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Resource stoichiometry shapes community invasion resistance via productivity-mediated species identity effects

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Resource stoichiometry shapes community invasion resistance via

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productivity-mediated species identity effects

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18

19 Abstract

20 The diversity-invasion resistance relationships are often variable and sensitive to
21 environmental conditions such as resource availability. Resource stoichiometry, the
22 relative concentration of different elements in the environment, has been shown to
23 have strong effects on the physiology and interactions between different species. Yet,
24 its role for diversity-invasion resistance relationships is still poorly understood. Here
25 we explored how the ratio of nitrogen and phosphorus affect the productivity and
26 invasion resistance of constructed microbial communities by plant pathogenic

27 bacterium, *Ralstonia solanacearum*. We found that resource stoichiometry and
28 species identity effects affected the invasion resistance of communities. Both high
29 nitrogen concentration and resident community diversity constrained invasions, and
30 two resident species, in particular, had strong negative effects on the relative density
31 of the invader and the resident community productivity. While resource stoichiometry
32 did not affect the mean productivity of the resident community, it favored the growth
33 of two species that strongly constrained invasions turning the slope of
34 productivity-invasion resistance relationship more negative. Together our findings
35 suggest that alterations in resource stoichiometry can change the community
36 resistance to invasions by having disproportionate effects on species growth
37 potentially explaining changes in microbial community composition under
38 eutrophication.

39

40 **Key words:**

41 Resource stoichiometry, diversity-invasion resistance relationship, nitrogen,
42 phosphorus, productivity, species identity effects

43

44 **1. Introduction**

45 Microbial biodiversity plays an important role in ecosystem functioning by offering
46 sets of functions that cannot be provided by single species [1–3]. For example,
47 host-associated microbial communities can ward off pathogens thereby protecting
48 their associated host organism [4–6]. This process can also be viewed from the
49 perspective of biological invasions where the members of resident microbial
50 communities facilitate or constrain the establishment of the invader [7,8]. Several
51 studies have shown that increasing community diversity reduces the likelihood of
52 invasions by promoting a more comprehensive use of available niches in the given
53 environment [3,9,10]. Such diversity-invasion resistance relationships are however
54 often sensitive to environmental conditions such as resource availability [11] or

55 temperature [12]. As a result, we still poorly understand how environmental contexts
56 shape diversity-invasion resistance relationships.

57 Resource availability has been shown to be an important factor affecting the
58 outcome of biological invasions [13–15]. Mechanistically, concentration or
59 composition of resources can alter the physiology and interactions between different
60 species within communities which can then lead to changes in community invasion
61 resistance. Moreover, changes in resource availability may change the significance of
62 species identity effects, i.e. the contribution of resident community members to the
63 invasion, by promoting the growth of species that grow either slow or fast [16,17].
64 Species identity effects could thus explain positive diversity-invasion resistance
65 relationships across environmental gradients where different species contribute to the
66 invasion resistance under specific environmental conditions [1,18]. Here we studied
67 how resource stoichiometry, the relative concentration of different elements in the
68 environment, shapes invasions via diversity, productivity and species identity effects.

69 Resource stoichiometry is a broad and active research field in ecology that has been
70 extensively used to understand predator-prey interactions [19,20]. While several
71 studies have highlighted the importance of resource stoichiometry for the ecology and
72 functioning of communities [21,22], its effects have been less studied in the context of
73 diversity-ecosystem functioning relationships. Environmental stoichiometry can be
74 used to link tissue composition of organisms with trophic level interactions [23–25]
75 and it plays important role in determining which species are able to grow in any given
76 ecosystem affecting consumer-resource interactions [26,27]. For example, low C:P
77 ratio has been shown to favour fast-growing species leading to an increase in
78 microbial diversity [28]. Resource stoichiometry can also affect invasions by altering
79 species biomasses and growth dynamics [29]. However, it is unclear how resource
80 stoichiometry shapes the diversity-invasion resistance and productivity-invasion
81 resistance relationships.

82 In the present study, we used experimental approach to directly expose model
83 microbial communities with varying levels of diversity to invasions by a single
84 invader species under different resource stoichiometry treatments. The model

85 'resident' community was constructed by using five different bacterial species that
86 have previously been shown to constrain invasions in a diversity-dependent manner
87 [4,17]. As an invader, we used a plant pathogenic *Ralstonia solanacearum* bacterium
88 whose life cycle is directly linked to biological invasions of the plant rhizosphere
89 microbiome. *Ralstonia solanacearum* causes bacterial wilt disease [30,31] and is a
90 major threat to global food production [32]. Before infecting its host, *R.*
91 *solanacearum* must first get through microbial communities surrounding the plant
92 roots. It has previously been shown that competition for resources between the
93 invader and resident community members is important for the outcome of invasions
94 [4,17]. How these invasion outcomes are affected by dynamic changes in nutrient
95 levels typical for rhizosphere microbiomes [33,34] and resident community diversity
96 remains unclear. To study this, we manipulated both resident community diversity
97 gradient (richness levels of 1 to 5 species in all possible combinations) and the
98 resource stoichiometry of the environment by changing the relative concentration and
99 ratio of nitrogen (N) and phosphorus (P) orthogonally by following the Redfield ratio.
100 Redfield ratio is the atomic ratio of carbon, nitrogen and phosphorus found in
101 phytoplankton and throughout the deep oceans [35] and a general baseline of element
102 composition for both aquatic and terrestrial ecosystems [36,37]. Communities were
103 then exposed to *R. solanacearum* invasions and the invasion success was determined
104 as the relative density of *R. solanacearum* invader after 72h growth in the resident
105 community (indicative of the reproductive success of the invader): higher the final
106 relative abundance of *R. solanacearum*, higher the invasion success. We expected that
107 resident community diversity-invasion resistance relationship could be sensitive to
108 resource stoichiometry having either positive or negative effects on invasions
109 depending on specific changes in species ability to grow under different N:P ratios.
110 Mechanistically, changes in invasion outcomes could potentially be explained via
111 effects on community productivity or changes in the relative contribution of
112 community members to invasions via species identity effects.

113

114 **2. Methods**

115 **(a) Bacterial strains and plasmids**

116 We used *Ralstonia solanacearum* species QL-Rs1115 tagged with pYC12-mCherry
117 plasmid as a model invader in our experiments [38]. Five avirulent, but closely
118 related, *Ralstonia spp.* isolates (*Ralstonia mannitolilytica* QL-A2, *Ralstonia*
119 *mannitolilytica* QL-A3, *Ralstonia pickettii* QL-A6, *Ralstonia taiwanensis* QL-117 and
120 *Ralstonia pickettii* QL-140) were used to construct our model resident communities
121 [4]. None of these bacteria showed direct antagonism towards each other or the
122 invader, which suggests that they likely interact indirectly through competition for
123 shared resources. A more detailed description of the bacteria and used plasmid can be
124 found in Table S1. All bacteria were stored at -80 °C in 20 % glycerol prior to the
125 experiments.

126 **(b) Assembly of resident communities**

127 The resident communities were assembled by using all five avirulent species in
128 substitutive design so that the final communities covered all possible species
129 combinations and richness levels (total of 31 communities with equal initial bacterial
130 biomasses, Table S2). Prior to the experiments, bacteria were pre-cultured from
131 frozen stocks on Nutrient Agar plates (NA, glucose 10.0 g L⁻¹, tryptone 5.0 g L⁻¹, beef
132 extract 3.0 g L⁻¹, yeast extract 0.5 g L⁻¹, agar 15.0 g L⁻¹, pH 7.0) and single colonies
133 were picked and re-grown in liquid nutrient broth (NA medium without agar) at 30 °C
134 for 12 h with 170 r.p.m. agitation. Bacterial isolates were washed three times in
135 0.85 % NaCl to remove nutrient residues and re-suspended in 0.85 % NaCl with final
136 densities of 10⁷ cells mL⁻¹.

137 **(c) Manipulation of resource stoichiometry**

138 To manipulate the resource stoichiometry, we first set up a minimal salt medium,
139 which did not contain carbon, nitrogen or phosphorus (MOPS 30mM, CaCl₂ 0.1mM,
140 FeSO₄ 3 mM, KCl 20mM, MgCl₂ 2mM, Na₂SO₄ 14mM and NaCl 51mM, pH 7.0).
141 The minimal medium was then supplemented with a mixture of carbons (fructose,

142 glucose, sucrose, maltose, arabinose and galactose) in equal concentrations to yield a
143 total concentration of 10 mM for all combined carbon resources as described
144 previously [4]. The concentration of total carbon resources (10 mM) was held
145 constant for all resource stoichiometry treatments. To manipulate the concentration
146 and ratio of nitrogen and phosphorus, we added NH_4Cl or $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ as the sole
147 nitrogen and phosphorus resource, respectively. A total of six resource stoichiometry
148 treatments with four unique N:P ratios were established for the experiment where low,
149 intermediate and high nitrogen levels were established within both low and high
150 phosphorus levels (Table 1). Each media was then used to establish replicate
151 treatments on 96-well microtiter plates in triplicate (18 microplates in total) for each
152 resident community combination.

153 **(d) Measuring resident community invasion resistance and productivity in**
154 **microcosms**

155 To quantify invasion resistance, all communities (10^6 cells mL^{-1} in 200 μL of final
156 volume) were exposed to invasion by *R. solanacearum* QL-Rs1115 (10^5 cells mL^{-1} in
157 200 μL of final volume) under different resource stoichiometry environments.
158 Replicate communities without invader were used as control treatments. All
159 communities were incubated for 72 h at 30 °C with 170 r.p.m. orbital agitation. To
160 measure invader density relative to resident community density, we measured the
161 mCherry fluorescence signal (Excitation: 587 nm, Emission: 610 nm, gain: 60) of the
162 invader and calculated the invasion success as mCherry relative fluorescence unit
163 against total bacterial density of the community (RFU, $\text{mCherry}/\text{OD}_{600}$) at the end of
164 the experiment (after 72 h of incubation). To quantify the total productivity of
165 different communities in each resource environment, we used optical density (OD_{600})
166 as a measure of total bacterial growth (invader and the resident community). We used
167 the control communities without the invader to blank the fluorescence signal
168 background and optical density of the culture media to blank the OD_{600} background.

169 In order to verify plasmid stability during the invasion experiments, we grew
170 gentamycin-tagged mCherry plasmid carrying *R. solanacearum* invader (10^6 cells
171 mL^{-1} in 200 μL of final volume) in four N:P ratios (0.16, 1.6, 16 and 160) in the

172 absence and presence of gentamycin antibiotic ($30 \mu\text{g mL}^{-1}$) for 72 h. Gentamycin
173 was added only at the beginning or at every 24 h to create a strong selective pressure
174 on the plasmid. The plasmid stability was determined as fluorescent signal intensity,
175 which is indicative of bacterial growth and expression of the plasmid-encoded
176 mCherry fluorescent protein (Figure S1). No difference was observed between
177 different antibiotic treatments in any of the N:P ratios after 72 h of incubation (Figure
178 S1; The main effect of antibiotic treatment in 0.16, 1.6, 16 and 160 N:P ratios,
179 respectively: $F_{1, 194} = 0.08$, $P = 0.77$; $F_{1, 381} = 0.2$, $P = 0.65$, $F_{1, 371} = 1.57$, $P = 0.21$ and
180 $F_{1, 190} = 0.08$, $P = 0.77$). This suggests that the plasmid was stably maintained during
181 the invasion experiments in the absence of gentamycin.

182 **(e) Measuring the growth and consumption of nitrogen and phosphorus by each**
183 **bacterial species**

184 The growth rate and productivity of all bacterial species were measured in
185 monoculture at four N:P ratios. Bacteria were inoculated at an initial density of 10^6
186 cells mL^{-1} in 96-well microtiter plates as described above. Each monoculture was
187 grown in triplicates under each of the N:P ratios at 30°C with agitation (170 r.p.m.)
188 for 72 h. To determine growth rates, we measured bacterial growth with
189 spectrophotometer (OD_{600}) at every 8 h to fit in Logistics model function
190 (“gcFitModel” in package “grofit” in R 3.3.1) [39] and the Maximum slope (μ , h^{-1}) of
191 the Logistic model was considered as the maximum growth rate [40]. The
192 productivity of each species was determined as the bacterial biomass after 72 h.
193 Culture media without bacteria was used to determine the background absorbance
194 before determining growth rates and productivity. To test the consumption of nitrogen
195 and phosphorus by all bacteria, we obtained cell-free supernatant by centrifugation
196 (10 000 rpm for 10 min) and filtration (0.22 μm filters) after 72 h incubation.
197 Nitrogen and phosphorus concentrations were measured using a continuous-flow
198 analyzer (AA3, SEAL, Germany) and compared with unconsumed media (no
199 bacterial inoculation).

200

201 (e) Statistical analyses

202 Invasion success (RFU, mCherry/OD₆₀₀) and resource stoichiometry ratios (N:P) were
203 log₁₀-transformed before statistical analyses to fulfill model assumptions. All
204 comparisons between the growth of individual species were analyzed using ANOVA
205 and linear regression. General Linear Mixed Models (GLMs) were used to examine
206 the resident species identity effects and resource stoichiometry as a function of
207 invader relative density (invasion success). Model 1 (“Richness-ratio”) and Model 2
208 (“Identity-ratio”) were used to identify how individual species contributed to the
209 invasion success as a function of resource stoichiometry. Model 3
210 (“Richness-concentration”, Table S3) was used to analyze whether N and P
211 concentrations had interactive effects with species richness in determining invasion
212 success and resident community productivity. Model 4 (“Productivity-ratio”, Table
213 S4) was used to study the interactive effects between productivity and resource
214 stoichiometry on the invasion success, while Models 5 (“Richness-ratio”, Table S4)
215 and 6 (“Identity-ratio”, Table S4) were used to explore the effects of community
216 diversity and species identity on the resident community productivity. In some cases,
217 we used the means of community treatment replicates for simplified analysis (e.g.
218 species presence effects in Figure 2).

219 Structural Equation Model (SEM; ‘lavaan’ package in R) was used to investigate
220 the relative importance of resource stoichiometry, species identity effects, species
221 maximum growth rates, nitrogen and phosphorus consumption and resident
222 community productivity on the invasion success (the *prior* model is shown in Figure
223 S2). All analyses were performed with R 3.3.1 [41].

224

225 3. Results

226 (a) Effects of resource stoichiometry on the diversity-invasion relationship

227 We found that increasing resident species richness had a negative effect on invasions
228 across all N:P ratios (Figure 1; Model 1 in Table 2, the main effect of species richness

229 on invader relative density). Similarly, the relative density of the invader decreased
230 with increasing N:P ratio and N concentration (Model 1 in Table 2, the main effect of
231 N:P ratio on invader relative density), while P concentration alone had no effect
232 (Model 3 in Table S3). No interactive effect between species richness and N:P ratio
233 on the relative density of the invader was found (Model 1 in Table 2). These results
234 suggest that both species richness and N:P ratio constrained invasions independently,
235 while N concentration alone had a stronger effect than P concentration.

236 **(b) The effect of resident species identities on resident community productivity**
237 **and invasions**

238 We found that *R. mannitolilytica* QL-A2, *R. mannitolilytica* QL-A3 and *R.*
239 *taiwanensis* QL-117 resident species had no significant species identity effects on the
240 relative density of the invader (Model 2 in Table 2). In contrast, *R. pickettii* QL-A6
241 and *R. pickettii* QL-140 resident species were very effective at reducing the relative
242 density of the invader (Model 2 in Table 2). To examine this in more detail, we
243 compared the invasion resistance of resident communities with and without these two
244 species. As expected, resident communities were less resistant to invasions in the
245 absence of these two species (Figure 2, comparison on communities with and without
246 *R. pickettii* QL-A6: panel a, $F_{1,184} = 400.4$, $P < 0.001$, comparison on communities
247 with and without *R. pickettii* QL-140: panel b, $F_{1,184} = 4.47$, $P = 0.036$).
248 Mechanistically, this could be explained by relatively more efficient consumption of
249 nitrogen and phosphorus compared to the other resident species (Figures S3 and S4;
250 Species main effects at 0.15, 1.5 and 15 mM nitrogen concentrations, respectively: $F_{6, 35} = 254.6$, $P < 0.001$; $F_{6, 35} = 3196$, $P < 0.001$ and $F_{6, 35} = 55.69$, $P < 0.001$. Species
251 main effects at 0.09 and 0.9 mM phosphorus concentrations, respectively: $F_{6, 56} =$
252 20.92 , $P < 0.001$ and $F_{6, 56} = 6.00$, $P < 0.001$). However, no clear differences were
253 found in comparison with the invader, which suggests that *R. pickettii* QL-A6 and
254 QL-140 were equally good at consuming nitrogen and phosphorus ($P > 0.05$ in all
255 pairwise comparisons).
256

257 Even though increasing N:P ratio constrained invasions, no significant

258 interactions with species identities were found in the full-scale invasion experiment
259 (Model 2 in Table 2). To explore this further we compared the growth of resident
260 species and the invader separately in monocultures. Growth rate of *R. pickettii*
261 QL-A6, *R. pickettii* QL-140 and the invader *R. solanacearum* did not increase linearly
262 with increasing N:P ratio. While *R. pickettii* QL-140 had the highest growth rate when
263 N:P ratio was 0.16 ($F_{5, 12} = 9.87$, $P < 0.001$, Figure S5), *R. pickettii* QL-A6 grew the
264 fastest at 16 and 160 N:P ratios ($F_{5, 30} = 24.87$, $P < 0.001$ and $F_{5, 12} = 26.77$, $P < 0.001$,
265 respectively in Figure S5). No difference in the maximum growth rate of these species
266 was found at 1.6 N:P ratio ($F_{5, 30} = 8.23$, $P < 0.001$, Figure S5).

267 However, clear positive correlations were found between the productivity
268 (population density after 72 h of growth) of the invader, QL-A6 and QL-140 species
269 and the increasing N:P ratio (Figure 3B). While *R. pickettii* QL-140 had the highest
270 productivity at the lowest N:P ratio (0.16), the *R. pickettii* QL-A6 became more
271 competitive relative to the invader at 16 and 160 N:P ratio treatments. This suggests
272 that differences in species ability to grow under increasing N:P ratios were likely
273 important in explaining diversity-productivity-invasion resistance relationships.

274

275 **(c) The effect of resource stoichiometry on the resident community productivity** 276 **and invasions**

277 We found that increasing N:P ratio had a hump-shaped (non-linear) relationship with
278 the resident community productivity (Figure S6, $F_{1, 184} = 1.28$, $P = 0.26$; the mean
279 productivity of all resident communities in all richness levels), and only the resident
280 community richness correlated positively with resident community productivity
281 (Model 5 in Table S4). However, resident community productivity was positively
282 affected by both *R. pickettii* QL-A6 and QL-140 species and QL-A6 had a positive
283 effect on community productivity with increasing N:P ratio (Model 6 in Table S4),
284 which is in line with their ability to efficiently consume nitrogen and phosphorus
285 (Figures S3 and S4) and to reach higher biomasses (productivity) with increasing N:P
286 ratios in monocultures (Figure 3B).

287 Resident community productivity had a clear negative effect on the relative
288 density of the invader (Figure 3A, Model 4 in Table S4), and crucially, the negative
289 relationship between the resident community productivity and the density of the
290 invader became stronger with increasing N:P ratio (Figure 3A, Model 4 in Table S4).
291 This can be explained by resident species identity effects, in particular, the ability of
292 *R. pickettii* QL-140 to increase its growth with increasing N:P ratio, which then turned
293 the productivity-invasion resistance relationship more pronounced.

294

295 **(d) Linking species identity and resource stoichiometry effects with** 296 **productivity-invasion resistance relationship**

297 To further study how invasions and community productivity were shaped by resource
298 stoichiometry and species identity effects, we built a Structural Equation Model
299 (SEM) describing direct and indirect relationships between these variables. The final
300 SEM model explained 76 % of the variance of the relative density of the invader
301 (Figure 4). The species *R. pickettii* QL-A6 had a significant negative effect on the
302 relative density of the invader, while the species *R. pickettii* QL-140 promoted both
303 community productivity and reduced the relative density of the invader. Similar to
304 previous analyses, resource stoichiometry did not change the species identity effects
305 in the SEM. However, resource stoichiometry had negative effects on both resident
306 community productivity and the relative density of invader, while the resident
307 community productivity itself had a negative effect on invasions. Together these
308 results suggest that species identity and resource stoichiometry had both direct and
309 indirect negative effects on invasions and that the indirect effects were mediated by
310 resident community productivity.

311

312 **4. Discussion**

313 Biodiversity is an important determinant of ecosystem functioning having significant
314 effects on community resistance to biological invasions [5,42,43]. Here we addressed

315 how changes in environmental stoichiometry (N:P ratio) affects invasion resistance of
316 model microbial communities. We found that changes in resource stoichiometry had
317 clear effects on invasion outcomes via productivity-mediated species identity effects.
318 First, increasing N:P ratio lowered the intercept of diversity-invasion resistance
319 relationship, which suggests that increasing the input of nitrogen reduced the
320 likelihood of invasions regardless of the community diversity. Second, two resident
321 species, *R. pickettii* QL-A6 and QL-140, played key roles in having negative effects
322 on the invader and positive effects on resident community productivity. Crucially,
323 increasing the N:P ratio turned the slope of productivity-invasion resistance
324 relationship much steeper because the species *R. pickettii* QL-A6 and *R. pickettii*
325 QL-140 grew better and constrained invasions more efficiently when nitrogen became
326 more abundant. Together these results suggest that resource stoichiometry can change
327 the outcome of microbial invasions via productivity-mediated species identity effects.

328 In line with the previous studies, increasing resident community diversity
329 decreased the likelihood of successful invasions [4,5,44]. While resource
330 stoichiometry did not interact with resident species richness, it lowered the intercept
331 of diversity-invasions resistance relationship. This suggests that an increase in the
332 relative concentration of nitrogen improved the resident community invasion
333 resistance regardless of the species richness but that this effect was stronger in more
334 diverse communities. One simple explanation for this is that increasing community
335 diversity increased the likelihood that one or both of the species that were effective at
336 constraining invasions (*R. pickettii* QL-A6 and QL-140) were included in
337 communities. To study this in more detail, we concentrated on exploring the relative
338 importance of resident species identities on invasions.

339 Two resident species, *R. pickettii* QL-A6 and QL-140, played key roles in having
340 negative effects on the invader relative density and positive effects on the resident
341 community productivity. Both of these species were effective at consuming nitrogen
342 and phosphorus across all N:P ratios (Figures S3 and S4), and hence, their
343 contribution to invasion resistance likely overshadowed the effects of the other
344 resident community members. However, no difference was found in the consumption

345 of nitrogen and phosphorus among the invader, *R. pickettii* QL-A6 and QL-140
346 (Figures S3 and S4), which suggests that these three species were equally efficient at
347 sequestering N and P. However, either the *R. pickettii* QL-A6 or QL-140 was clearly
348 faster at growing than the invader in three out of four N:P ratios used in our
349 experiments (Figure S5), which could have helped them to outcompete the invader in
350 these conditions. Moreover, while these species constrained invasions across all N:P
351 ratios (Figure 4), we found that the importance of species QL-A6 on community
352 productivity increased along increasing N:P ratio, while species QL-140 had the
353 highest productivity at low N:P ratios (Figure 3B). Together these results suggest that
354 resource stoichiometry changed invasion outcomes via productivity-mediated species
355 identity effects by favoring resident species that were efficient at growing when the
356 nitrogen was abundant. This finding is in line with a previous study where these two
357 species were observed to have highly negative effects on the same invader used in this
358 study due to high catabolic similarity [17] and supports the idea that individual
359 contribution of resident community members on invasions can change according to
360 resource availability [17]. In the future, it would be interesting to study if
361 environmental stoichiometry can drive changes in the elemental stoichiometry of
362 bacterial and other microbial cells. For example, it has been shown that the effects of
363 resource stoichiometry can be species-specific [46] where environmental
364 stoichiometry favor species with similar biomass composition [25,26]. In this case,
365 the similarity in biomass composition between the resident species and the invader
366 could be important determinant for invasions.

367 Furthermore, we found that increasing N:P ratio had a hump-shaped effect on
368 community productivity that peaked at intermediate N:P ratios, which can optimize
369 species coexistence or community productivity [19,22,47]. However, this relationship
370 was not very strong and was only visible when all the communities with different
371 richness levels were included in the analysis. Interestingly, resident community
372 productivity correlated negatively with the relative density of the invader only within
373 16 and 160 N:P ratios. Mechanistically, this could be explained by the fact that the
374 importance of *R. pickettii* QL-A6 on resident community productivity and invasions

375 resistance increased along increasing N:P ratio (Figure 3B and Model 2 in Table 2).
376 This suggests that increasing the input of N can increase the invasion resistance of
377 communities via productivity, but that these effect might be driven by certain
378 important ‘key stone’ species instead of changes in the total community productivity
379 [9,17]. Several previous studies have suggested that resource stoichiometry of the
380 environment is a good predictor of species growth capacity [46,48]. For example, both
381 N and P are important for species growth via effects on production and expression of
382 proteins, enzymes and cell structures [27,49,50] and could often be limiting resources
383 in the environment. Furthermore, it is possible that invasion resistance is not only
384 mediated by nitrogen uptake but also by carbon metabolism, which is known to be
385 interconnected with nitrogen regulation [51]. In support for this, a previous study has
386 shown that the same resident species used in this study had higher growth rates,
387 productivity and high resource niche overlap with the same invader used in this
388 experiment when measured in various carbon media [4,17]. As a result, it is possible
389 that competition for both carbon and nitrogen affected the observed invasion
390 outcomes also in this experiment.

391 Here we link the high N:P ratio to improved community functioning in terms of
392 increased invasion resistance. Our results suggest that resource stoichiometry can
393 have positive effects on resident community productivity by favoring species that are
394 very efficient at constraining invasions. Interestingly, resource stoichiometry did not
395 change the shape of diversity-invasion resistance relationship even though invasions
396 were less successful in more diverse communities in general. In contrast, resource
397 stoichiometry turned the slope of the productivity-invasion resistance more negative,
398 because increase in nitrogen availability potentially intensified the competitive
399 interactions between resident community members and the invader by favoring the
400 growth of certain resident community members (*R. pickettii* QL-A6 and *R. pickettii*
401 QL-140). This is in line with studies showed that competition becomes stronger under
402 higher N:P ratio [28,52]. In the future, it will be important to better understand the
403 effects of resource stoichiometry on invasions in more natural environments. For
404 example, more information is needed how the presence of more complex microbial

405 community, multi-trophic interactions with predators and parasites, root exudation
406 and spatially uneven distribution of particulate organic matter shape the elemental
407 stoichiometry and their effects on invasions in complex plant-soil ecosystems.

408 We conclude that resource stoichiometry is an important determinant of
409 community invasion resistance. Human activities continue to have a huge effect on
410 global elemental cycling [53], nitrogen leaching and eutrophication, which are
411 causing growing problems and having devastating effects on the functioning of
412 ecosystems [54,55]. In the case of eutrophication, our results suggest that resource
413 stoichiometry could drive changes in microbial community composition potentially
414 affecting the likelihood of biological invasions. In the agricultural context, resource
415 stoichiometry could affect the severity of disease epidemics via effects on microbial
416 competition. A better understanding of this process could potentially help to control
417 plant pathogen invasions via modulation of soil nutrient availability and balance (N:P
418 ratios) to maintain relatively stable and invasion resistant microbial community. In
419 broader perspective, understanding how changes in global element balances affect the
420 interactions within and between communities is crucial for predicting ecosystem-level
421 responses to environmental change.

422

423 **Authors contributions.** Alexandre Jousset, Zhong Wei and Tianjie Yang designed
424 the experiment and Tianjie Yang carried out the laboratory work with the help of
425 Gang Han and Qingjun Yang and analyzed all the data. All authors wrote the
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427

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443

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624 Table 1. Concentration of nitrogen (NH_4Cl) and phosphorus (NaH_2PO_4) and their ratios (N:P) in
 625 different treatments

Treatment	Nitrogen (mM)	Phosphorus (mM)	N:P ratio
1	1.5	0.09	16:1 (Redfield ratio)
2	15	0.09	160:1
3	0.15	0.09	1.6:1
4	1.5	0.9	1.6:1
5	15	0.9	16:1
6	0.15	0.9	1.6:10

626

627 **Table 2.** ANOVA table summarizing the species richness, N:P ratio and species identity effects on the
 628 relative density of the invader (Models 1 – 2). Significant effects ($P < 0.05$) are highlighted in bold and
 629 the “up” and “down” arrows denote for positive and negative effects on the relative density of invader,
 630 respectively. Non-significant terms were not retained in the final models (“Not retained”).

	Relative density of the invader		
	Df	F	P
Model 1 (“Richness-ratio”)			
Species richness (Richness)	1	28.50	< 0.001 ↓
N:P ratio (Ratio)	1	9.71	0.0021 ↓
Richness * Ratio	1	0.0072	0.93
Residuals	182		
Model summary		AIC: 368.45, $R^2 = 0.17$	

Model 2 (“Identity-ratio”)

QL-A2			Not retained
QL-A3			Not retained
QL-A6	1	524.41	< 0.001 ↓
QL-117			Not retained
QL-140	1	25.27	< 0.001 ↓
N:P ratio (Ratio)	1	33.74	< 0.001 ↓
QL-A2 * Ratio			Not retained
QL-A3 * Ratio			Not retained
QL-A6 * Ratio			Not retained
QL-117 * Ratio			Not retained
QL-140 * Ratio			Not retained
Residuals	182		
Model summary		AIC: 136.72, $R^2 = 0.76$	

631

632 **Figure 1.** The effects of species richness and N:P ratio on invasion success (relative invader density).

633 The relative density of the invader was defined as log-10 transformed relative mCherry fluorescence

634 unit (RFU, mCherry/OD₆₀₀) after 72 h incubation.

635

636 **Figure 2.** The effect of *R. pickettii* QL-A6 and QL-140 species on the relative density of the invader

637 (panel a and b, respectively). Relative density of the invader was defined as log-10 transformed relative

638 mCherry fluorescence unit (RFU, mCherry/OD₆₀₀) after 72 h incubation. The 0 and 1 on X-axes denote639 for the presence and absence of *R. pickettii* QL-A6 and QL-140 species in the bacterial community and640 bars show ±1 standard error (n=186). Asterisks indicate significant differences (*, $P < 0.05$; **, $P <$ 641 0.01; ***, $P < 0.001$).

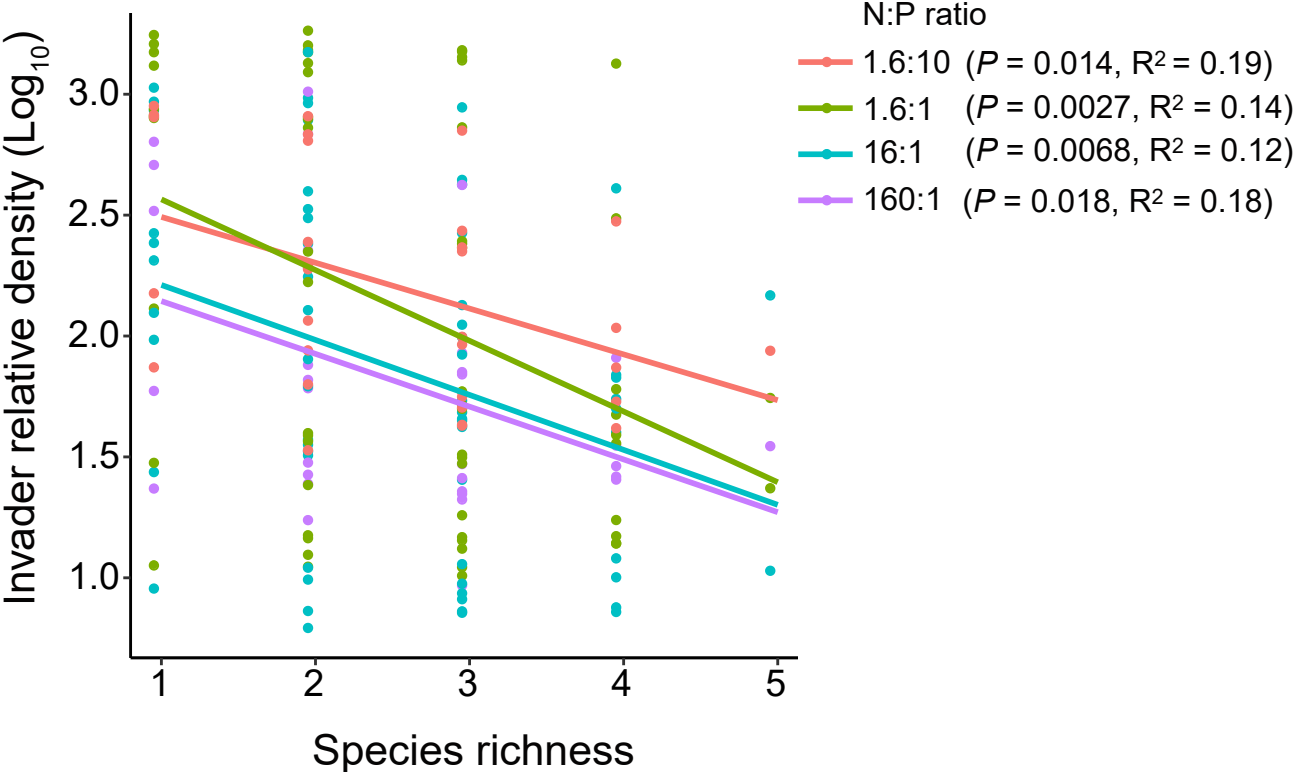
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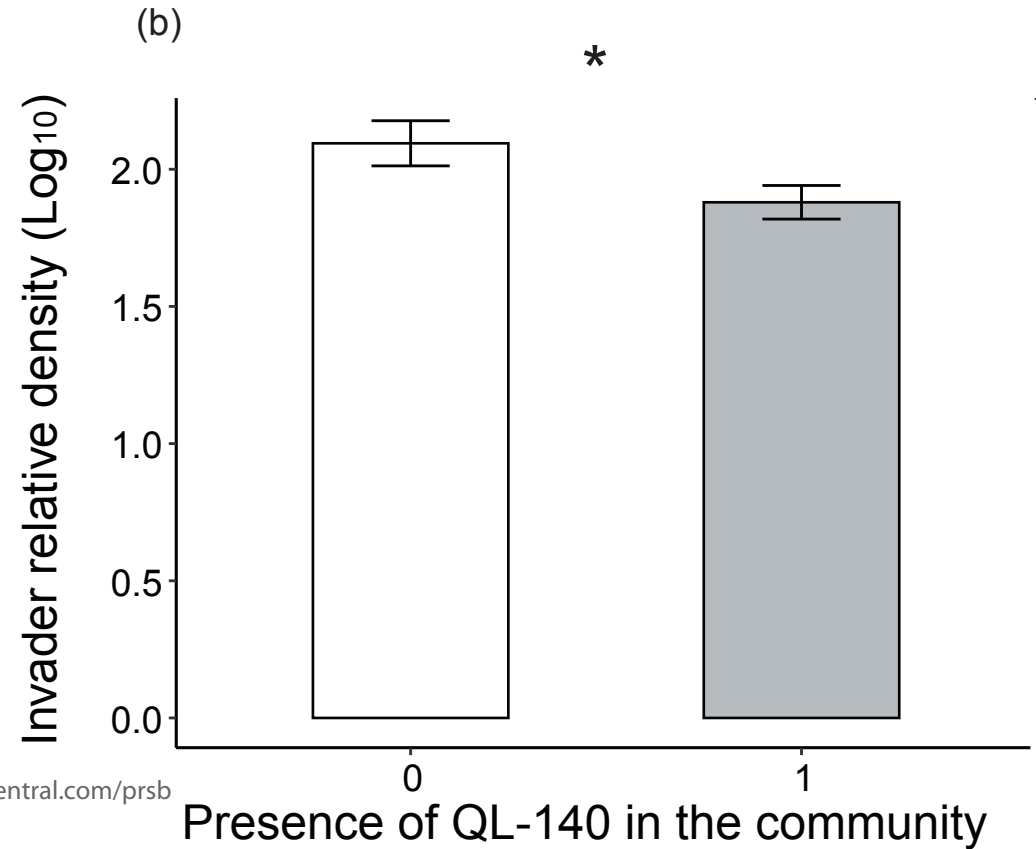
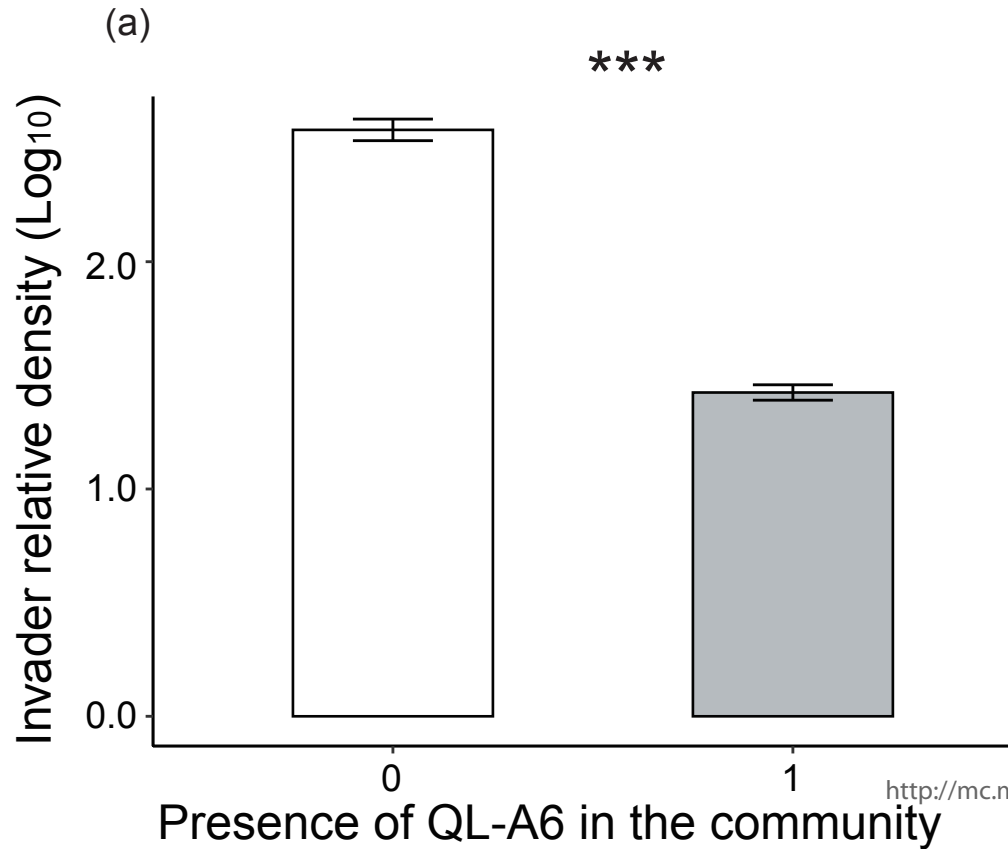
643 **Figure 3.** (A) The effect of N:P ratio on the resident community productivity-invasion resistance
644 relationship. Resident community productivity was defined as optical density (OD_{600}) after 72 h
645 incubation and shows the mean of all resident communities across all richness levels. The relative
646 density of invader was defined as log-10 transformed relative mCherry fluorescence unit (RFU,
647 $mCherry/OD_{600}$) after 72 h incubation. (B) The productivity of resident species and the invader at
648 different N:P ratios measured in bacterial monocultures (OD_{600} at 72 h of incubation).

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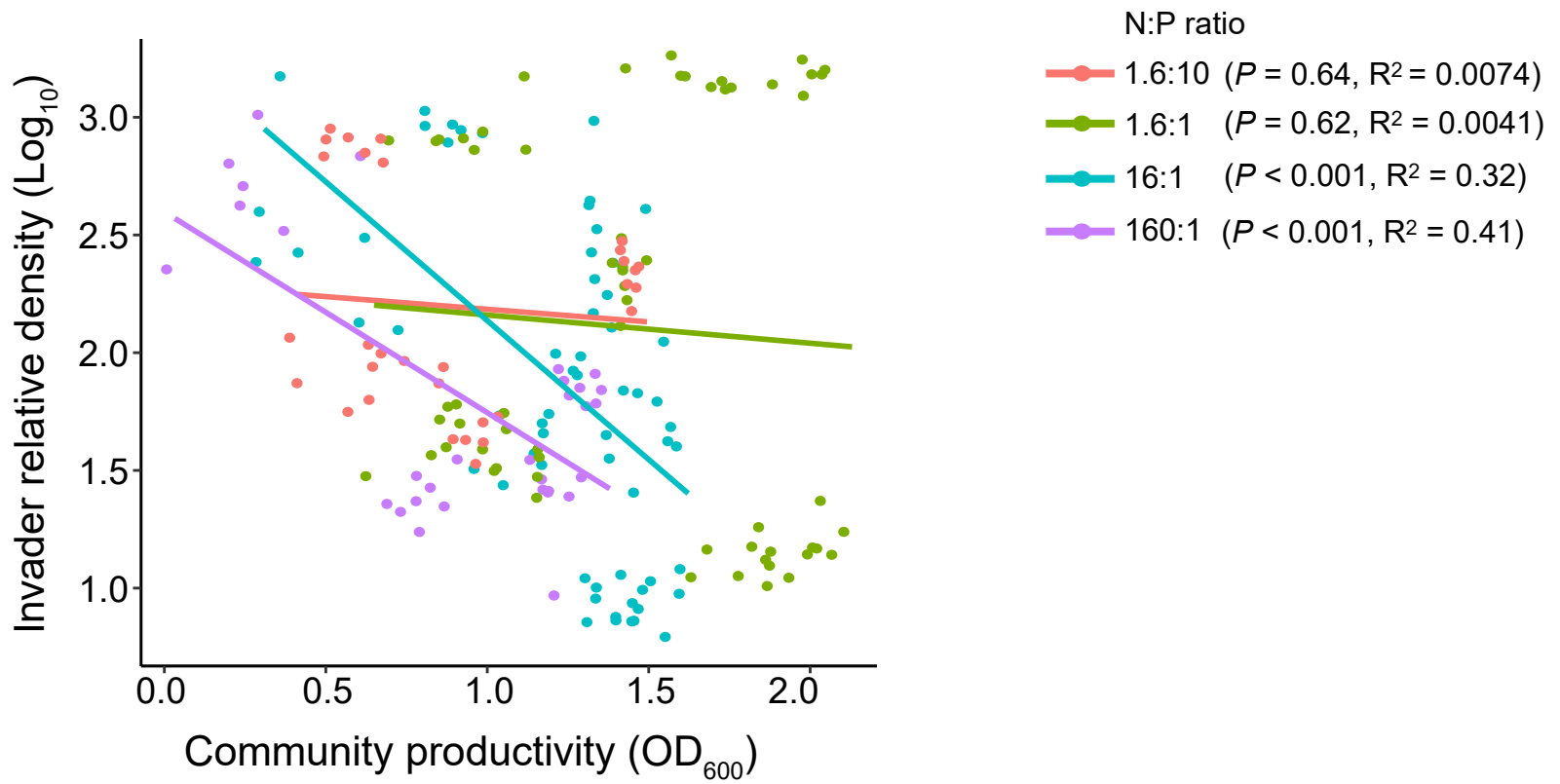
650 **Figure 4.** Structural Equation Model presenting direct and indirect effects of species identities and
651 resource stoichiometry on resident community productivity and relative density of the invader.
652 Resident community productivity was defined as optical density (OD_{600}) after 72 h incubation. Invasion
653 success was defined as log-10 transformed relative mCherry fluorescence unit (RFU, $mCherry/OD_{600}$)
654 after 72 h incubation. Continuous and dashed arrows indicate positive and negative effects, respectively,
655 and the width of the arrows indicate relative effect sizes. Black circles indicate the proportion of the
656 total variance explained and asterisks indicate significant effects (*, $P < 0.05$; **, $P < 0.01$; ***, $P <$
657 0.001).

658

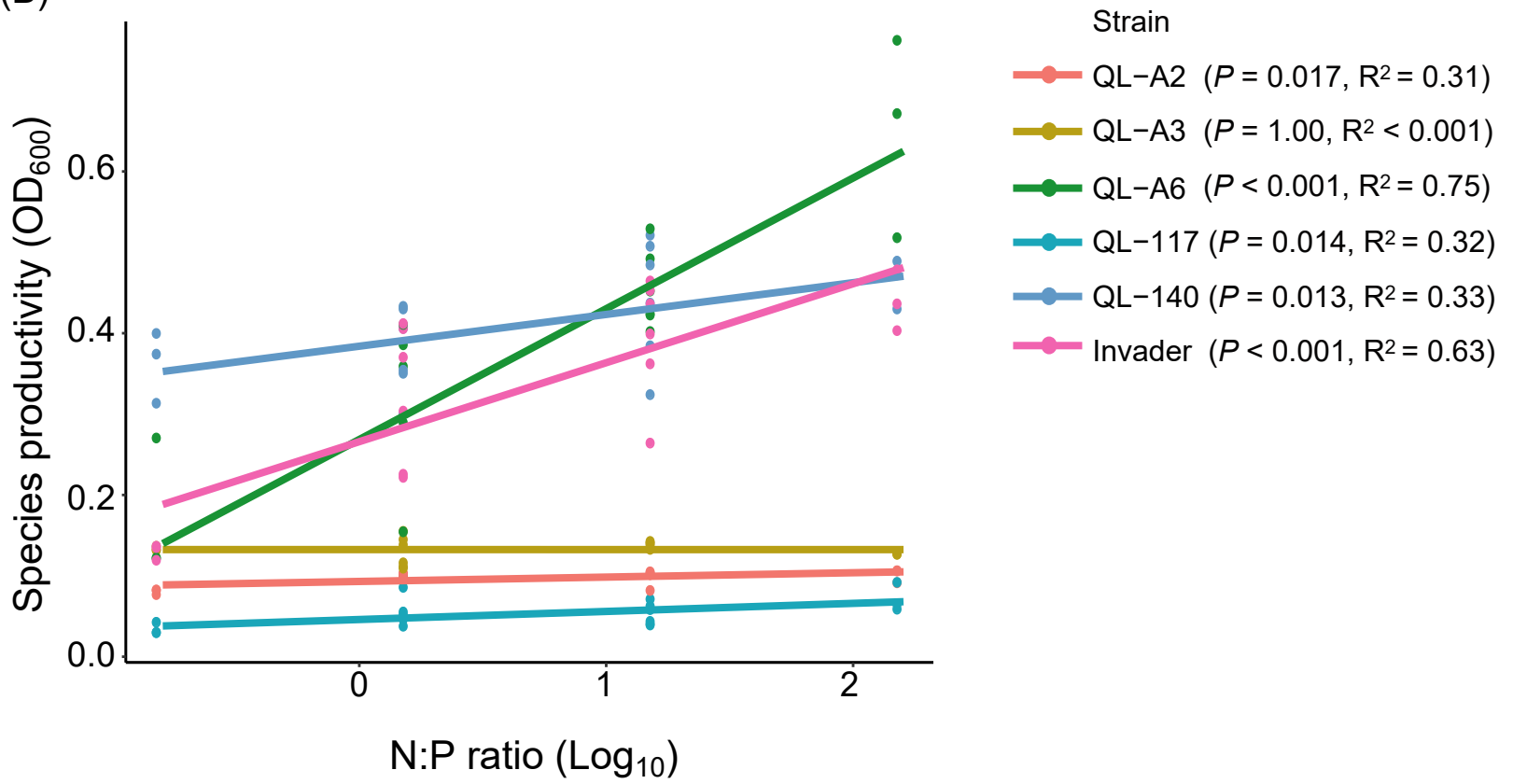




(A)



(B)



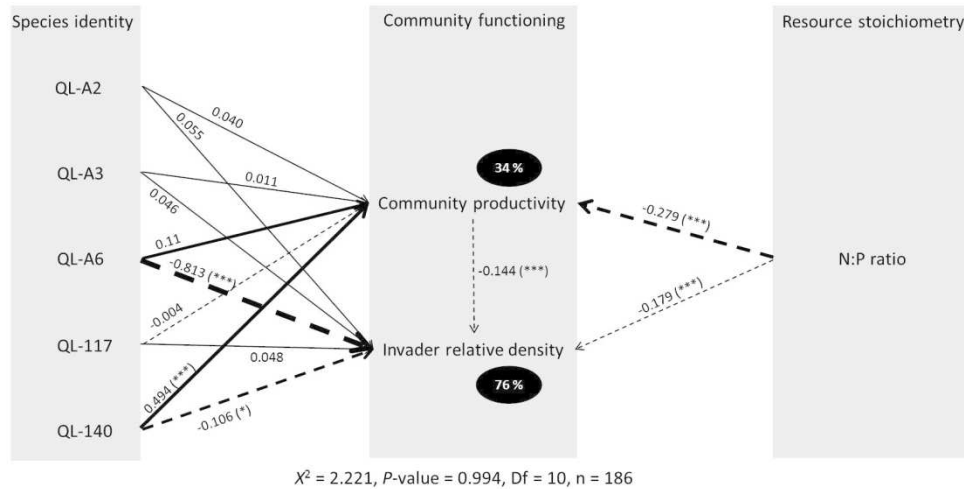
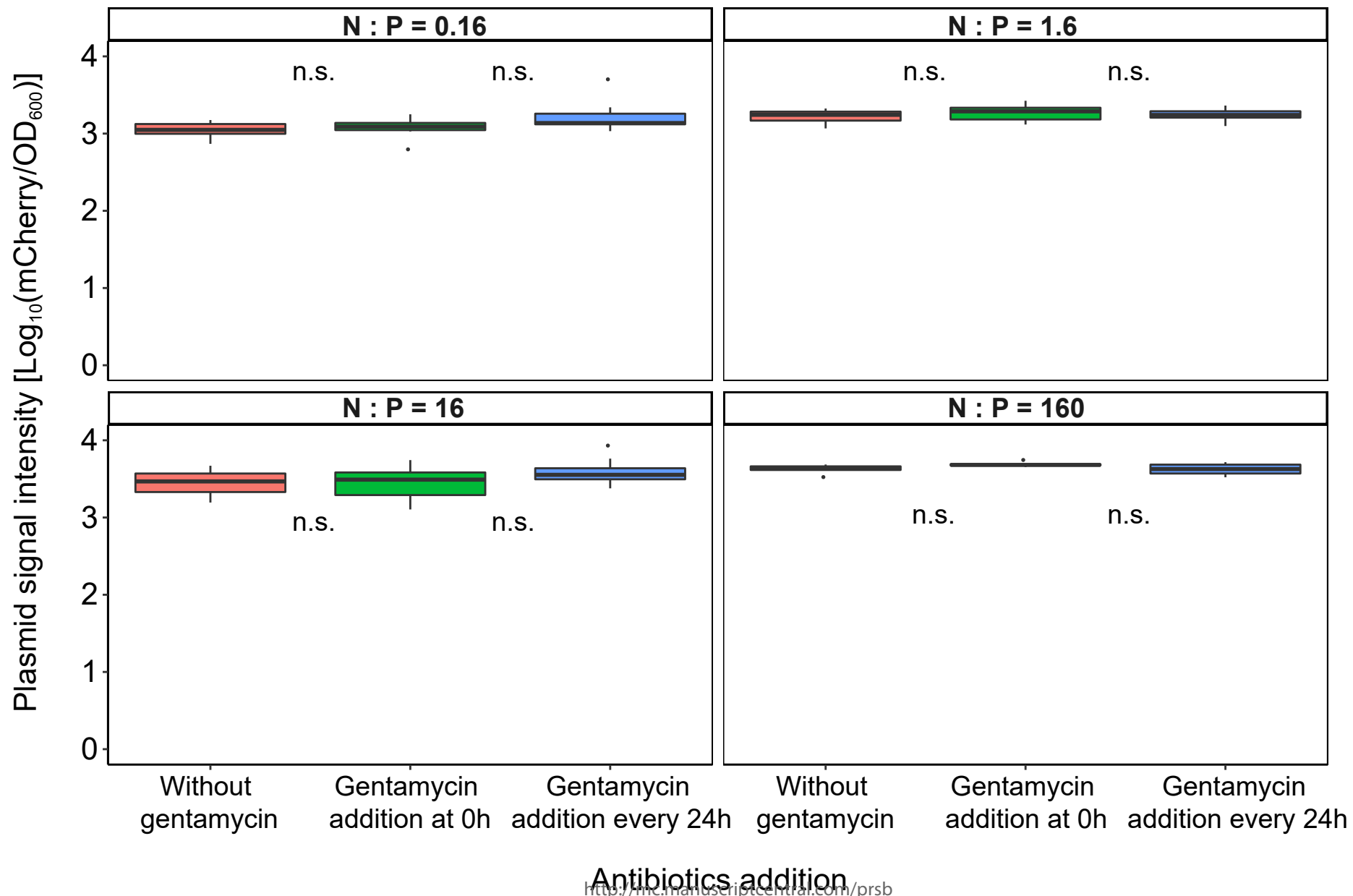


Figure 4. Structural Equation Model presenting direct and indirect effects of species identities and resource stoichiometry on resident community productivity and relative density of the invader. Resident community productivity was defined as optical density (OD600) after 72 h incubation. Invasion success was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD600) after 72 h incubation.

Continuous and dashed arrows indicate positive and negative effects, respectively, and the width of the arrows indicate relative effect sizes. Black circles indicate the proportion of the total variance explained and asterisks indicate significant effects (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

294x146mm (150 x 150 DPI)



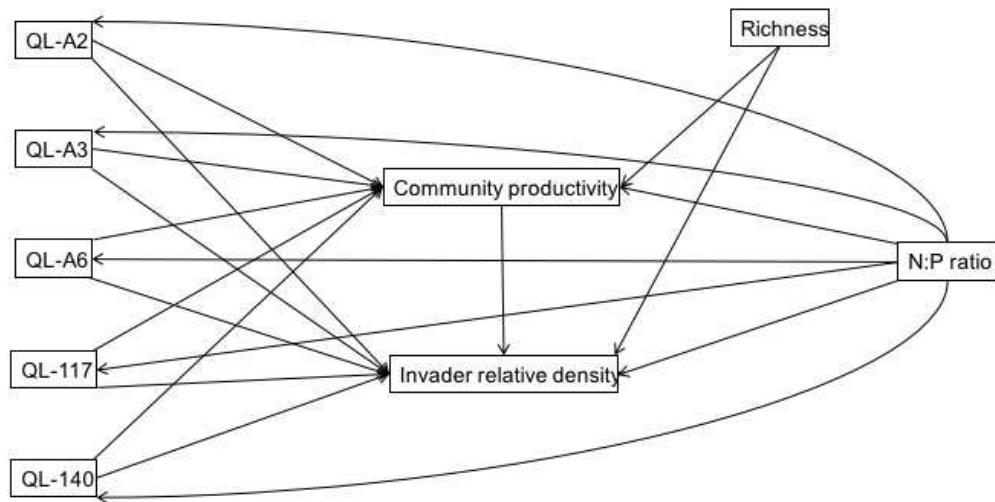


Figure S2. A priori structural equation models including species identity, N:P ratio, community productivity and the relative density of the invader.

230x115mm (72 x 72 DPI)

