increased competition did not cause any
353 significant shifts in isotopic niche sizes, contrary to Prediction 1. Instead, divergence
354 in the isotopic niches of the fishes occurred, with this independent of shifts in somatic
355 growth rates. Both increased intra- and inter-specific competition impacted the
356 isotopic niche of the model native species, as per Prediction 2. However, the impact
357 on the isotopic niche differed between the competition types. Increased inter-specific

358 competition resulted in the niche shifting to a significantly higher trophic position,
359 whereas increased intra-specific competition caused the niche to shift towards
360 elevated $\delta^{13}\text{C}$. Finally, there were similar patterns of trophic niche divergence
361 between the model species in the experiment and invaded communities, as per
362 Prediction 3. This suggests that experimental predictions can help the understanding
363 of how trophic relationships develop in invaded communities in the wild.

364

365 Ecological theory relating to invasions posits that invaders can out-compete similar
366 native competitors by occupying a broader niche (Elton 1958). This has been
367 supported empirically in studies involving non-native taxa such as invasive crayfish
368 (e.g. Olsson et al. 2009; Ercoli et al. 2014). By occupying a broad niche, theory
369 suggests that the invader suppresses the niche size of trophically analogous native
370 species (Thomson 2004; Jackson et al. 2016a). Conversely, other studies have
371 suggested that when in sympatry, the trophic niche of both the invader and native
372 species will constrict due to dietary specialisations (Tran et al. 2015; Jackson et al.
373 2016a). In the experiment, there were no significant shifts in the trophic niche sizes of
374 each species between their treatments. This finding is, therefore, contrary to these
375 aspects of invasion theory. Where an invader competes for the same resources as
376 being used by a native species then, if that invader is a superior competitor, it has
377 been predicted that their interactions will competitively exclude the native species.
378 This can result in a shift in the position of the niche of the native species, potentially
379 resulting in reduced food intake and suppressing growth rates (Bøhn, Amundsen &
380 Sparrow 2008). The experiment results had some consistency with this prediction, as
381 all treatments resulted in a change in the position of the trophic niche of *T. tinca*. As
382 invader abundance increased in the inter-specific competition treatments, the extent of

383 niche divergence also increased. Whilst this suggests some competitive exclusion
384 driven by *C. carpio* and *C. auratus*, some niche shifts were also apparent in the intra-
385 specific competition treatment, suggesting the presence of some density dependent
386 effects. However, the divergent niches developed independently of changes in fish
387 growth rates, suggesting they enabled the fishes to maintain their food intake rates.

388

389 The results of the experiment were also consistent with patterns of inter-specific
390 trophic niche divergence detected in other invasive fishes. For example, Tran et al.
391 (2015) revealed that in allopatry, the diet of the Asian invasive fish *Pseudorasbora*
392 *parva* had the potential to overlap with some native fishes, but this never occurred in
393 sympatry. Niche divergence was also apparent between non-native pumpkinseed
394 *Lepomis gibbosus* and native fishes in both rivers and ponds (Jackson et al. 2016b;
395 Copp et al. 2017). Jackson and Britton (2014) detected partitioning between the
396 trophic niches of sympatric *P. parva*, *C. carpio* and signal crayfish *Pacifastacus*
397 *leniusculus* in ponds. In entirety, these results suggest that, in freshwater fishes at
398 least, the initial response to an invasion is trophic niche divergence, leading to niche
399 partitioning. This response reduces the strength of the competitive interactions, and
400 can occur independently of shifts in niche size and growth rates. This response also
401 occurs despite the high functional similarity of many of these fishes. Their traits must
402 thus be sufficiently different or plastic between the species to enable these dietary
403 specialisations to develop in sympatry (Jackson & Britton 2014; Tran et al. 2015;
404 Jackson et al. 2016b).

405

406 Recently, studies have suggested that ‘native invasions’, such as where wild
407 populations are supplemented by hatchery-reared conspecifics, can result in similar,

408 and sometimes stronger, ecological impacts than those caused by non-native invasions
409 (Carey et al. 2012; Buoro et al. 2016). In the experiment, the comparison of intra-
410 versus inter-specific competition across the treatments was also a simulation of a
411 ‘native’ versus ‘non-native’ invasion, where the driver of impact was from increased
412 competitive interactions. Whilst increased intra- and inter-specific competition both
413 impacted the isotopic niche of *T. tinca*, there were differences in how these impacts
414 manifested. Increased intra-specific competition caused the isotopic niche of *T. tinca*
415 to shift to a significantly lower trophic position that was significantly carbon enriched.
416 In contrast, increased inter-specific competition resulted in the isotopic niche of *T.*
417 *tinca* shifting to a significantly higher trophic position, with this also apparent in the
418 invaded communities. Whilst these results suggest that both ‘native’ and ‘non-native’
419 invasions can indeed impact native species (*cf.* Buoro et al. 2016), they indicate that
420 the impacts might differ between the invasion types (i.e. native vs. non-native). This
421 finding has potential implications for understanding how the model fishes can be
422 better used in fishery enhancement schemes, particularly regarding the numbers being
423 introduced in relation to the ecological effects they might incur (Bašić & Britton
424 2016). The experiment could not, however, determine how these differences between
425 intra- and inter-specific competitive differences developed temporally. It is therefore
426 recommended that this is explored in future work, such as through more controlled
427 experiments using a wider range of model species (e.g. Dick et al. 2017).

428

429 Each natural fish community had been invaded by either *C. carpio* or *Carassius* spp.
430 Within these multi-species communities, each sympatric combination of the model
431 species had isotopic niches that were divergent, with no sharing of isotopic space
432 between them. This might have been the result of differences in the length ranges of

433 the fishes in each site, especially in Site 3, resulting in the different size classes of fish
434 exploiting different food resources. However, these patterns were relatively consistent
435 with those detected in the experiment. They were also consistent with other studies on
436 invasive fishes that suggest isotopic niche partitioning, rather than niche convergence,
437 is the general pattern within invaded fish communities (e.g. Jackson & Britton 2014;
438 Tran et al. 2015; Basic & Britton 2016), except perhaps where the invader has
439 attained high population abundances (e.g. Britton, Davies & Harrod 2010). The data
440 from the invaded communities have the caveat that they were non-replicated wild
441 samples that were only sampled once, and were subject to uncontrolled environmental
442 conditions. Comparison of the results between the experiment and the invaded
443 communities did, however, indicate that when the model fishes were in the wild they
444 exhibited complete partitioning in their isotopic niches, a contrast to the experiment..
445 This might relate to the experiment being completed in relatively enclosed spaces,
446 resulting in reduced opportunities for exploiting different food resources. The invaded
447 communities were also more complex with higher species richness and so might have
448 contributed to their niche partitioning through stronger inter-specific competition.
449 Also, the experimental data were collated in relatively controlled conditions and over
450 shorter timeframes than the invaded communities. Indeed, ecological experiments
451 often have ‘scaling-up’ issues that arise from their limited timeframes (Korsu, Huusko
452 & Muotka 2009; Spivak, Vanni & Mette 2011). Mesocosm approaches have,
453 however, been used successfully for understanding the trophic relationships of
454 freshwater fishes (e.g. Jackson et al. 2013; Bašić and Britton 2016), with these
455 successfully extrapolated to wild populations to help explain ecological patterns (e.g.
456 Tran et al. 2015; Copp et al. 2017).

457

458 In summary, the experiment revealed isotopic niche divergence developed between
459 the model fishes when intra- and inter-specific competition was elevated. The
460 magnitude and direction of niche divergence in the model native fish did, however,
461 differ between intra- and inter-specific competition. Patterns of trophic niche
462 partitioning were also strongly apparent between the model fishes in the invaded
463 communities. As isotopic niche divergence occurred in the experiment in isolation
464 from niche constriction and impacts on growth rates, this suggests the initial
465 ecological response to an introduction is the trophic re-organisation of the food web to
466 minimise the interactions between the competing species.

467

468 **Author contributions**

469

470 RB and AN conceived the ideas and designed methodology; RB and HV collected the
471 data; RB and FAT analysed the data; RB and FAT led the writing of the manuscript.
472 All authors contributed critically to the manuscript and gave final approval for
473 publication.

474

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476

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481

482

483 **Data accessibility**

484

485 Data available from the Dryad Digital Repository: Experiment:
486 <http://dx.doi.org/10.5061/dryad.qn186> (Britton et al. 2017); Invaded communities:
487 <http://dx.doi.org/10.5061/dryad.12344> (Tran et al. 2015).

488

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619

620

621 **Supporting information**

622

623 Additional supporting information can be found in the online version of this article.

624 Appendix S1: Supplementary tables and figures

625 Table S1, S2, S3: Linear mixed model results for $\delta^{13}\text{C}$ and trophic position in the
626 experiment for *Tinca tinca*, *Cyprinus carpio*, and *Carassius auratus* respectively.

627 Table S4: Linear mixed model results for $\delta^{13}\text{C}$ and trophic position in the experiment
628 for intra- versus inter-specific experimental treatments of *Tinca tinca*.

629 Figures S1, S2, S3: Stable isotope bi-plots of the distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ per
630 replicate and treatment for *Tinca tinca*, *Cyprinus carpio*, and *Carassius auratus*
631 respectively.

632 Figure S4: Isotopic niche and niche overlap of the model species between allopatry
633 and sympatry in the experiment.

634 Figure S5: Isotopic niche and niche overlap of *Tinca tinca* between allopatry and the
635 competitive treatments in the experiment.

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Table 1. Structure of the treatments used in the experiment, showing the number of fish per species per treatment (n), and the total number of fish per treatment (N).

Treatment	<i>T. tinca</i> (n)	<i>C. carpio</i> (n)	<i>C. auratus</i> (n)	N
Allopatry (<i>T. tinca</i>)	8	0	0	8
Allopatry (<i>C. carpio</i>)	0	8	0	8
Allopatry (<i>C. auratus</i>)	0	0	8	8
Sympatry (<i>T. tinca</i> + <i>C. carpio</i>)	4	4	0	8
Sympatry (<i>T. tinca</i> + <i>C. auratus</i>)	4	0	4	8
Sympatry (<i>C. carpio</i> + <i>C. auratus</i>)	0	4	4	8
Intra-specific competition	12	0	0	12
Inter-specific competition (4)	4	4	4	12
Inter-specific competition (8)	8	8	8	24
Inter-specific competition (12)	12	12	12	36

Table 2. Details of the invaded communities (Sites 1 to 3), including their locations, sizes and information on the fish populations present.

Site	Country	Location	Size (m ⁻²)	Comparator model species (mean length ± 95% CI, mm)	Other fishes present	Reference
1	Wales	N: 51°41'10.0" W: 4°12'06.00"	3000	<i>T. tinca</i> (96 ± 20) <i>C. auratus</i> (60 ± 4)	<i>Scardinius erythrophthalmus</i> ; <i>Pseudorasbora parva</i>	Tran et al. 2015
2	Belgium	N:50°2'59'3.35" E: 5°20'10.52"	1900	<i>C. carpio</i> (70 ± 6) <i>Carassius</i> spp. (86 ± 10)	<i>S. erythrophthalmus</i> ; <i>Blicca bjoerkna</i> ; <i>Rutilus rutilus</i> <i>Leucaspis delineates</i> ; <i>Rhodeus amarus</i>	Tran et al. 2015
3	England	*N: 51°12" W: 0°34"	3000	<i>T. tinca</i> (174 ± 20) <i>C. carpio</i> (218 ± 72)	<i>P. parva</i>	Jackson & Britton (2013)

*Approximate location as exact location unable to be provided for business confidentially reasons relating to *P. parva* invasion and subsequent eradication

Table 3. Isotopic niche size (as lower and upper 95 % confidence intervals of SEA_B) of each species per treatment in the experiment.

Treatment	Species		
	<i>Tinca tinca</i>	<i>Cyprinus carpio</i>	<i>Carassius auratus</i>
Allopatry	0.63 - 1.48	0.42 - 1.19	0.74 - 1.84
Sympatry (<i>T. tinca</i> + <i>C. carpio</i>)	0.50 - 1.40	1.08 - 3.88	-
Sympatry (<i>T. tinca</i> + <i>C. auratus</i>)	0.29 - 1.06	-	0.32 - 1.07
Sympatry (<i>C. carpio</i> + <i>C. auratus</i>)	-	0.73 - 2.96	0.69 - 2.33
Inter-specific competition (4)	0.41 - 1.37	0.39 - 1.33	0.29 - 1.01
Inter-specific competition (8)	0.47 - 1.46	0.73 - 2.10	0.48 - 1.35
Inter-specific competition (12)	0.51 - 1.22	1.01 - 2.48	0.50 - 1.23

Figure captions

Figure 1. Mean $\delta^{13}\text{C}$ (clear circle) and trophic position (filled circle) per experimental treatment for *Tinca tinca* (A), *Cyprinus carpio* (B) and *Carassius auratus* (C). *difference between Allopatry and the treatment is significant at $P < 0.05$. Error bars represent 95 % confidence limits.

Figure 2. Comparison of the isotopic niche (as SEA_c) of *Tinca tinca* in the experiment according to: Allopatry (black circles, solid black line), intra-specific competition (grey circles, dashed black line), inter-specific competition (4) (clear circles, grey line), and inter-specific competition (8) (grey triangles, grey dashed line) (*cf.* Table 1).

Figure 3. Mean specific growth rate by experimental treatment (adjusted for the effect of starting mass in *T. tinca* and *C. carpio* in the generalised linear models) for: (A) *Tinca tinca*, (B) *Cyprinus carpio* and (C) *Carassius auratus*. The treatments on the X axis are as per Table 1. *difference between Allopatry and the treatment is significant at $P < 0.01$. Error bars represent 95% confidence limits.

Figure 4. Stable isotope bi-plots comparing the isotopic niches (as SEA_c) of sympatric *T. tinca*, *C. carpio* and *Carassius* spp. between the invaded communities and the experiment. Plots A, C, E are Field sites 1, 2 and 3 respectively. Plots B, D and F are the comparator sympatric treatments from the field experiment. *T. tinca*: clear circles and solid black line; *C. carpio*: grey circles, grey line; and *C. auratus*: black circles, black dashed line. Error bars represent 95% confidence limits. Note differences in the X and Y axes in A, C and E.

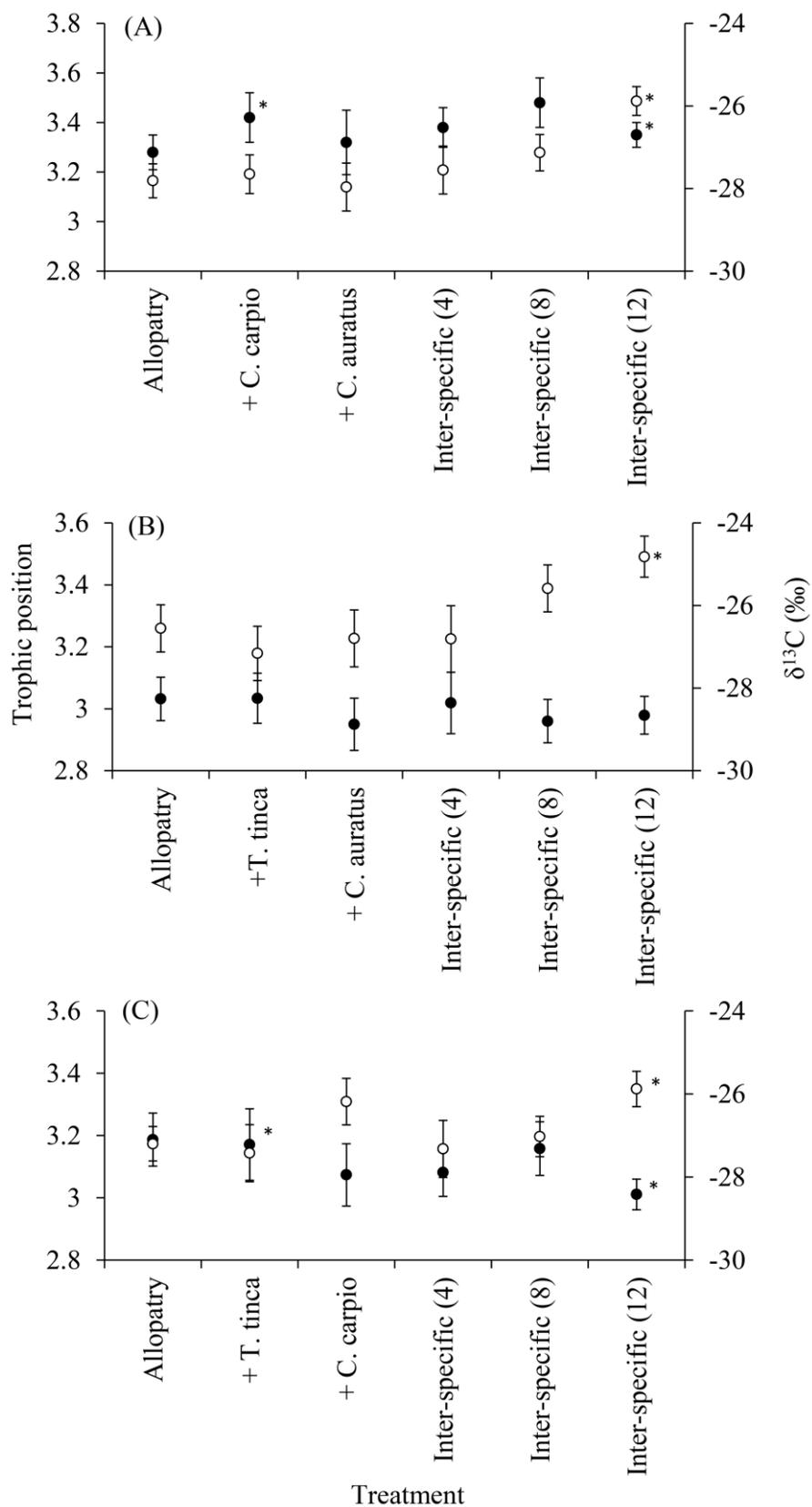


Figure 1.

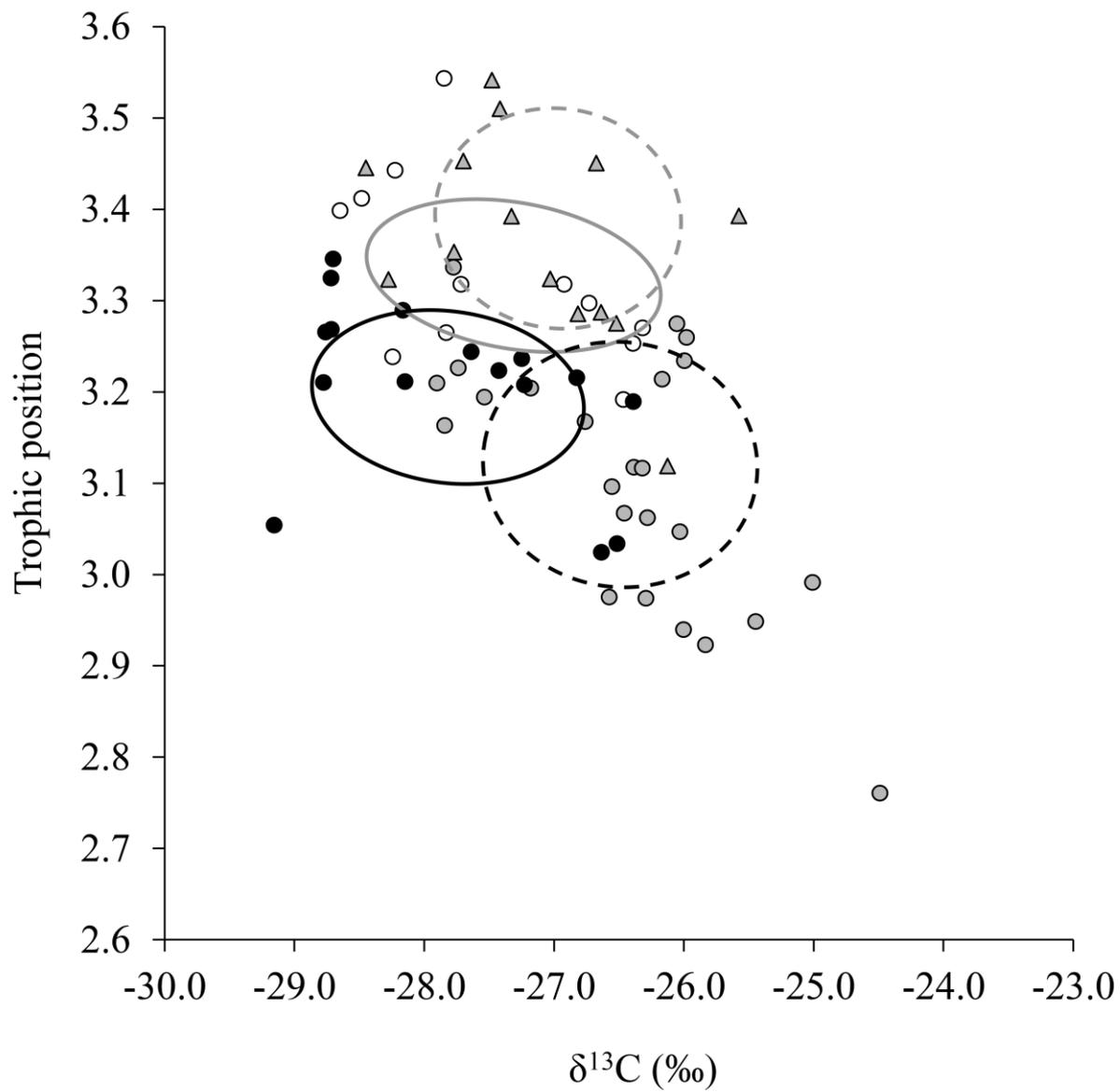


Figure 2.

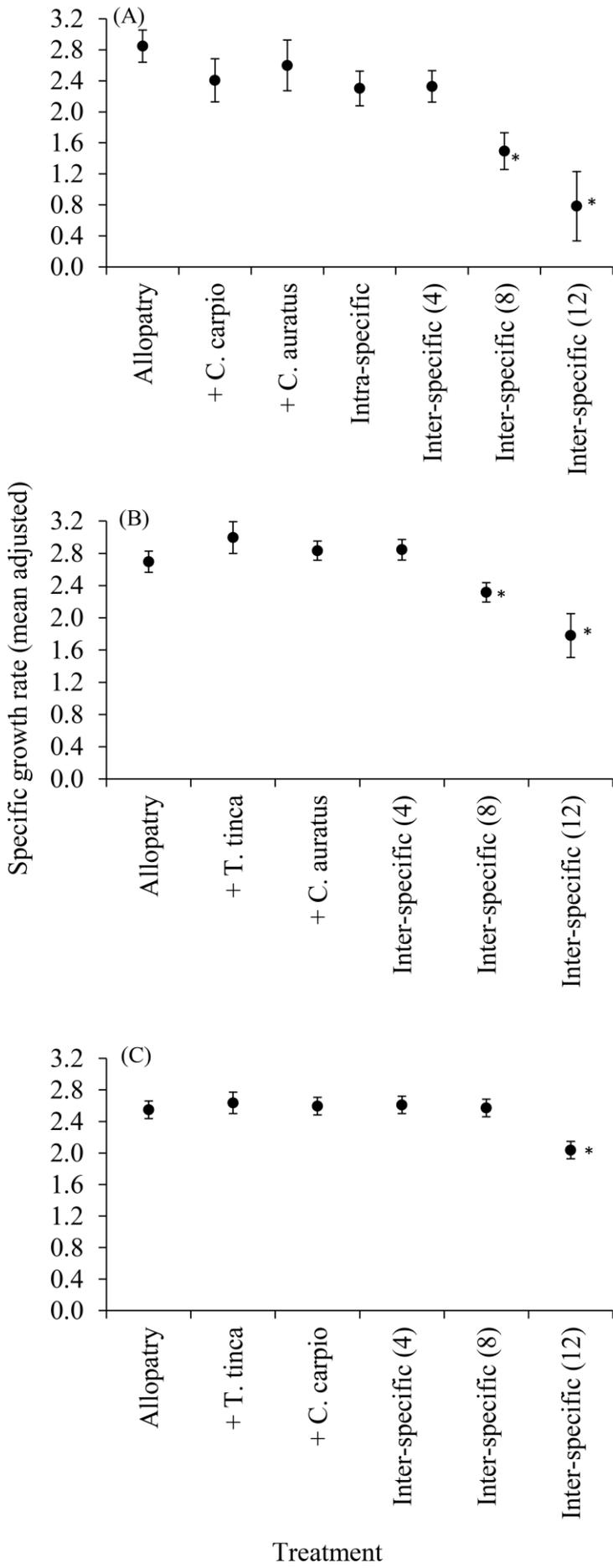


Figure 3.

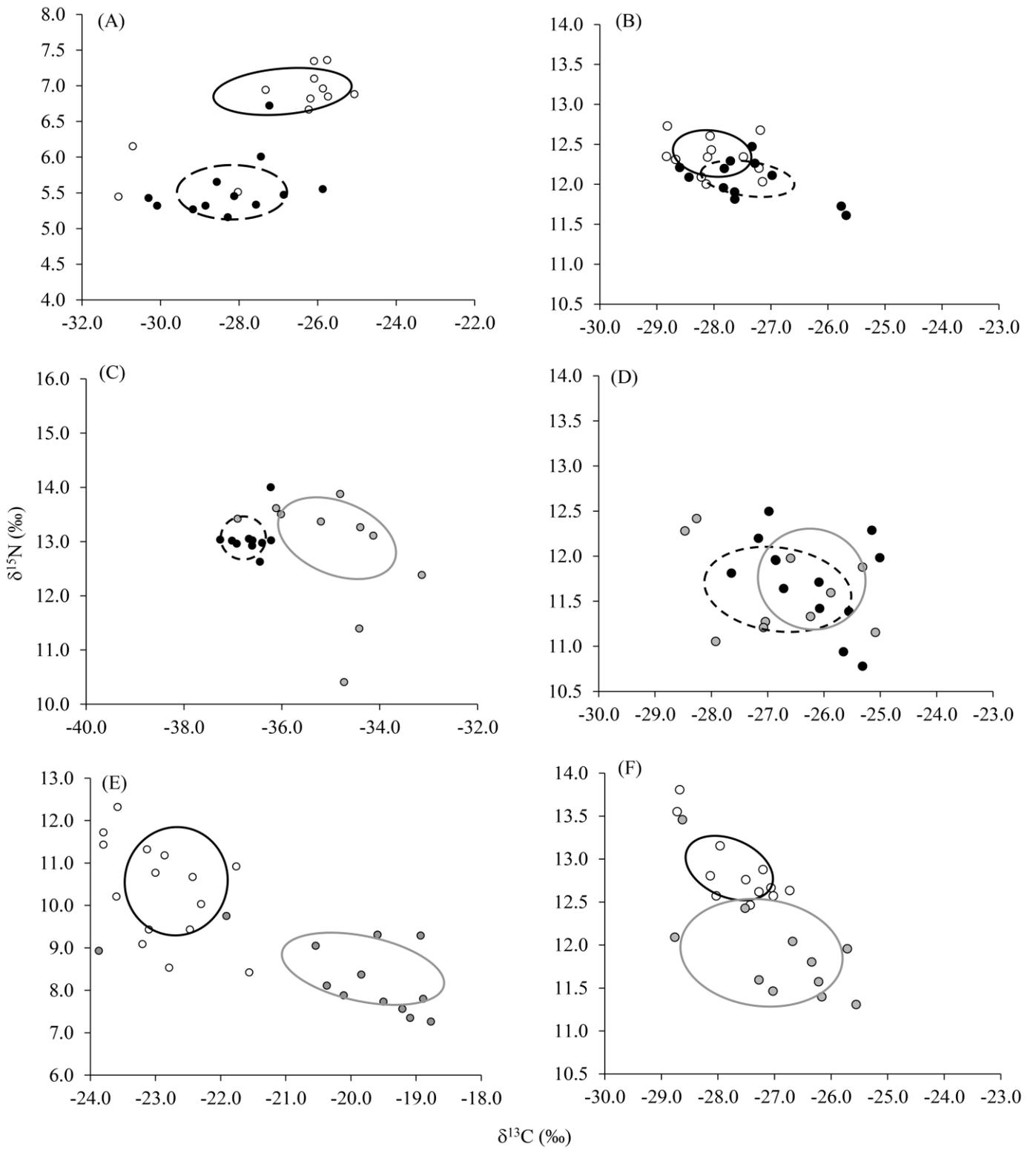


Figure 4.