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Resource composition mediates the effects of intraspecific variability in nutrient recycling on ecosystem processes

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Abstract:	Despite the growing evidence for individual variation in trophic niche within populations, its potential indirect effects on ecosystem processes remains poorly understood. Here, we first quantified the level of intraspecific trophic variability in 11 wild populations of the omnivorous fish Lepomis gibbosus. Outputs from stomach content and stable isotope analyses revealed that the degree of trophic specialization and trophic positions were highly variable between and within these wild populations. There was intrapopulation variation in trophic position of more than one trophic level, suggesting that individuals consumed a range of plant and animal resources. We then experimentally assessed how intraspecific trophic variability modulates consumer-mediated nutrient effects on relevant processes of ecosystem functioning. This was completed by manipulating intraspecific trophic variability through changes in the degree of specialization (i.e. specialist, intermediate and generalist) and using three food sources varying in nutrient quality (e.g. plant material, macro-invertebrate and fish meat). Food items were used individually (specialist individuals) or in combination (intermediate and generalist individuals) leading to the use of seven dietary experimental treatments. Results indicated that intraspecific variability in growth and nitrogen excretion rates were more related to the composition of the diet rather than the degree of specialization, and increased with the trophic position of the diet consumed. In contrast, phosphorous excretion rates did not change in accordance with these variables. We subsequently used microcosms in which excretory products were introduced and showed that critical ecosystem functions, such as primary production and community respiration, were affected by the variability in excretory products caused by the manipulation of intraspecific trophic variability, and this effect was biomass-dependent. These results highlight the importance of the interaction between individual trophic niche and consumer-mediated nutrient recycling in modulating ecosystem processes.

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1 **Resource composition mediates the effects of intraspecific variability in nutrient**
2 **recycling on ecosystem processes**
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15 **Abstract**

16 Despite the growing evidence for individual variation in trophic niche within populations, its
17 potential indirect effects on ecosystem processes remains poorly understood. In particular,
18 few studies have investigated how intraspecific trophic variability can modulate the effects of
19 consumers on ecosystems through potential changes in nutrient excretion rates. Here, we first
20 quantified the level of intraspecific trophic variability in 11 wild populations of the
21 omnivorous fish *Lepomis gibbosus*. Outputs from stomach content and stable isotope analyses
22 revealed that the degree of trophic specialization and trophic positions were highly variable
23 between and within these wild populations. There was intrapopulation variation in trophic
24 position of more than one trophic level, suggesting that individuals consumed a range of plant
25 and animal resources. We then experimentally manipulated intraspecific trophic variability to
26 assess how it can modulate consumer-mediated nutrient effects on relevant processes of
27 ecosystem functioning. Specifically, three food sources varying in nutrient quality (e.g. plant
28 material, macro-invertebrate and fish meat) were used individually or in combination to
29 simulate seven diet treatment. Results indicated that intraspecific variability in growth and
30 nitrogen excretion rates were more related to the composition of the diet rather than the
31 degree of specialization, and increased with the trophic position of the diet consumed. We
32 subsequently used microcosms and showed that critical ecosystem functions, such as primary
33 production and community respiration, were affected by the variability in excretory products,
34 and this effect was biomass-dependent. These results highlight the importance of considering
35 variation within species to better assess the effects of individuals on ecosystems and, more
36 specifically, the effects of consumer-mediated nutrient recycling because the body size and
37 the trophic ecology of individuals are affected by a large spectrum of natural and human-
38 induced environmental changes.

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40 **INTRODUCTION**

41

42 Within populations, trophic niches vary among individuals (e.g. Bolnick et al. 2003), which
43 can have strong consequences on population dynamics and community structure (Araújo et al.
44 2011). Intraspecific trophic variability may also alter the extent to which and how species
45 influence ecosystem functioning (Harmon et al. 2009), but the mechanisms underpinning
46 these changes remain relatively unknown. Consumers can exert top-down control through
47 resource consumption, potentially causing cascading effects to lower trophic levels via
48 density-mediated interactions (Pace et al. 1999). Simultaneously, they selectively sequester
49 consumed nutrients into their body to meet their requirements for growth and reproduction
50 (Sterner and Elser 2002). Excess nutrients and metabolic by-products are released via
51 excretion and egestion, potentially enhancing the "bottom-up" control of ecosystem processes
52 (Glaholt and Vanni 2005, Knoll et al. 2009). Consequently, consumer-mediated nutrient
53 recycling is an essential process within ecosystem dynamics, and predicting patterns in
54 variation of excretion rates is relevant to better understanding the effects of consumers on
55 ecosystem functioning (Taylor et al. 2015).

56 Early studies on consumer-mediated nutrient recycling have uncovered substantial
57 interspecific variations in rates of nitrogen and phosphorus excretion, ultimately depending on
58 elemental imbalance between consumers and their resources (Vanni et al. 2002, McIntyre et
59 al. 2007, Small et al. 2011). High variations in excretion rates among conspecific individuals
60 have also been reported (Villéger et al. 2012, El-Sabaawi et al. 2015a), with this potentially
61 affecting ecosystem processes (Bassar et al. 2010, Taylor et al. 2012, El-Sabaawi et al.
62 2015b). Intraspecific variability in excretion rates is difficult to predict, as it might result from
63 differences in both metabolic rate and trophic niche among individuals (Vrede et al. 2011).
64 Intraspecific trophic variability may create variations in nutrient excretion rates of consumers

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65 by acting on the degree of specialization and the quality of the resource consumed, with the
66 two factors potentially interacting. However, the relative importance of these two parameters
67 is poorly understood, notably because trophic specialization and diet composition are difficult
68 to assess in wild populations (e.g. Bolnick et al. 2003). By foraging on a single prey, specialist
69 individuals should display strongest nutrient imbalance with their resources compared to
70 generalists, particularly if the mismatch is large (Frost et al. 2005). Therefore, individuals
71 specialized on nutrient-rich resources are expected to release nutrient at higher rate than
72 individuals foraging on nutrient-poor resources (Sterner and Elser 2002). In addition,
73 specialist individuals are also predicted to have superior fitness than generalists (Bolnick et al.
74 2003). Alternatively, generalists might maximize their fitness by foraging on a resource
75 assemblage made of nutritionally complementary resources (DeMott 1998). Diet mixing, by
76 altering resource-consumer elemental imbalance, may lead to antagonistic or synergistic
77 effects on nutrient excretion rates in generalist individuals.

78 Fishes are important regulators of biogeochemical cycles in freshwater ecosystems,
79 due to their excretion of potentially limiting nutrients which are essential to support primary
80 producers and heterotrophic microbes (Small et al. 2011, Capps and Flecker 2013). Fish diet
81 composition varies considerably across trophic levels, ranging from herbivores consuming
82 plant, phytoplankton and algal detritus to apex-predators consuming vertebrates, and
83 including omnivores that feed across multiple trophic levels. These trophic groups often
84 display striking differences in nutrient excretion rates, which are apparent at both the inter-
85 and intraspecific levels (Villéger et al. 2012). Fishes are relevant examples of animals whose
86 populations show considerable intraspecific trophic variability (Smith and Skúlason 1996,
87 Bolnick et al. 2003), with individuals within populations often exhibiting a diversity of dietary
88 strategies, from generalist to specialist (Quevedo et al. 2009, Svanbäck et al. 2015). For
89 instance, the ratio of nitrogen and phosphorus excreted by Grizzard shad (*Dorosoma*

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90 *cepedianum*) was found to vary over nearly an order of magnitude among individuals, with
91 dietary shifts suggested as mainly being responsible for this intraspecific variability in
92 nutrient excretion rates (Pilati and Vanni, 2007). This highlights the requirement to account
93 for intraspecific trophic variability to better understand how fish can mediate nutrient
94 recycling and, ultimately, affect the functioning of aquatic ecosystems.

95 The aim of our study was to quantify the effects of intraspecific trophic variability on
96 consumer-mediated nutrient recycling and determine its potential consequences on relevant
97 ecosystem processes. We selected the omnivorous fish *Lepomis gibbosus* (Linnaeus, 1758) as
98 model species since it is an opportunistic omnivore that varies in its intensity of feeding on
99 animals (e.g. macro-invertebrates and fish) and plant material (e.g. algae, macrophytes, wind-
100 spread terrestrial seeds and detritus) (García-Berthou and Moreno-Amich 2000, Rezsu and
101 Specziar 2006). It also displays a high level of intraspecific trophic variability (McCairns and
102 Fox 2004, Bhagat et al. 2011). Using field and experimental approaches, the study was
103 conducted in three phases: a field survey to quantify trophic specialization and diet
104 composition in wild populations, a feeding experiment designed to test how specialization and
105 diet composition influenced nutrient excretion rates and growth rates of fish, and a laboratory
106 microcosm experiment used to assess effects of intraspecific variability of nutrient excretion
107 rates on ecosystem processes. Stomach content and stable isotope analyses (SCA and SIA,
108 respectively) were used in conjunction and performed on individuals of *L. gibbosus*
109 originating from 11 lakes to determine extent of population heterogeneity as revealed by
110 trophic specialization and difference in trophic position. In the laboratory, individuals of *L.*
111 *gibbosus* were provided with diet differing in the number and type of food items to test for
112 differences in nutrient excretion rates between specialists and generalists (Frost et al. 2005).
113 We hypothesized that, independently of the diet quality, high level of specialization would
114 lead to higher range of nutrient excretion rates within a population, given that diet diversity

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115 enables generalist individuals to cope better with nutrient imbalances than specialists (Frost et
116 al. 2005). We also predicted that individuals feeding on nutrient-rich resource would excrete
117 more nutrients (Sterner and Elser 2002, McIntyre and Flecker 2010). Finally, microbial
118 microcosms supplied with excretion products released by fish during the feeding experiment
119 were used to assess how intraspecific variation in nutrient excretion rates affected whole-
120 system metabolism and litter decomposition. Increased nutrient availability through consumer
121 recycling should stimulate rates of both autotrophic (primary production) and heterotrophic
122 (respiration and litter decomposition) processes. Moreover, as decomposers are better
123 competitors for nutrient resources than producers (Currie and Kalff 1984), it is also possible
124 that heterotrophic processes respond more to fish excretion products addition than autotrophic
125 processes.

126

127 **MATERIAL & METHODS**

128

129 *Field survey of intraspecific trophic variability*

130 To assess inter-individual variability in the trophic niches of *L. gibbosus*, populations were
131 sampled from 11 lakes that were former gravel pits in the flood plain of the Garonne River,
132 France (Zhao et al. 2016). Sampling was completed in similar weather conditions (21 to 24°C,
133 mixed cloud cover) between mid-September and mid-October 2012 by electrofishing along
134 the littoral shoreline (Evangelista et al. 2015). To reduce biases between lakes in SCA related
135 to the feeding period of consumers, one lake was sampled per day and electrofishing was
136 conducted during the same period of time in each lake (between 1:00 to 3:30 pm). Captured
137 individuals were immediately euthanized using an overdose of anaesthetic, stored on ice and
138 frozen in the laboratory (-20°C) until subsequent processing. After defrosting, a subsample of
139 28 adult individuals (mean fork length = 79.0 mm ± 1.4 SE) was selected in each population

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140 when available (mean = 27.6 ± 1.2 individuals per population; Table 1), measured for fork
141 length (FL ± 1 mm) and weighted (W ± 0.1 g). Stomach contents were dissected under a
142 microscope and prey items were counted and identified to the lowest taxonomic level (mostly
143 family level) for SCA (Appendix A). Importantly, although present in stomach contents, plant
144 debris (i.e. wind-spread seeds, algae, terrestrial detritus) could not be counted and were thus
145 excluded from SCA. Whilst stomach content data provide information on the taxonomy of
146 prey items, it has important limitations arising from their representation of the diet of an
147 individual as a single snapshot (i.e. several hours only); it can also underestimate the
148 consumption of highly digestible prey. Consequently, the use of a complementary, temporally
149 integrative approach to measure of intraspecific trophic variability was required, with this
150 provided by SIA (Bolnick et al. 2003, Layman et al. 2012). The SIA was performed on the
151 same individuals to assess trophic variability over a longer time period than SCA (Layman et
152 al. 2012). Specifically, dorsal muscle samples were collected for stable isotope analyses of
153 carbon ($\delta^{13}C$ value) and nitrogen ($\delta^{15}N$ value) which provide information on the origin of the
154 resource consumed (e.g. littoral versus pelagic) and the trophic position of the consumers,
155 respectively (Post 2002). Concomitantly, the putative prey resources of the fish were sampled
156 from the littoral and pelagic habitats of the lakes with a pond net and with a 100-µm mesh net
157 and an Ekman dredge, respectively (Appendix A). Prior to analyses, all stable isotope samples
158 were oven dried (48h at 60°C), ground in a fine powder and subsequently analyzed at the
159 Cornell Isotope Laboratory (Ithaca, New York, USA). The analytical precision for all
160 samples, calculated as the standard deviation of an internal mink standard, was 0.11 and 0.12
161 ‰ for $\delta^{13}C$ and $\delta^{15}N$ values respectively.
162
163 ***Experimental approaches***
164 *Collection and rearing of experimental fish*

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165 Based on the field results, *L. gibbosus* were then collected from a single lake (area = 20.8 ha,
166 mean depth = 3.7 m) whose trophic outputs indicated an intermediate level of trophic
167 specialization, with a relatively large spectrum of prey consumed. On October 3rd 2013, 81
168 individuals (FL: 65 to 75 mm) were captured in the littoral habitat using a seine net (5-mm
169 mesh size) and then acclimated to laboratory conditions in three tanks (200 L, photoperiod:
170 12/12 h; water temperature: 17 to 18.5 °C) over six weeks. The individual fish were fed *ad*
171 *libitum* with commercial red maggots (*Diptera*) until the beginning of the experiment to
172 minimize background variability in body elemental composition among individuals. During
173 the mid-acclimation period, *L. gibbosus* were anaesthetized with eugenol (0.1 $mL.L^{-1}$),
174 measured for initial fork length (FL_i ± 1 mm), weighed (W_i ± 0.1 g), individually tagged with
175 passive integrated transponder (FDX PIT-tags, Oregon RFID, Portland, Oregon), and released
176 into the 200 L-tanks after recovery in well-aerated water. At the end of the acclimation period,
177 48 individuals of the similar length range and age class were selected (FL_i range: 79 - 98 mm,
178 mean ± SE 89.0 ± 0.8; age 1+ and 2+ years; Evangelista et al. *unpublished data*) for use in the
179 experiment. The use of fish of similar lengths and ages limited the potential effects of
180 ontogeny on the experimental data. The fish were transferred individually to 48 tanks filled
181 with 50-L of dechlorinated tap water. Each tank was equipped with a filtration system, a
182 plastic plant and a shelter. The 48 experimental units were distributed among 6 vertical
183 shelving units (6 blocks of 8 treatments).

184

185 *Effects of intraspecific trophic variability on nutrient recycling rates*

186 During the laboratory experiment (9 weeks), fish were provided with one of three diets that
187 comprised of one, two, or three food items in order to simulate three levels of decreasing
188 trophic specialization. Using the diet data from experiment, trophic specialization was
189 calculated as the diet overlap between an individual i and all individuals used in the

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190 experiment (i.e. the population) using the proportional similarity index PS_i, calculated
191 following Bolnick et al. (2002) (Appendix B). PS_i varies from 0 (no overlap) to 1 (total
192 overlap). Here, PS_i ranged from 0.33 for specialist individuals and 0.99 for generalist
193 individuals, and the PS_i of intermediate individuals was 0.66 (Fig. 1). The items represented
194 vegetable matter (cooked white rice [R]), macro-invertebrates (chironmonid larvae [C]) and
195 fish (grounded rainbow trout dorsal muscle with skin [F]). These items were used as they
196 represented the three different reported trophic levels of *L. gibbosus* prey (García-Berthou and
197 Moreno-Amich 2000) and cover a broad range of elemental composition (Fig. 1). White rice
198 was selected as a plant-based source because it is readily available in a standardized size and
199 quality and could mimic the quality of plant seeds and angling bait that are consumed by *L.*
200 *gibbosus*. Rice was cooked to obtain a texture similar to the texture of plant seeds and angling
201 bait after they have spent several days in water. Where mixtures of two and three items were
202 used as the diet, their total wet mass partitioned equally among the items. The diets were
203 hand-fed to *L. gibbosus* using a daily ration of 3% of individual initial body mass (Glaholt
204 and Vanni 2005). Mixed diets (i.e. intermediate and generalist) were homogenized manually
205 to reduce potential bias towards the consumption of potential preferred item(s), while also
206 maintaining item size.

207 The experimental design composed of seven treatments (Fig. 1): three types of
208 specialists feeding on a single diet item (cooked rice [R], chironomid larvae [C] or fish meat
209 [F]), three types of intermediates feeding on a mixture of two diet items (cooked rice ×
210 chironomid larvae [RC], cooked rice × fish meat [RF] or chironomid larvae × fish meat [CF]),
211 and one generalist type feeding on an even mixture of all the diet items (cooked rice ×
212 chironomid larvae × fish meat [RCF]). There were six replicates for each specialist and
213 intermediate treatment, and twelve replicates for the generalist treatment to fully account for
214 higher variability in the mixture that arose from the homogenization of the dietary items. The

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215 individual *L. gibbosus* were randomly assigned to each treatment and there was no significant
216 difference in the mean W_i and FL_i between treatments at the start of the experiment
217 (ANOVA, $P = 0.178$ and $P = 0.07$ respectively).

218 Ammonium ($N\text{-}NH_4^+$, hereafter referred to as N) and soluble reactive phosphorus
219 (SRP, hereafter referred to as P) excretion rates of *L. gibbosus* were quantified at the
220 beginning of the experiment, just prior to the individuals being transferred in their
221 experimental tanks (November 15th 2013) and at the end of the experiment (January 16th
222 2014). *Per capita* excretion rates (ER, hereafter referred to as 'excretion rate'; µmol ind.$^{-1}$ h^{-1})
223 were quantified, following Vanni et al. (2002). Two hours after feeding, *L. gibbosus* were
224 incubated individually for 1.5 h in a plastic bag containing 0.8 L of spring water (Glaholt and
225 Vanni 2005; see details in Appendix B). Filtered water samples (80 mL filtered using
226 Whatman GF/C, pore size 1.2 µm) were analyzed for N and SRP concentrations (Appendix
227 B) and excretion rates of N and P (µmol ind.$^{-1}$ h^{-1}) were calculated for each individual
228 following Vanni et al. (2002):

229 $$ER_I = (([I]_{ind} - [I]_{control}) \times V) / t$$

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230 where $[I]_{ind}$ and $[I]_{control}$ are the molar concentration (µmol L^{-1}) of the element I observed for
231 fish and control, respectively, V is the volume (L) of spring bottled water in the plastic bag
232 and t is the duration of the incubation (h) (Apendix B). For each block, one control bag filled
233 with bottled water but without fish was used to assess background levels of ammonium and
234 phosphorus at the end of the excretion trials. Importantly, these excretion trials were
235 performed every two weeks over the whole experimental period to renew the microcosm
236 water but without quantifying nutrient concentrations (see details below). At the end of the
237 experiment, after the final excretion trial, *L. gibbosus* were euthanized using an overdose of
238 anesthetic and weighed ($W_f \pm 0.1$ g). Specific growth rate (SGR; % week^{-1}) during the
239 experiment was calculated as follows:

240 $$SGR = 100 \times (\ln W_f - \ln W_i) / t$$

241 where t is the duration of the experiment (9 weeks).

242

243 *Effects of consumer-mediated nutrient recycling on ecosystem functioning*

244 Laboratory microcosms were used to assess the indirect effects of intraspecific trophic
245 variability on aquatic ecosystem processes through changes in fish-mediated nutrient
246 recycling. Microcosms were used to mimic relevant processes occurring in the benthic littoral
247 zone occupied by *L. gibbosus* in the wild, because the ability to measure these processes *in-*
248 *situ* is inherently challenging. Microcosms (n = 54, 48 for each experimental individual and 6
249 for each control) consisted of one-liter cylindrical containers initially filled with 0.25 L of
250 dechlorinated water and initiated with 0.5 L unfiltered lake water containing an inoculum of
251 autotrophic and heterotrophic microorganisms. They were supplied with oak (*Quercus robur*
252 L.) leaf litter collected at abscission and cut into leaf discs of 15 mm diameter using a cork
253 borer. Each microcosm received a set of 10 leaf discs that were previously weighed to the
254 nearest 0.1 mg (mean = 164.83 mg ± 0.41 SE). Microbial communities were allowed to
255 develop from November 7th to 22th 2013 before the microcosms were gently emptied until
256 there was 0.1 L of water left in order to avoid losing particulate matter that remained on the
257 bottom of the microcosm. Microcosms were then immediately supplied with 0.8 L of water
258 containing fish excretory products (or clean spring water for controls) from fish excretion
259 trials of 22th November 2013. During the experiment (54 days), microcosm water was
260 renewed on four occasions with excretion products from trials spaced two weeks apart (i.e.
261 22th November, 5th and 19th December, and 2nd January 2014), and following the same
262 procedure as described above. Microcosms were exposed to a 12 h light : 12 h dark
263 photoperiod (mean instantaneous light intensity = 252.1 μmol m^{-2} s^{-1} ± 10.3 SE) at the
264 laboratory temperature (17.0 - 18.5°C). They were assigned to the 48 experimental

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265 individuals and replicates were arranged in six blocks, corresponding to the level and the side
266 (left or right) of a three-shelf unit.

267 Whole-microcosm metabolism, the standing biomass of algae and leaf-decaying fungi,
268 litter mass loss rate and particulate nutrient concentration were assessed at the end of the
269 experiment. The side of each microcosm was gently brushed to remove the biofilm, content
270 was homogenized with blender and 0.06 L of water was filtered onto a Whatman GF/C filter.
271 Filters were then oven-dried (60°C for 48h) and used to quantify the amounts of particulate
272 nutrients (N and P; μmol). Gross primary productivity (GPP) and community respiration (CR)
273 were quantified using diurnal changes in oxygen levels (following Harmon et al. 2009). Using
274 an optical DO probe, Dissolved oxygen (DO) was measured (optical DO probe; Hach HQ10,
275 LDO) right after the light was switched on (t_0, sunrise), and after 12 h (t_1, sunset) and 24 h (t_2,
276 following sunrise) to capture day-time and night-time variations. Daily CR and GPP (mg O_2)
277 was calculated as follows:

278 $$CR = (DO_{t1 - t2}) \times 2 \times V \text{ and } GPP = (CR + DO_{t1 - t0}) \times V$$

279 where V was the volume of water in microcosms (0.9 L). Total algal standing biomass (μg)
280 was assessed based on the chlorophyll-a concentration and a subsample of 0.1 L of
281 homogenized water was filtered onto a Whatman GF/F filter (pore size 0.45 μm) stored in the
282 dark at -20°C until analysis. Chlorophyll-a was extracted in 90% acetone for 24h and its
283 concentration was determined with a spectrophotometer (HITACHI U-1100) following
284 Steinman et al. (2006). Before quantifying algal biomass, the leaf discs were gently removed
285 from the microcosms, washed with deionized water, and freeze-dried to estimate the final
286 litter mass. The remaining leaf material was coarsely crushed and an aliquot (mean = 21.60
287 mg ± 0.13 SE) was used to determine the ergosterol content in the leaf litter as a surrogate of
288 fungal biomass (mg g^{-1} of litter). Ergosterol was determined using high-performance lipid

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289 chromatography and was converted into dry mass using a factor of 182 (Gessner and Chauvet
290 1993). The rate of litter decomposition (k in day^{-1}) was calculated as follows:

291 $$k = -\ln (M_f / M_i) / t$$

292 where M_f and M_i are the final and initial freeze-dried mass of leaf litter remaining in the
293 microcosm, respectively, and t is the duration of the microcosm experiment (54 days).

294

295 ***Statistical analyses***

296 *Intraspecific trophic variability in wild populations*

297 To ensure long-term comparisons of diet variability between and within populations, $\delta^{13}C$ and
298 $\delta^{15}N$ values of each *L. gibbosus* were used to calculate a measure of trophic position (TP) and
299 the corrected carbon isotope ratio ($\delta^{13}C_{cor}$) adjusted for between-population variation in stable
300 isotope baselines following Post (2002) (Appendix A):

301 $$TP = \lambda_{base} + (\delta^{15}N_{ind} - [\delta^{15}N_{lit} \times \delta^{13}C_{cor} + \delta^{15}N_{pel} \times (1 - \delta^{13}C_{cor})]) / \Delta_N$$

302 $$\delta^{13}C_{cor} = (\delta^{13}C_{ind} - \delta^{13}C_{pel}) / (\delta^{13}C_{lit} - \delta^{13}C_{pel})$$

303 where λ_{base} is the trophic position of the littoral and pelagic baseline ($\lambda_{base} = 2$), $\delta^{15}N_{ind}$ is the
304 stable isotope value of the *L. gibbosus*, $\delta^{15}X_{lit}$ and $\delta^{15}X_{pel}$ are the stable isotope values of the
305 littoral and pelagic baselines and Δ_N is the trophic enrichment factor obtained from previous
306 studies ($\Delta_N = 3.4$; Post 2002). Using these baseline-corrected isotope values that allow
307 comparison between populations, the size of the isotopic niche of each population was
308 calculated using Bayesian standard ellipse area (SEA_b, 10 000 simulations) using Stable
309 Isotope Bayesian Ellipse in R (SIBER; Jackson et al. 2011) from the SIAR package (Parnell
310 et al. 2010). This ellipse-based metric focuses on the core area of the isotopic niche and low
311 values (i.e. low stable isotope area) indicate small isotopic niches. In addition, Kruskall-
312 Wallis test was used to test for significant differences in trophic position between wild
313 populations of *L. gibbosus*.

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314 Based on SCA, PS_i was calculated (Appendix A) and the overall degree of individual
315 specialization (IS) in each population was then determined as the average PS_i of all
316 individuals (Bolnick et al. 2002). For the sake of clarity, the index V = 1 - IS was used in the
317 present study, with values closer to 1 indicating a high level of trophic specialization in the
318 population. Significant differences in the level of specialization between wild populations
319 were tested using Kruskall-Wallis test. Generalized linear mixed-effects model (package lme4
320 v.1.1.10; Bates *et al.* 2015), with site as a random factor, tested the effect of body size on diet
321 variation, with PS_i and FL used as independent and dependent variables respectively. Since
322 PS_i varied between 0 and 1, the model specified a binomial family with a logistic link
323 function.
324
325 *Effects of intraspecific trophic variability on nutrient recycling rates*
326 Two-way nested analysis of variance (ANOVA) tested the response of fish (growth and
327 excretion rates) to the experimental treatments. In the experimental design, diet composition
328 (defined as the number and types of food items: each item singly: [R]-[C]-[F], each item pair:
329 [RC]-[RF]-[CF], and the three-items combination: [RCF]; Fig. 1) was nested in trophic
330 specialization (the number of food items supplied to fish: 1-2-3; Fig. 1). The nested design
331 was analyzed using the linear model:
332
$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk}$$
333 where Y_{ijk} is the rate of growth or excretion of the kth fish fed with the jth type of diet within
334 the ith level of specialization, α_i is the effect of ith level of specialization, $\beta_{j(i)}$ is the effect of the
335 j^{th} diet nested in the ith level of specialization, and μ and ε_{ijk} are the intercept and residual error
336 of the model respectively. Two error terms are required for significance testing: specialization
337 was tested against diet composition and diet composition against residual error (Quinn and
338 Keough, 2002). Tukey's post-hoc test was used to determine which pairs of diet type

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339 significantly differed. Because excretion rates are a function of size, allometry was integrated
340 into the analyses of nutrient excretion rates by dividing *per capita* excretion rates by W_f raised
341 to the ¾ power (mass-normalized *per capita* excretion rates; Torres and Vanni 2007). Non-
342 additive effects of diet mixing on response variables were evaluated through a comparison of
343 the observed versus expected values using one-sample paired t-test. Specifically, for each
344 mixed-diet treatment (3 intermediates and 1 generalist), the expected value was calculated as
345 the mean value of specialist fish fed with either one of the food items. Given the multiplicity
346 of comparisons involved, the false discovery rate (FDR; Benjamini and Hochberg 1995)
347 procedure was applied to correct for alpha inflation using the *p.adjust* function (base-package
348 v.3.1.2; R Development Core Team 2013). The significant results after the FDR procedure are
349 reported.
350
351 *Effects of consumer-mediated nutrient recycling on ecosystem functioning*
352 The general effects of the presence of excretory products on ecosystem processes were
353 evaluated by comparing nutrient-less (i.e. control) with all treatments that contained fish
354 excretory products using t-tests. Linear models were used to examine effects of amounts of
355 excretory products (*per capita* N and P excretion rates) on ecosystem processes measured in
356 the microcosms (i.e. particulate nutrient content, gross primary productivity, community
357 respiration, algal standing biomass, fungal biomass on leaves and litter decomposition rates).
358 To assess the mass dependence of nutrient excretion effects on ecosystem processes, linear
359 models were also performed with mass-normalized *per capita* excretion rates and ecosystem
360 processes as independent and dependent variables, respectively. Linear models were built
361 with a block effect as covariate to control for potential variation in the experimental set-up.
362 The assumption of homoscedasticity and the normality of the residuals for linear models and
363 nested ANOVA were checked graphically using Tukey Ascombe and Q-Q plots, respectively.

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364 Prior to all statistical analyses, nutrient excretion rates and fungal biomass were log_{10}
365 transformed and growth rate was square-root transformed. Highly influential data points were
366 identified by the Cook's distance (D_i) plot and values were considered as critical for $D_i > 4/N$,
367 where N is the number of observations (Bollen and Jackman 1990). Critical values were
368 removed from the dataset prior the model was refitted. All statistical analyses were performed
369 using R v.3.1.2 (R Development Core Team 2013).

370

371 **RESULTS**

372

373 *Intraspecific trophic variability in wild populations*

374 Isotopic niche size (SEA_b, mean = 0.33 ± 0.02 SE) could increase up to two times between
375 populations, ranging from 0.25 up to 0.48 (Table 1; Appendix A, Fig. A1), indicating that
376 variations in isotopic niche across populations were apparent when the temporally integrated
377 SIA was used. In addition, trophic position significantly differed between populations
378 (Kruskal-Wallis, H = 127.31, $P < 0.001$). Within-populations, SIA also indicated relatively
379 high variability in trophic position, with the range of trophic position extending over more
380 than one trophic level in several cases (mean TP = 3.37 ± 0.06 SE, mean within-population
381 range = 1.32 ± 0.05 SE; Table 1; Appendix A, Fig. A1). These results suggested that
382 individuals of *L. gibbosus* integrate across a wide range of resources with different trophic
383 positions (i.e. from primary producer to secondary consumer), as individuals feeding
384 exclusively on invertebrates would have a trophic position of 3.

385 SCA revealed that the level of trophic specialization (V ranging from 0.17 to 0.64;
386 mean = 0.38 ± 0.05 SE) was significantly different between populations (Kruskal-Wallis, H =
387 144.62, $P < 0.001$). Within populations, individual specialization (PS_i) was highly variable

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388 (Table 1; Appendix A, Fig. A2) and increased nine-fold on average among individuals (Table
389 1). The relationship between FL and PS_i was not significant (GLMM, z = 0.55, *P* = 0.581).
390
391 *Effects of intraspecific trophic variability on nutrient recycling rates*
392 Laboratory experiment revealed that individual growth rate did not vary significantly with the
393 degree of specialization but was significantly affected by diet composition (nested ANOVAs,
394 *P* = 0.627 and *P* < 0.001, respectively; Table 2). Specialists feeding on fish meat exhibited
395 higher individual growth rates than individuals feeding on rice (Fig. 2A) and values obtained
396 from mixed diet were not significantly different from predicted values based on mixing of
397 single values (*P* > 0.227).
398 At the end of the experiment, the excretion rates of the individual *L. gibbosus*
399 displayed a wide range of variation among individuals, ranging from 4.12 to 22.61 μmol N
400 $ind.^{-1}$ h^{-1} and from 0.04 to 0.29 μmol P $ind.^{-1}$ h^{-1}, but mass-normalized excretion rates did not
401 significantly differ between the degrees of diet specialization (nested ANOVAs, *P* = 0.897
402 and *P* = 0.164, respectively; Table 2). Diet composition significantly affected mass-
403 normalized N excretion rate (nested ANOVA, *P* < 0.001; Table 2, Fig. 2B) whereas it did not
404 significantly affect mass-normalized P excretion rate (nested ANOVA, *P* = 0.072; Table 2,
405 Fig. 2C). Mass-normalized N excretion rate was significantly different for the three specialist
406 treatments, with the highest and lowest excretion rates for fish specialized on fish meat (mean
407 = 19.11 μmol N $ind.^{-1}$ h^{-1} ± 1.11 SE) and rice (mean = 5.94 μmol N $ind.^{-1}$ h^{-1} ± 0.47 SE),
408 respectively (Fig. 2B). In general, these results suggested that the presence of fish meat within
409 a diet also containing rice (i.e. [RF] and [RCF]) increased mass-normalized N excretion rate
410 when compared to specialists feeding on rice (Fig. 2B). In parallel, intermediate individuals
411 feeding on rice and chironomids excreted N nutrients at similar rates to specialists feeding on

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412 rice (Fig. 2B). For both N and P mass-normalized excretion rates, additive effects were
413 observed for all mixed-diet treatment ($P > 0.059$).
414
415 *Effects of consumer-mediated nutrient recycling on ecosystem functioning*
416 Particulate N and P contents, gross primary production (GPP), community respiration (CR),
417 and algal standing biomass (chlorophyll-a) were higher in microcosms supplied with fish
418 excretory products than in control microcosms (t-tests, $P < 0.05$). Linear models showed that
419 particulate N content, GPP and CR increased significantly with N excretion rate and that
420 particulate P content and algal standing biomass increased significantly with P excretion rate
421 (Table 3, Fig. 3). In contrast, whole-system metabolism did not change with P excretion rate
422 (linear models, GPP: $F = 2.25$, $P = 0.142$ and CR: $F = 1.83$, $P = 0.184$; Table 3, Fig. 3B and
423 3D) and differences in algal standing biomass among microcosms were inconsistent with
424 intraspecific variation in N excretion rate (linear model, $F = 0.12$, $P = 0.732$; Table 3, Fig.
425 3E). No difference was detected for the biomass of leaf-associated fungi and litter
426 decomposition rate between treatment and control microcosms (t-tests, $P = 0.656$ and $P =$
427 0.672, respectively; Fig. 3K and 3L). Intraspecific variation in nutrient excretion rates did not
428 explain differences in these ecosystem properties among microcosms that were supplied with
429 fish excretory products (linear models, $P > 0.367$; Table 3, Fig. 3G to 3J). Except for algal
430 standing biomass (linear model, $F = 6.08$, $P = 0.018$; Table 3), effects of N and P excretion
431 rates on ecosystem properties were not statistically significant with mass-normalized
432 excretion rates (linear models, $P > 0.071$; Table 3), indicating that consumer-mediated effects
433 on ecosystem processes were primarily driven by the effects of diet treatment on individual
434 body mass through differences in growth rate.
435
436 **DISCUSSION**

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437

438 The widespread occurrence of diet variability within populations is now recognized, although
439 its incidence and implications at the higher levels of biological organization remain poorly
440 understood (Bolnick et al. 2003, Araújo et al. 2011). Here, we observed that intraspecific
441 trophic variability occurred between and within wild populations of *L. gibbosus*. This was
442 partly due to high variability in both the degree of individual specialization and their trophic
443 position quantified using SCA and SIA. Variability in trophic position also suggested that
444 individuals consumed a range of plant and animal resources. The experimental approaches
445 then indicated that the rate of nutrient excretion was influenced by diet composition but did
446 not change with the degree of specialization. Specifically, N excretion rate increased with diet
447 quality but P excretion rate did not change with diet composition. Finally, we found that
448 increased nutrient excretion rates potentially enhanced integrative ecosystem processes
449 through biomass-dependent effects.

450 Generalist populations with wide trophic niches are composed of more heterogeneous
451 individuals using only a subset of the available prey (Bolnick et al. 2003). Here, we found that
452 trophic specialization differed widely among coexisting individuals of *L. gibbosus* and such
453 trophic variability was consistently detected within the eleven wild populations surveyed. In
454 addition, omnivorous *L. gibbosus* individuals did not occupy the same trophic position, as
455 evidenced by stable isotope analyses that reflect dietary information over several months
456 (Layman et al. 2012). Clearly, individuals within populations differed in respect of the
457 relative contribution of plant- versus animal-derived resources to diet, as supported by the
458 wide range of observed trophic position values. Plant material was commonly found in the
459 stomach content of *L. gibbosus* in the present and previous studies (García-Berthou and
460 Moreno-Amich 2000, Rezsu and Specziar 2006) and may be ingested while fish foraged on
461 benthic prey embedded within sediments or macrophytes. While SCA suggested that *L.*

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462 *gibbosus* relied exclusively on invertebrate prey as animal food source, we cannot rule out
463 that some individuals displaying a high trophic position also consumed fish-derived prey such
464 as eggs and larvae (García-Berthou and Moreno-Amich 2000). For instance, in the present
465 study, fish were sampled in late summer, i.e. at a time when fish eggs and larvae had become
466 scarce since all co-occurring species have already spawned, which likely explained the
467 absence of fish-derived prey in the stomach contents. Indeed, SCA provide only a snapshot of
468 individuals' diets, unless repeated non-lethal stomach flushing are performed from the same
469 individuals (Araújo et al. 2011). Nevertheless, SCA provided dietary information at the
470 taxonomic level, with these data used to calculate trophic specialization based on count data.
471 Since plant debris could not be quantified in the same way as animal prey, however, they
472 were excluded from SCA and could not be including in the quantification of trophic
473 specialization, which represents a limitation of this approach when using omnivorous model
474 species. In contrast, SIA provides temporally-integrative information about individual diet,
475 but precise quantification of the trophic niche can be difficult, particularly if putative
476 resources are taxonomically distinct but isotopically similar. Therefore, the combined use of
477 SIA and SCA is an appropriate way to counterbalance the limitations of each method to
478 investigate the trophic ecology of omnivores. Variation in trophic enrichment factor can also
479 be a source of uncertainty when quantifying the trophic position of wild organisms (Vander
480 Zanden and Rasmussen 2001, Busst et al. 2015). Indeed, in omnivorous species, high
481 variation in TEF has been reported between individuals consuming plant-based or animal-
482 based diet (Busst and Britton 2016). However, in the present study, such potential variations
483 in TEF among individuals were unlikely to affect our findings of the existence of strong
484 variations in trophic position within and between wild populations.

485 The balanced diet hypothesis stipulates that generalist individuals consuming multiple
486 prey have access to a more complete range of nutrients than specialist individuals, which

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487 could provide fitness benefits (DeMott 1998). Our laboratory experiment did not support this
488 hypothesis, as diet mixing did not increase individual growth. By contrast, growth rates were
489 higher for individuals feeding on the single best-quality food item, whereas specialization on
490 poorer-quality diet might induce low or negative growth rate (Lefcheck et al. 2013). These
491 findings thus indicate that diet specialization toward high quality food may confer fitness
492 advantages in generalist populations. However, in natural situations, consumers specializing
493 on high food quality may expend more energy to capture their prey. For instance, predation on
494 fish eggs can induce fighting costs and reciprocal predation, particularly in nest-guarding
495 species (Baldridge and Lodge 2013). In parallel, species providing parental care, such as *L.*
496 *gibbosus*, can also display cannibalism, particularly when guarding is costly (Manica 2002).
497 Thus, specializing on high quality food may induce contradictory effects in fish in natural
498 environments, although this requires further investigations.

499 Nutrient cycling measured as *per capita* excretion rates was highly variable among
500 individuals (ranging from to 4.12 to 22.61 µmol N ind.$^{-1}$ h^{-1} and from 0.04 to 0.29 µmol P
501 ind.$^{-1}$ h^{-1}, respectively). These values were within the range reported by Villéger et al. (2012)
502 for wild populations of freshwater fish species (ranging from 0.20 to 518 µmol N ind.$^{-1}$ h^{-1}
503 and from 0.03 to 29.34 µmol P ind.$^{-1}$ h^{-1}). They were, however, slightly lower than the values
504 observed for *L. gibbosus* (ranging from 13.46 to 26.12 µmol N ind.$^{-1}$ h^{-1} and from 0.13 to 1.74
505 µmol P ind.$^{-1}$ h^{-1}; Villéger et al. 2012). Although the effects of diet composition on consumer
506 excretion rates can be difficult to predict (but see Moody et al. 2015), our findings revealed
507 that individuals feeding at higher trophic position (i.e. those with an animal-based diet)
508 excreted N at higher rates (Bassar et al. 2010). This is probably because animal items used in
509 this study were nutrient-rich compared to rice and, correspondingly, consumers would release
510 these nutrients at higher rates (Sterner and Elser 2002). Surprisingly, and contrary to findings
511 reported in literature (Moody et al. 2015), we did not detect significant changes in P excretion

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512 rate in relation to intraspecific trophic variability. Fish require large amount of P that is
513 allocated to the formation of bones and scales, and to somatic growth (Pilati and Vanni 2007,
514 McIntyre and Flecker 2010). The diet items used in the present study were relatively low in P
515 (from 0.2 to 0.9 % dry mass; Fig. 1) and this could explain the high level of P retention by
516 fish. For instance, the mean C:P ratios of diet items used in the experiment were higher (mean
517 = 311.8 ± 221.8 SD) than the mean threshold elemental ratio of carbon and phosphorus (i.e.
518 the nutrient ratio of an organism's diet where growth limitation of this organism switches from
519 one element to another; Sterner and Elser 2002) of nine freshwater fish species reported in the
520 literature (mean = 135.4 ± 44.4 SD; Frost et al. 2006). This indicates that fast growth *L.*
521 *gibbosus* species (Copp and Fox 2007) was probably P-limited in our experiment, suggesting
522 that consumers with an r- or k-strategy would potentially have different role in nutrient
523 recycling.

524 Some of the integrative ecosystem processes measured during the present study
525 differed substantially among microcosms (i.e. gross primary productivity and community
526 respiration) and a significant fraction of this variability was driven by intraspecific variation
527 in the rate of nitrogen excreted by fish. Because we also demonstrated that diet composition
528 determined nitrogen excretion rate, it indicated that intraspecific trophic variability can alter
529 ecosystem functioning through consumer-mediated nutrient recycling (Bassar et al. 2010,
530 Taylor et al. 2015). Based on our findings, specialization toward resources with higher trophic
531 level should exacerbate the effects of individual fish excretion on ecosystem functioning.
532 Previous studies have demonstrated that increased nutrient quantity through loading can shape
533 consumer-mediated nutrient recycling, probably through changes in population biomass and/
534 or community structure (Vanni et al. 2005, Wilson and Xenopoulos 2011). Here, we found
535 that the biomass dependence of fish excretion can also occur within the same cohort and
536 mediate the effects of consumers on ecosystem functioning. This highlights the importance of

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537 integrating body size variation in assessments of the effects of intraspecific variability on
538 ecosystem functioning (Rudolf and Rasmussen 2013). In addition, since human activities can
539 affect body size distribution in wild population (e.g. Evangelista et al. 2015), it would be of
540 interest to assess how they affect the relative importance of consumer-driven nutrient
541 recycling along a gradient of perturbation, such as anthropogenic eutrophication.

542 The amount of N and P associated with fine particulate organic matter increased with
543 the inputs of fish excretory products, but also as a result of microbial immobilization of
544 dissolved inorganic nutrients. Primary producers and decomposers rely on inorganic nutrients
545 for growth and metabolism (Daufresne and Loreau 2001). In our experiment, neither
546 decomposition rate of coarse particulate organic matter nor fungal biomass were modified by
547 the addition of excretory products, suggesting that litter-associated decomposers did not use
548 fish-derived nutrients. Therefore, and contrary to our prediction, producers may have
549 contributed substantially to nutrient immobilization in microcosms as evidenced by the
550 positive response of GPP and algal biomass to fish excretory products. Finally, the
551 asymmetric responses between autotroph and heterotroph organisms observed here
552 highlighted the complexity of ecosystem processes responses to nutrient loading. Although
553 these results are not trivial, we argue that future investigations are needed in less contrived
554 experimental environments. This would help to fully encompass other additional effects that
555 might modulate and interact with the relationship between nutrient recycling and ecosystem
556 processes (Knoll et al. 2009, Bassar et al. 2010, Taylor et al. 2012).

557 A large number of studies have demonstrated that changes in community structure or
558 population size can influence consumer-mediated nutrient recycling (Vanni et al. 2002,
559 McIntyre et al. 2007, Pilati and Vanni 2007, Villéger et al. 2012, Allgeier et al. 2016). In the
560 present study, we demonstrated that changes within populations can also induce variation in
561 consumer nutrient excretion rates and ecosystem processes, providing a deeper understanding

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562 of the indirect role of consumers in regulating ecosystem functioning. Together, these
563 findings highlight that the ecological effects induced by intraspecific variability in consumers
564 may be strong compared to those induced by interspecific variability (Palkovacs et al. 2015).
565 This would be particularly relevant in the current context of global changes in general and
566 biological invasions in particular, as they can alter the diversity patterns of consumers both at
567 the intraspecific and interspecific levels, affecting native organisms and recipient ecosystems
568 across levels of biological organization (Buoro et al. 2016). We also argue that future studies
569 would benefit to quantify the relative effects of top-down (direct and consumptive) and
570 bottom-up (indirect and nutrient-mediated) mechanisms in controlling the effects of
571 intraspecific variability on ecosystem functioning (Knoll et al. 2009, Taylor et al. 2015).
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729 **Table 1** Mean value (± 95% CI or ± SE) and range of trophic niche (SEA_b), trophic position
730 (TP) and individual specialization (PS_i) in each studied wild population (n = 11). The
731 significance of PS_i was calculated using a Monte Carlo procedure: *** $P < 0.001$; ** $P <$
732 0.01.

	SEA_b		TP			PS_i		
Lake	Mean (± 95% CI)	n‡	Mean (± SE)	Range	n‡	Mean (± SE)	Range	n‡
A	0.27 (0.18 - 0.37)	28	3.10 (0.02)	2.82 - 3.43	28	0.36 (0.05) ***	0.09 - 0.62	12
B	0.38 (0.24 - 0.52)	28	3.13 (0.04)	2.85 - 3.72	28	0.83 (0.03) **	0.27 - 0.97	26
C	0.32 (0.21 - 0.44)	28	3.41 (0.03)	3.13 - 3.64	28	0.77 (0.03) ***	0.22 - 0.92	26
D	0.30 (0.20 - 0.42)	28	3.40 (0.04)	2.89 - 3.94	28	0.59 (0.04) ***	0.08 - 0.82	25
E	0.27 (0.18 - 0.37)	28	3.56 (0.03)	3.19 - 3.86	28	0.73 (0.04) ***	0.15 - 0.92	28
F	0.32 (0.21 - 0.44)	27	3.29 (0.04)	2.67 - 3.74	27	0.67 (0.04) **	0.07 - 0.85	26
G	0.40 (0.26 - 0.55)	28	3.65 (0.06)	2.74 - 4.56	28	0.75 (0.05) ***	0.05 - 0.95	26
H	0.35 (0.23 - 0.48)	28	3.61 (0.06)	3.16 - 4.34	28	0.42 (0.03) ***	0.05 - 0.72	23
I	0.48 (0.31 - 0.67)	28	3.21 (0.07)	2.37 - 3.64	28	0.40 (0.03) ***	0.10 - 0.77	28
J	0.32 (0.20 - 0.45)	24	3.35 (0.04)	3.01 - 3.63	24	0.81 (0.04) ***	0.08 - 0.94	22
K	0.25 (0.17 - 0.35)	28	3.36 (0.02)	3.13 - 3.52	28	0.50 (0.04) ***	0.08 - 0.78	26

733 ‡ Differences between the numbers of individuals used for stomach content and for stable isotope
734 analyses were caused by the presence of empty stomachs.
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746 **Table 2** Results of the nested ANOVAs used to assess the effects of diet composition nested
747 under degree of specialization on growth (% week^{-1}; square-root transformed) and the mass-
748 normalized N and P *per capita* excretion rates (µmol g$^{-3/4}$ h^{-1}; $\log_{10}$ transformed) of *Lepomis*
749 *gibbosus*. Significant *P*-values are in bold.

Response variables	Source	df	Mean squares	F	*P*	Eta-squared
Growth rate	Degree of specialization	2	0.476	0.34	0.627	0.13
	Diet composition	4	1.415	87.72	**< 0.001**	0.78
	Error	38	0.016			
N excretion rate	Degree of specialization	2	0.011	0.07	0.897	0.03
	Diet composition	4	0.156	36.01	**< 0.001**	0.76
	Error	41	0.004			
P excretion rate	Degree of specialization	2	0.064	1.66	0.164	0.14
	Diet composition	4	0.039	2.34	0.072	0.17
	Error	39	0.016			

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766 **Table 3** Results of the linear models assessing the relationships between N and P *per capita*
767 (µmol ind.$^{-1}$ h^{-1}; log$_{10}$ transformed) and mass-normalized *per capita* excretion rates (µmol g$^{-3/4}$
768 h^{-1}; log$_{10}$ transformed) and ecosystem processes: nutrient particulate content (µmol), gross
769 primary productivity (mg O_2), community respiration (mg O_2), algal standing biomass (µg),
770 fungal biomass (mg; log$_{10}$ transformed) and litter decomposition rate (day^{-1}). Significant *P*-
771 values are in bold.

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Response variables	Source	df	*Per capita* excretion rates			Mass-normalized *per capita* excretion rates		
			Mean squares	**F**	***P***	**Mean squares**	**F**	***P***
Particulate N concentration	Block	5	24490	4.84	**0.002**	24491	4.43	**0.003**
	N excretion rate	1	34660	6.85	**0.013**	19123	3.46	0.071
	P excretion rate	1	2070	0.41	0.526	219	0.04	0.843
	Residuals	37	5058			5528		
Particulate P concentration	Block	5	12.58	33.5	**< 0.001**	12.61	29.16	**< 0.001**
	N excretion rate	1	0.93	2.48	0.124	0.003	0.01	0.934
	P excretion rate	1	2.67	7.11	**0.011**	1.06	2.45	0.126
	Residuals	37	0.38			0.43		
Gross primary productivity	Block	5	6.63	2.34	0.061	6.32	2.16	0.080
	N excretion rate	1	15.07	5.32	**0.027**	3.22	1.10	0.302
	P excretion rate	1	6.39	2.25	0.142	5.20	1.77	0.191
	Residuals	37	2.84			108.51		
Community respiration	Block	5	4.20	2.36	0.058	4.05	2.10	0.087
	N excretion rate	1	8.20	4.62	**0.038**	4.64	2.41	0.129
	P excretion rate	1	3.26	1.83	0.184	1.76	0.91	0.345
	Residuals	38	1.77			1.93		
Algal standing biomass	Block	5	2115	0.27	0.927	3695	0.46	0.805
	N excretion rate	1	935	0.12	0.732	12327	1.53	0.224
	P excretion rate	1	88245	11.21	**0.002**	49079	6.08	**0.018**
	Residuals	38	7871			8072		
Fungal biomass	Block	5	0.17	3.72	**0.008**	0.17	3.72	**0.008**
	N excretion rate	1	0.01	0.19	0.664	0.02	0.39	0.538
	P excretion rate	1	0.02	0.46	0.500	0.01	0.26	0.610
	Residuals	37	0.05			0.05		
Litter decomposition rate	Block	5	1.33e-06	0.42	0.829	1.33e-06	0.42	0.829
	N excretion rate	1	2.91e-07	0.09	0.763	2.12 e-06	0.67	0.417
	P excretion rate	1	2.62e-06	0.83	0.367	6.94 e-07	0.22	0.642
	Residuals	37	3.15e-06			3.15 e-06		

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777 **Figure legends**

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779 **Figure 1** Schematic representation of the experimental design used to test the indirect effects
780 (i.e. through nutrient recycling) of inter-individual trophic variability on individuals and
781 ecosystem functioning. Trophic variability was manipulated with diet elemental composition
782 nested under degree of specialization. Replicated treatments were dispatched in 6 vertical
783 shelving units (blocks). The degree of individual trophic specialization (PS_i) was 0.33, 0.66
784 and 0.99 for specialist, intermediate and generalist treatments, respectively. Abbreviations: R:
785 cooked rice; C: chironomid larvae; F: fish meat.

786

787 **Figure 2** Effects of diet elemental composition treatment nested under degree of
788 specialization treatment on mean (± SE) **(A)** growth rate (% month^{-1}) and **(B)** N and **(C)** P
789 mass-normalized *per capita* excretion rates (µmol g$^{-3/4}$ h^{-1}). Colored dots represent rice
790 specialists (yellow), chironomids specialists (blue), fish specialists (red), intermediates rice ×
791 chironomids (green), intermediates rice × fish (orange), intermediates chironomid × fish
792 (purple) and generalists (dark green). Different letters indicate significant differences among
793 these means (Tukey's HSD, $P < 0.05$).

794

795 **Figure 3** Relationship between N (left panels) and P (right panels) *per capita* excretion rates
796 (µmol ind.$^{-1}$ h^{-1}; $\log_{10}$ transformed) and **(A-B)** gross primary productivity (n = 45), **(C-D)**
797 respiration (n = 46), **(E-F)** algal standing biomass (µg; n = 46), **(G-H)** fungal biomass (mg; n
798 = 45) and **(I-J)** litter decomposition rate (day^{-1}; n = 45). Regression lines are displayed using
799 continuous black lines with equations above each panel when significant. The dotted lines
800 represent the mean value of the control microcosms.

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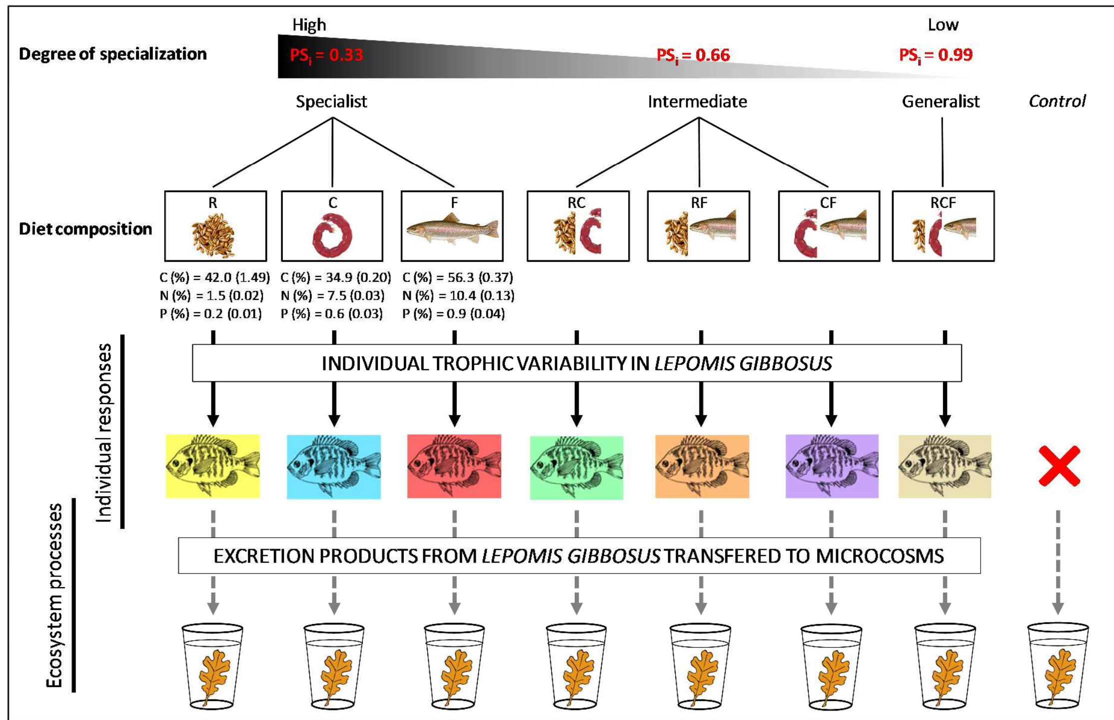

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803 **Figure 1**

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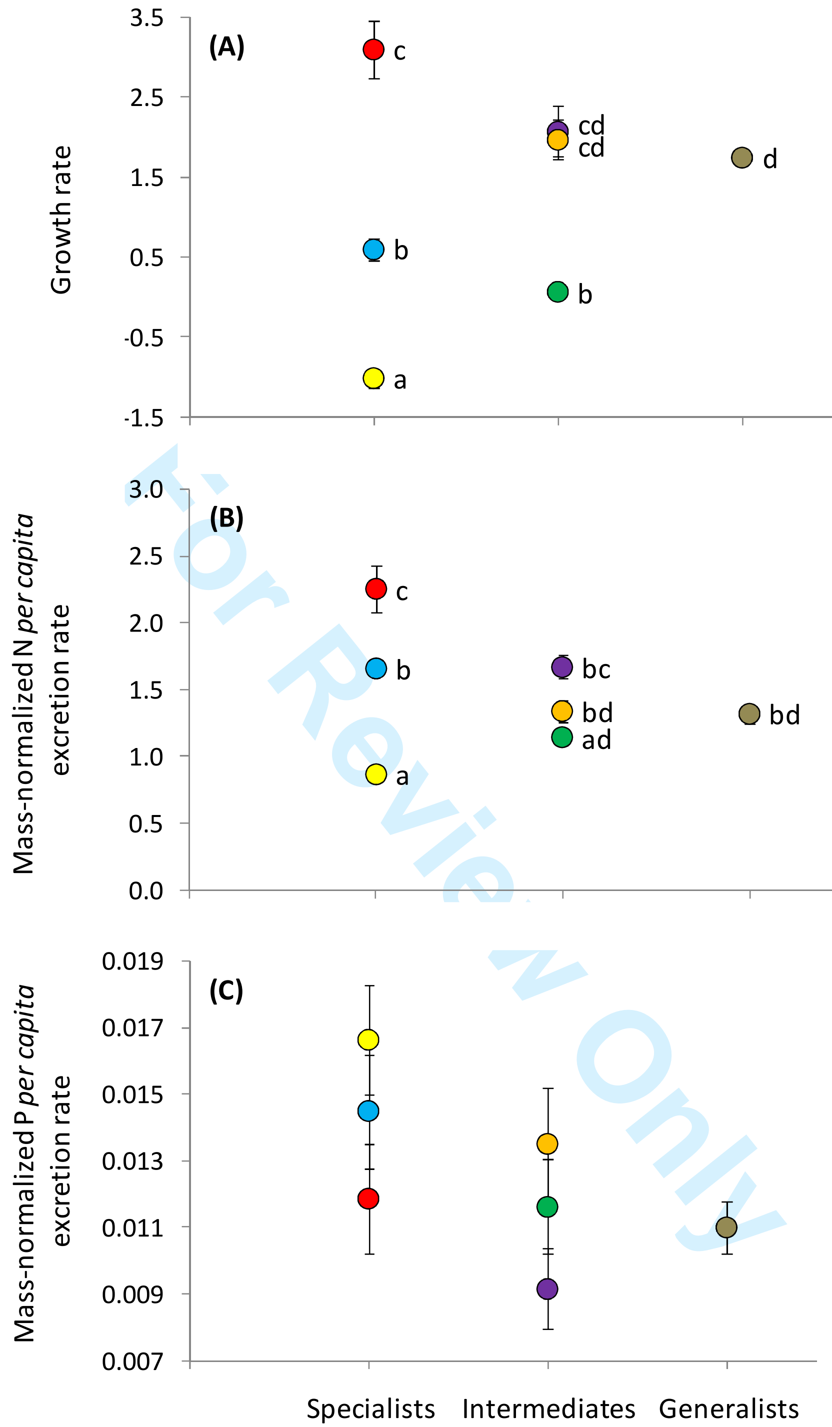

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806 **Figure 2**

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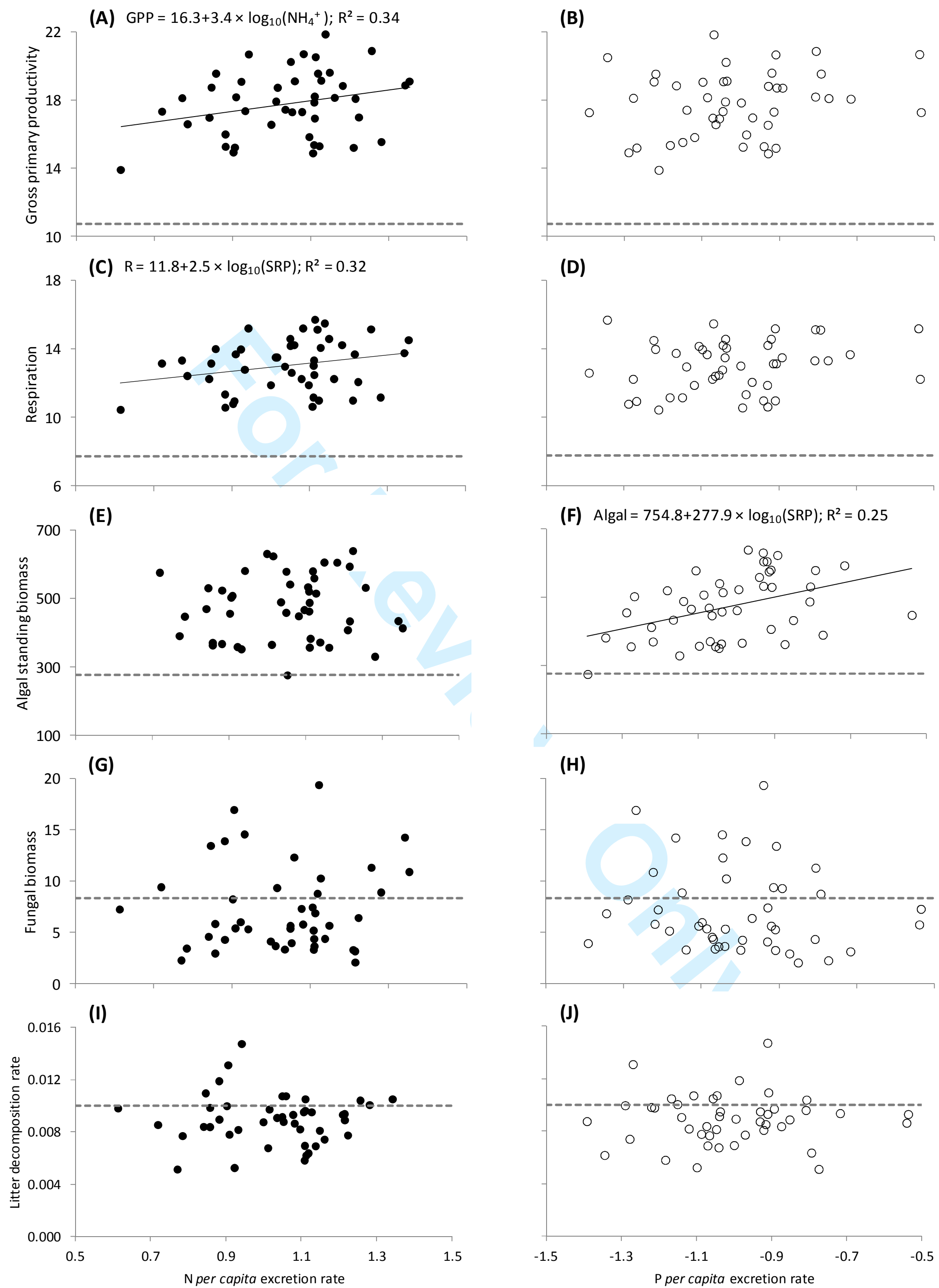

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812 **Figure 3**

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814 Supplementary material to:

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817 Resource composition mediates the effects of intraspecific variability in nutrient

818 recycling on ecosystem processes

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821 C. Evangelista, A. Lecerf, J.R. Britton, J. Cucherousset

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844 ***Supplementary material***

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846 **C. Evangelista, A. Lecerf, J.R. Britton, J. Cucherousset. Resource composition mediates**
847 **the effects of intraspecific variability in nutrient recycling on ecosystem processes**

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849 **Appendix A:** Stomach content and stable isotope analyses.

850

851 ***Stomach content analyses***

852 Stomach content analyses are traditional approaches used to examine the feeding ecology of
853 consumers in wild populations (Svanbäck and Bolnick 2007, Robinson et al. 1993). In the
854 present study, stomach contents of wild *L. gibbosus* were dissected under microscope and
855 prey items were counted and identified to the lowest taxonomic level (mostly family level). A
856 total of 70 prey taxa were found, including numerous invertebrate families and eggs. To assist
857 analysis, within orders, several families were grouped into similar morphotype groups
858 (aquatic larvae, emergent invertebrates or terrestrial invertebrates) and ultimately provided 29
859 prey categories for SCA. Importantly, although present in stomach content, plant detritus
860 could not be counted and were thus excluded from subsequent analyses.

861 Indices were developed to calculate individual specialization using the population's
862 total diet to define resource availability, involving that individuals are compared to their
863 population niche rather than to the food availability (Bolnick et al. 2002). In the present study,
864 we used the proportional similarity index (PS) to measure diet overlap between individual i
865 and its population following Bolnick et al. (2002):

866 $$PS_i = 1 - 0.5 \sum_j |p_{ij} - q_{ij}|$$

867 "where p_{ij} is the proportion of the *j*th resource category in individual *i*'s diet, q_{ij} is the
868 proportion of the *j*th resource category in the population's niche and is calculated as:

869 $$q_{ij} = \frac{\sum_i n_{ij}}{\sum_i \sum_j n_{ij}}$$

870 where n_{ij} represent the number of diet items in individual *i*'s diet that fall in category *j*. For
871 individuals that specialize on a single diet item *j*, PS_i takes on the value q_j. For individuals that
872 consume resources in direct proportion to the population as a whole, PS_i will equal 1."
873 (Bolnick et al. 2002). PS_i were calculated using the package RInSp (Zaccarelli et al. 2013).
874 For each *L. gibbosus*, the count of prey items was converted to proportions and averaged

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875 across all individuals for each resource (Bolnick et al. 2002). The significance of PS_i was
876 evaluated using resampling methods based on Monte Carlo procedures (using 10 000
877 replicates). The null hypothesis was that any observed diet variation arose from individuals
878 sampling stochastically from a shared distribution (Araújo et al. 2011).
879
880 ***Stable isotope analyses***
881
882 Stable isotope analyses are useful tools to investigate the trophic ecology of consumers in
883 wild populations with the particularity to integrate dietary information through time (Layman
884 et al. 2012). For instance, stable isotope values in fish muscle samples provide dietary
885 information over 2 to 8 weeks (Boecklen et al. 2011). The stable isotope ratio of carbon and
886 nitrogen (noted $\delta^{13}C$ and $\delta^{15}N$, respectively) are commonly used to diet reconstruction due to
887 their abilities to discriminate between different origin of resources (e.g. pelagic *versus*
888 littoral), and differential trophic position (Post 2002).
889 $\delta^{13}C$ and $\delta^{15}N$ values were measured on muscle samples from individuals *Lepomis*
890 *gibbosus* selected in each population. In addition, the stable isotope values of putative prey
891 resources were analyzed in each population during fish sampling (mid-September to mid-
892 October 2012) to inform on the diet of *Lepomis gibbosus* during the period of their maximal
893 growth rate (summer period). Specifically, littoral and pelagic prey resources were collected
894 in different locations when available to account for potential spatial variability within each
895 lake. The $\delta^{13}C$ and $\delta^{15}N$ values of littoral prey consisted of mean values of the most common
896 invertebrates sampled in the littoral habitat with a pond net (Chironomidae, Ephemeroptera,
897 Gastropoda, Assellidae and Oligochaeta; n = 1 to 4 samples per lake; n = 1 to 6 individuals
898 per sample). Isotope analyses for Gastropoda were performed on the soft muscle tissue. Stable
899 isotope values of pelagic prey were obtained from zooplankton and Chironomidae samples (n
900 = 3 - 4 samples per lake; n = 2 - 3 chironomids per sample) collected in the pelagic habitat
901 with a 100-µm mesh net and an Ekman dredge, respectively.
902 Prior to all analyses, stable isotope samples were oven dried for 48 h at 60 °C, ground
903 in a fine powder using a mortar and pestle and then analyzed at the Cornell Isotope
904 Laboratory (Ithaca, New York, USA). The analytical precision for all samples, calculated as
905 the standard deviation of an internal mink standard, was 0.11 and 0.12 ‰ for $\delta^{13}C$ and $\delta^{15}N$
906 values, respectively. Since the C:N ratio of prey (littoral prey : mean = 4.60 ± 0.10 SE;
907 pelagic prey : mean = 4.15 ± 0.12 SE) were higher than the suggested limits (3.5 for aquatic

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908 organisms; Post et al. 2007), the stable isotope values of prey were lipid-corrected before
909 subsequent analyses.
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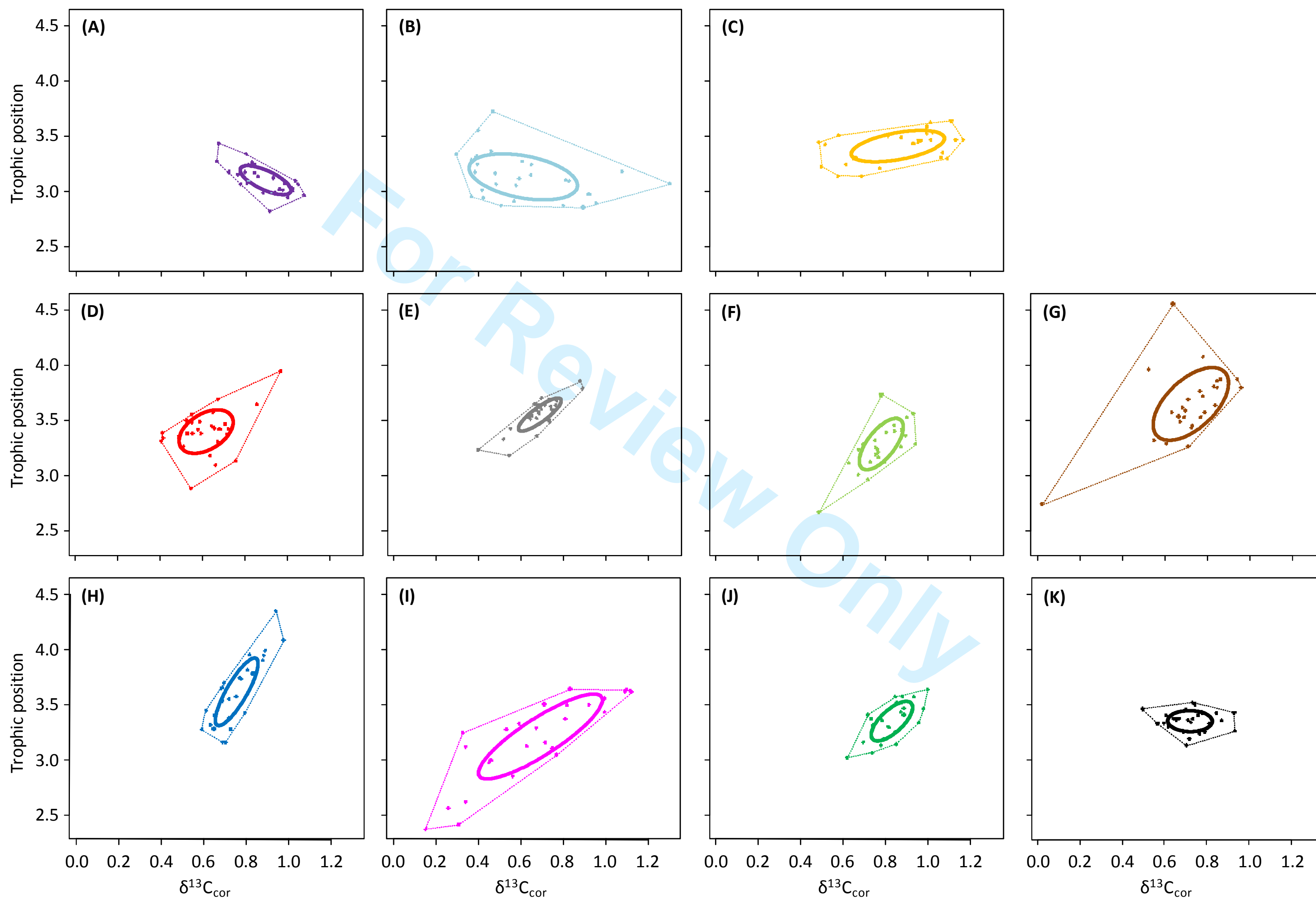

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913 **Figure A1** Bi-plots of the trophic position and the corrected $\delta^{13}C$ values ($\delta^{13}C_{cor}$) for each
914 population (A – K, n = 11). Each dot represents a *Lepomis gibbosus* individual. For each
915 population, the stable isotope niche is represented by the convex hull area (TA, dashed line;
916 Layman et al. 2007) and the standard ellipse area (SEA, continuous line). Statistical analyses
917 indicate a strong and positive correlation between SEA_b and TA (Spearman: rho = 0.81, S =
918 42, 9df, $P = 0.004$).
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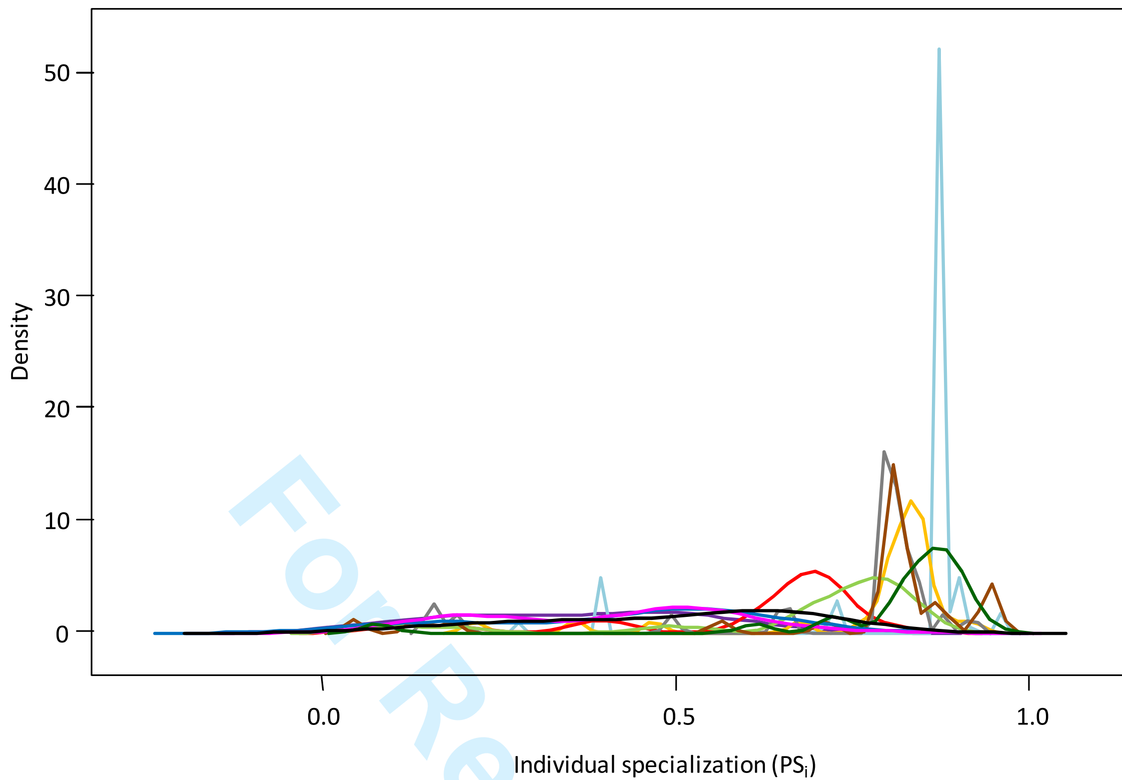

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939 **Figure A2** Kernel distribution of the individual specialization index (PS_i) calculated for the
940 11 wild populations of *Lepomis gibbosus* and based on stomach content analyses. Population
941 colors are indicated in Fig. A1.

942

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967 *Supplementary material*

968

969 **C. Evangelista, A. Lecerf, J.R. Britton, J. Cucherousset. Resource composition mediates**
970 **the effects of intraspecific variability in nutrient recycling on ecosystem processes**

971

972 **Appendix B:** Experimental protocol of nutrient excretion rates and results of the effects of
973 intraspecific trophic variability on consumers and their subsequent indirect effects on
974 ecosystem functioning.

975

976 Nutrient excretion rates of *L. gibbosus* were quantified in the laboratory following the
977 protocol of Vanni et al. (2002). Two hours after feeding (Glaholt and Vanni 2005), *L.*
978 *gibbosus* were placed individually into a translucent plastic bag containing 0.8 L of spring
979 water and stored in a covered bucket to minimize visual contact and reduce physiological
980 stress during incubation (Whiles et al. 2009). The spring water was low in soluble N and P but
981 had similar chemical characteristics, especially pH, to the water used in the experiments. An
982 incubation time of 1h30 was used to ensure concentrations of ammonium and phosphorus in
983 the water were above quantification levels (Glaholt and Vanni 2005). Filtered water samples
984 (80 mL filtered using Whatman GF/C, pore size 1.2 µm) were analyzed for ammonium (N-
985 NH_4^+, hereafter referred to as N) and soluble reactive phosphorus (SRP, hereafter referred to
986 as P) concentrations using the phenol-hypochlorite and molybdenum blue methods,
987 respectively, run by an automated continuous-flow colorimetric analyzer (ALPKEM
988 Corporation, Clackamas, OR, U.S.A.). *Per capita* excretion rates (ER; hereafter referred to as
989 'excretion rate') of N and P (µmol ind.$^{-1}$ h^{-1}) were calculated for each individual following
990 Vanni et al. (2002):

991 $$ER_I = (([I]_{ind} - [I]_{control}) \times V) / t$$

992 where $[I]_{ind}$ and $[I]_{control}$ are the molar concentration (µmol L^{-1}) of the element I observed for
993 fish and control, respectively, V is the volume (L) of spring bottled water in the plastic bag
994 and t is the duration of the incubation (hours). At the start of the experiment, initial *per capita*
995 excretion rates did not differ significantly between treatments (ANOVAs, $P = 0.154$ for N and
996 $P = 0.341$ for P). At the end of the experiment, after the final excretion trial, *L. gibbosus* were
997 euthanized using an overdose of anesthetic and weighted individually ($W_f \pm 0.1$ g).

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