UNIVERSITY of York

This is a repository copy of *Resource stoichiometry shapes community invasion resistance via productivity-mediated species identity effects*.

White Rose Research Online URL for this paper: <u>https://eprints.whiterose.ac.uk/138880/</u>

Version: Accepted Version

#### Article:

Yang, Tianjie, Han, Gang, Yang, Qingjun et al. (7 more authors) (2018) Resource stoichiometry shapes community invasion resistance via productivity-mediated species identity effects. Proceedings of the Royal Society B: Biological Sciences. ISSN 1471-2954

https://doi.org/10.1098/rspb.2018.2035

#### Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

#### Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk https://eprints.whiterose.ac.uk/

### PROCEEDINGS OF THE ROYAL SOCIETY B

BIOLOGICAL SCIENCES

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

Journal:	Proceedings B
Manuscript ID	RSPB-2018-2035.R1
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Yang, Tian-jie; Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University, 210095, Nanjing, PR China.; Institute for Environmental Biology, Ecology & Biodiversity, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands. Han, Gang; Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University Yang, Qingjun; Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University Yang, Qingjun; Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University, 210095, Nanjing, PR China. Friman, Ville-Petri; Department of Biology, Wentworth Way, Y010 5DD, University of York, York, UK.; Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University, 210095, Nanjing, PR China. Gu, Shao-hua; Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University, 210095, Nanjing, PR China. Wei, Zhong; Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center
	Xu, Yang-chun; Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for

	Organic-based Fertilizers, Nanjing Agricultural University, 210095, Nanjing, PR China. Shen, Qi-rong; Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University, 210095, Nanjing, PR China. Jousset, Alexandre; Institute for Environmental Biology, Ecology & Biodiversity, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.; Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University, 210095, Nanjing, PR China.
Subject:	Ecology < BIOLOGY
Keywords:	Resource stoichiometry, Diversity-invasion resistance relationship, Nitrogen, Phosphorus, Productivity, Species identity effects
Proceedings B category:	Ecology

### SCHOLARONE<sup>™</sup> Manuscripts

1 Resource stoichiometry shapes community invasion resistance via 2 productivity-mediated species identity effects 3 4 Tianjie Yang<sup>1,2</sup>, Gang Han<sup>1</sup>, Qingjun Yang<sup>1</sup>, Ville-Petri Friman<sup>1,3</sup>, Shaohua Gu<sup>1</sup>, 5 Zhong Wei<sup>1\*</sup>, George A. Kowalchuk<sup>1,2</sup>, Yangchun Xu<sup>1</sup>, Qirong Shen<sup>1</sup>, Alexandre 6 Jousset<sup>1,2</sup> 7 <sup>1</sup> Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu 8 Collaborative Innovation Center for Solid Organic Waste Resource Utilization, 9 National Engineering Research Center for Organic-based Fertilizers, Nanjing 10 Agricultural University, 210095, Nanjing, PR China. 11 <sup>2</sup> Institute for Environmental Biology, Ecology & Biodiversity, Utrecht University, 12 Padualaan 8, 3584 CH Utrecht, The Netherlands. 13 <sup>3</sup> Department of Biology, Wentworth Way, YO10 5DD, University of York, York, 14 15 UK. 16 Email: weizhong@njau.edu.cn 17 18 Abstract 19 The diversity-invasion resistance relationships are often variable and sensitive to 20 environmental conditions such as resource availability. Resource stoichiometry, the 21 relative concentration of different elements in the environment, has been shown to 22 have strong effects on the physiology and interactions between different species. Yet, 23 its role for diversity-invasion resistance relationships is still poorly understood. Here 24 we explored how the ratio of nitrogen and phosphorus affect the productivity and 25 invasion resistance of constructed microbial communities by plant pathogenic 26

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

39

#### 40 Key words:

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

43

#### 44 **1. Introduction**

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

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

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

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

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

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

113

#### 114 2. Methods

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

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

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

The resident communities were assembled by using all five avirulent species in 127 substitutive design so that the final communities covered all possible species 128 combinations and richness levels (total of 31 communities with equal initial bacterial 129 biomasses, Table S2). Prior to the experiments, bacteria were pre-cultured from 130 frozen stocks on Nutrient Agar plates (NA, glucose 10.0 g L<sup>-1</sup>, tryptone 5.0 g L<sup>-1</sup>, beef 131 extract 3.0 g L<sup>-1</sup>, yeast extract 0.5 g L<sup>-1</sup>, agar 15.0 g L<sup>-1</sup>, pH 7.0) and single colonies 132 were picked and re-grown in liquid nutrient broth (NA medium without agar) at 30 °C 133 for 12 h with 170 r.p.m. agitation. Bacterial isolates were washed three times in 134 0.85 % NaCl to remove nutrient residues and re-suspended in 0.85 % NaCl with final 135 densities of 10<sup>7</sup> cells mL<sup>-1</sup>. 136

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

To manipulate the resource stoichiometry, we first set up a minimal salt medium,
which did not contain carbon, nitrogen or phosphorus (MOPS 30mM, CaCl<sub>2</sub> 0.1mM,
FeSO<sub>4</sub> 3 mM, KCl 20mM, MgCl<sub>2</sub> 2mM, Na<sub>2</sub>SO<sub>4</sub> 14mM and NaCl 51mM, pH 7.0).
The minimal medium was then supplemented with a mixture of carbons (fructose,

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

# (d) Measuring resident community invasion resistance and productivity in microcosms

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

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

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

# (e) Measuring the growth and consumption of nitrogen and phosphorus by each bacterial species

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

200

#### 201 (e) Statistical analyses

Invasion success (RFU, mCherry/OD<sub>600</sub>) and resource stoichiometry ratios (N:P) were 202 log<sub>10</sub>-transformed before statistical analyses to fulfill model assumptions. All 203 comparisons between the growth of individual species were analyzed using ANOVA 204 205 and linear regression. General Linear Mixed Models (GLMs) were used to examine the resident species identity effects and resource stoichiometry as a function of 206 invader relative density (invasion success). Model 1 ("Richness-ratio") and Model 2 207 ("Identity-ratio") were used to identify how individual species contributed to the 208 209 invasion success as а function of resource stoichiometry. Model 3 ("Richness-concentration", Table S3) was used to analyze whether N and P 210 concentrations had interactive effects with species richness in determining invasion 211 success and resident community productivity. Model 4 ("Productivity-ratio", Table 212 213 S4) was used to study the interactive effects between productivity and resource stoichiometry on the invasion success, while Models 5 ("Richness-ratio", Table S4) 214 and 6 ("Identity-ratio", Table S4) were used to explore the effects of community 215 diversity and species identity on the resident community productivity. In some cases, 216 we used the means of community treatment replicates for simplified analysis (e.g. 217 species presence effects in Figure 2). 218

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

225 **3. Results** 

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

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

<sup>224</sup> 

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

### (b) The effect of resident species identities on resident community productivityand invasions

We found that R. mannitolilytica QL-A2, R. mannitolilytica QL-A3 and R. 238 taiwanensis QL-117 resident species had no significant species identity effects on the 239 relative density of the invader (Model 2 in Table 2). In contrast, R. pickettii QL-A6 240 and R. pickettii QL-140 resident species were very effective at reducing the relative 241 density of the invader (Model 2 in Table 2). To examine this in more detail, we 242 243 compared the invasion resistance of resident communities with and without these two species. As expected, resident communities were less resistant to invasions in the 244 absence of these two species (Figure 2, comparison on communities with and without 245 *R. pickettii* QL-A6: panel a,  $F_{1,184} = 400.4$ , P < 0.001, comparison on communities 246 with and without *R. pickettii* QL-140: panel b,  $F_{1,184} = 4.47$ , *P* = 0.036). 247 Mechanistically, this could be explained by relatively more efficient consumption of 248 nitrogen and phosphorus compared to the other resident species (Figures S3 and S4; 249 Species main effects at 0.15, 1.5 and 15 mM nitrogen concentrations, respectively:  $F_6$ 250  $_{35} = 254.6$ , 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 254 255 QL-140 were equally good at consuming nitrogen and phosphorus (P > 0.05 in all pairwise comparisons). 256

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

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

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

274

## (c) The effect of resource stoichiometry on the resident community productivity and invasions

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

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

294

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

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

311

#### 312 4. Discussion

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

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

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

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

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

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

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

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

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.

We conclude that resource stoichiometry is an important determinant of 408 community invasion resistance. Human activities continue to have a huge effect on 409 global elemental cycling [53], nitrogen leaching and eutrophication, which are 410 causing growing problems and having devastating effects on the functioning of 411 412 ecosystems [54,55]. In the case of eutrophication, our results suggest that resource stoichiometry could drive changes in microbial community composition potentially 413 affecting the likelihood of biological invasions. In the agricultural context, resource 414 stoichiometry could affect the severity of disease epidemics via effects on microbial 415 competition. A better understanding of this process could potentially help to control 416 plant pathogen invasions via modulation of soil nutrient availability and balance (N:P 417 ratios) to maintain relatively stable and invasion resistant microbial community. In 418 broader perspective, understanding how changes in global element balances affect the 419 420 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 426 manuscript and gave final approval for publication. We have no competing interests.

427

Acknowledgements. We thank Yi'an Gu for the advices on data analysis and all
authors' contribution on manuscript writing.

430

Funding statement. This research was financially supported by the National Natural
Science Foundation of China (41471213 to Yangchun Xu; 41671248 to Zhong Wei;
41807045 to Tianjie Yang), National Key Basic Research Program of China
(2015CB150503, Qirong Shen), the Natural Science Foundation of Jiangsu Province

(Grants No BK20170085, Zhong Wei and Grants No BK20180527, Tianjie Yang), 435 the 111 project (B12009, Qirong Shen), Young Elite Scientist Sponsorship Program 436 by CAST (2015QNRC001 to Zhong Wei), and the Qing Lan Project (funding to 437 Yangchun Xu and Zhong Wei), and the Chinese Scholarship Council (CSC) joint PhD 438 scholarship. Ville-Petri Friman is supported by the Wellcome Trust [ref: 105624] 439 through the Centre for Chronic Diseases and Disorders (C2D2) and Royal Society 440 Research Grant (RSG\R1\180213) at the University of York. Alexandre Jousset was 441 442 supported by the Dutch Science foundation NWO (870.15.050).

443

444	Refer	ences
445	1.	Eisenhauer N, Scheu S, Jousset A. 2012 Bacterial diversity stabilizes
446		community productivity. PLoS One 7, 1–5.
447		(doi:10.1371/journal.pone.0034517)
448	2.	Jousset A, Schulz W, Scheu S, Eisenhauer N. 2011 Intraspecific genotypic
449		richness and relatedness predict the invasibility of microbial communities.
450		ISME J. 5, 1108–1114. (doi:10.1038/ismej.2011.9)
451	3.	Mallon CA, Van Elsas JD, Salles JF. 2015 Microbial invasions: The process,
452		patterns, and mechanisms. Trends Microbiol. 23, 719-729.
453		(doi:10.1016/j.tim.2015.07.013)
454	4.	Wei Z, Yang T, Friman V-P, Xu Y, Shen Q, Jousset A. 2015 Trophic network
455		architecture of root-associated bacterial communities determines pathogen
456		invasion and plant health. Nat. Commun. 6, 8413. (doi:10.1038/ncomms9413)
457	5.	van Elsas JD, Chiurazzi M, Mallon CA, Elhottova D, Kristufek V, Salles JF.
458		2012 Microbial diversity determines the invasion of soil by a bacterial
459		pathogen. Proc. Natl. Acad. Sci. 109, 1159-1164.
460		(doi:10.1073/pnas.1109326109)
461	6.	Hu J, Wei Z, Friman V-P, Gu S. 2016 Probiotic Diversity Enhances
462		Rhizosphere Microbiome Function and Plant Disease Suppression. MBio 7,
463		e01790-16. (doi:10.1128/mBio.01790-16.Editor)
464	7.	Becker J, Eisenhauer N, Scheu S, Jousset A. 2012 Increasing antagonistic
465		interactions cause bacterial communities to collapse at high diversity. Ecol. Lett.
466		<b>15</b> , 468–474. (doi:10.1111/j.1461-0248.2012.01759.x)
467	8.	Vivant AL, Garmyn D, Maron PA, Nowak V, Piveteau P. 2013 Microbial
468		Diversity and Structure Are Drivers of the Biological Barrier Effect against
469		Listeria monocytogenes in Soil. PLoS One 8, 1-11.
470		(doi:10.1371/journal.pone.0076991)
471	9.	Eisenhauer N, Schulz W, Scheu S, Jousset A. 2012 Niche dimensionality links
472		biodiversity and invasibility of microbial communities. Funct. Ecol. 27, 282-
473		288. (doi:10.1111/j.1365-2435.2012.02060.x)

474	10.	Elton CS. 1958 The ecology of invasions by plants and animals. Methuen,
475		London 18.
476	11.	Davis MA, Grime JP, Thompson K. 2000 Fluctuating resources in plant
477		communities: a general theory of invasibility. J. Ecol. 88, 528-534.
478		(doi:10.1046/j.1365-2745.2000.00473.x)
479	12.	Wei Z, Huang JF, Hu J, Gu YA, Yang CL, Mei XL, Shen QR, Xu YC, Friman
480		VP. 2015 Altering transplantation time to avoid periods of high temperature
481		can efficiently reduce bacterial wilt disease incidence with tomato. PLoS One
482		10, 1–14. (doi:10.1371/journal.pone.0139313)
483	13.	Mallon C., Poly F, Le Roux X, Marring I, van Elsas JD, Salles JF. 2015
484		Resource pulses can alleviate the biodiversity – invasion relationship in soil
485		microbial communities. <i>Ecology</i> 96, 915–926. (doi:10.1890/14-1001.1)
486	14.	Li W, Stevens MHH. 2012 Fluctuating resource availability increases
487		invasibility in microbial microcosms. Oikos 121, 435-441.
488		(doi:10.1111/j.1600-0706.2011.19762.x)
489	15.	Kuebbing S, Rodriguez-Cabal MA, Fowler D, Breza L, Schweitzer JA, Bailey
490		JK. 2013 Resource availability and plant diversity explain patterns of invasion
491		of an exotic grass. J. Plant Ecol. 6, 141-149. (doi:10.1093/jpe/rts018)
492	16.	Fridley J. 2002 Resource availability dominates and alters the relationship
493		between species diversity and ecosystem productivity in experimental plant
494		communities. Oecologia 132, 271–277. (doi:10.1007/s00442-002-0965-x)
495	17.	Yang T, Wei Z, Friman V, Xu Y, Shen Q, George A. 2017 Resource
496		availability modulates biodiversity-invasion relationships by altering
497		competitive interactions. Environ. Microbiol. 19, 2984–2991.
498		(doi:10.1111/1462-2920.13708)
499	18.	Loreau M. 2000 Biodiversity and ecosystem functioning: recent theoretical
500		advances. Oikos 91, 3–17. (doi:doi:10.1034/j.1600-0706.2000.910101.x)
501	19.	Grover JP. 2004 Predation, competition, and nutrient recycling: a
502		stoichiometric approach with multiple nutrients. J. Theor. Biol. 229, 31-43.
503		(doi:10.1016/j.jtbi.2004.03.001)

504	20.	Moe SJ, Stelzer RS, Forman MR, Harploe WS, Daufresne T, Yoshida T. 2005
505		Recent advances in ecological stoichiometry: Insights for population and
506		community ecology. Oikos 109, 29-39.
507		(doi:10.1111/j.0030-1299.2005.14056.x)
508	21.	Sterner RW, Elser JJ. 2002 Ecological stoichiometry: the biology of elements
509		from molecules to the biosphere.
510	22.	Hillebrand H, Cowles JM, Lewandowska A, Van de Waal DB, Plum C. 2014
511		Think ratio! A stoichiometric view on biodiversity-ecosystem functioning
512		research. Basic Appl. Ecol. 15, 465-474. (doi:10.1016/j.baae.2014.06.003)
513	23.	Aubert AB, Svensen C, Hessen DO, Tamelander T. 2013 CNP stoichiometry of
514		a lipid-synthesising zooplankton, Calanus finmarchicus, from winter to spring
515		bloom in a sub-Arctic sound. J. Mar. Syst. 111-112, 19-28.
516		(doi:10.1016/j.jmarsys.2012.09.004)
517	24.	Vecchio-Pagan B, Bewick S, Mainali K, Karig DK, Fagan WF. 2017 A
518		stoichioproteomic analysis of samples from the Human Microbiome Project.
519		Front. Microbiol. 8, 1119. (doi:10.3389/fmicb.2017.01119)
520	25.	Naddafi R, Eklöv P, Pettersson K. 2009 Stoichiometric Constraints Do Not
521		Limit Successful Invaders: Zebra Mussels in Swedish Lakes. PLoS One 4,
522		e5345. (doi:10.1371/journal.pone.0005345)
523	26.	Hall SR. 2009 Stoichiometrically Explicit Food Webs: Feedbacks between
524		Resource Supply, Elemental Constraints, and Species Diversity. Annu. Rev.
525		Ecol. Evol. Syst. 40, 503–528.
526		(doi:10.1146/annurev.ecolsys.39.110707.173518)
527	27.	Hood JM, Sterner RW. 2014 Carbon and phosphorus linkages in Daphnia
528		growth are determined by growth rate, not species or diet. Funct. Ecol. 28,
529		1156–1165. (doi:10.1111/1365-2435.12243)
530	28.	Delgado-Baquerizo M et al. 2017 It is elemental: soil nutrient stoichiometry
531		drives bacterial diversity. Environ. Microbiol. 19, 1176-1188.
532		(doi:10.1111/1462-2920.13642)
533	29.	González AL, Kominoski JS, Danger M, Ishida S, Iwai N, Rubach A. 2010

534		Can ecological stoichiometry help explain patterns of biological invasions?
535		Oikos 119, 779–790. (doi:10.1111/j.1600-0706.2009.18549.x)
536	30.	Jiang G, Wei Z, Xu J, Chen H, Zhang Y, She X, Macho AP, Ding W, Liao B.
537		2017 Bacterial wilt in China: History, current Status, and future perspectives.
538		Front. Plant Sci. 8, 1-10. (doi:10.3389/fpls.2017.01549)
539	31.	Salanoubat M et al. 2002 Genome sequence of the plant pathogen Ralstonia
540		solanacearum. Nature 415, 497-502. (doi:10.1038/415497a)
541	32.	Yabuuchi E, Nishiuchi Y, Kosako Y, Wako O, August R, August A. 1995
542		Transfer of Two Burkholderia and An Alcaligenes Species to Ralstonia Gen.
543		Nov.: Proposal of Ralstonia pickettii (Ralston, Palleroni and Doudoroff 1973)
544		Comb. Nov., Ralstonia solanacearum (Smith 1896) Comb. Nov. and Ralstonia
545		eutropha (Davis 1969) Comb. No. <b>39</b> , 897–904.
546	33.	Badri D V., Vivanco JM. 2009 Regulation and function of root exudates. Plant,
547		Cell Environ. 32, 666–681. (doi:10.1111/j.1365-3040.2009.01926.x)
548	34.	Walker TS, Bais HP, Grotewold E, Vivanco JM. 2003 Root Exudation and
549		Rhizosphere Biology. Plant Physiol. 132, 44-51.
550		(doi:10.1104/pp.102.019661.Although)
551	35.	Redfield AC. 1958 The biological control of chemical factors in the
552		environment. Am. Sci. 46, 205–221.
553	36.	Tian H, Chen G, Zhang C, Melillo JM, Hall C a S. 2010 Pattern and variation
554		of C:N:P ratios in China's soils: A synthesis of observational data.
555		Biogeochemistry 98, 139–151. (doi:10.1007/s10533-009-9382-0)
556	37.	Cleveland CC, Liptzin D. 2007 C:N:P stoichiometry in soil: is there a
557		"Redfield ratio" for the microbial biomass? Biogeochemistry 85, 235-252.
558		(doi:10.1007/s10533-007-9132-0)
559	38.	Tan S, Gu Y, Yang C, Dong Y, Mei X, Shen Q, Xu Y. 2015 Bacillus
560		amyloliquefaciens T-5 may prevent Ralstonia solanacearum infection through
561		competitive exclusion. Biol. Fertil. Soils 52, 341-351.
562		(doi:10.1007/s00374-015-1079-z)
563	39.	Kacena MA, Merrell GA, Manfredi B, Smith EE, Klaus DM, Todd P. 1999

564		Bacterial growth in space flight: logistic growth curve parameters for
565		Escherichia coli and Bacillus subtilis. <i>Appl. Microbiol.</i> <b>51</b> , 229–234.
566	40.	Kahm M, Hasenbrink G, Ludwig J. 2010 grofit : Fitting Biological Growth
567		Curves with R. <b>33</b> .
568	41.	R Core Team. 2013 R: A language and environment for statistical computing.
569	42.	Loreau M et al. 2001 Biodiversity and ecosystem functioning: current
570		knowledge and future challenges. Science 294, 804-808.
571		(doi:10.1126/science.1064088)
572	43.	Byrnes JEK et al. 2014 Investigating the relationship between biodiversity and
573		ecosystem multifunctionality: Challenges and solutions. Methods Ecol. Evol. 5,
574		111-124. (doi:10.1111/2041-210X.12143)
575	44.	Symstad AJ. 2000 A test of the effects of functional group richness and
576		composition on grassland invasibility. Ecology 81, 99-109.
577		(doi:doi:10.1890/0012-9658(2000)081[0099:ATOTEO]2.0.CO;2)
578	45.	Klausmeier CA, Litchman E, Daufresne T, Levin1 SA. 2004 Optimal
579		nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 429, 171-174.
580	46.	Cardinale BJ, Hillebrand H, Harpole WS, Gross K, Ptacnik R. 2009 Separating
581		the influence of resource 'availability' from resource 'imbalance' on
582		productivity-diversity relationships. Ecol. Lett. 12, 475-487.
583		(doi:10.1111/j.1461-0248.2009.01317.x)
584	47.	Moorthi SD, Schmitt JA, Ryabov A, Tsakalakis I, Blasius B, Prelle L,
585		Tiedemann M, Hodapp D, Moorthi SD. 2016 Unifying ecological
586		stoichiometry and metabolic theory to predict production and trophic transfer in
587		a marine planktonic food web.
588	48.	Keiblinger KM et al. 2010 The effect of resource quantity and resource
589		stoichiometry on microbial carbon-use-efficiency. FEMS Microbiol. Ecol. 73,
590		430-440. (doi:10.1111/j.1574-6941.2010.00912.x)
591	49.	Roscher C, Beßler H, Oelmann Y, Engels C, Wilcke W, Schulze E-D. 2009
592		Resources, recruitment limitation and invader species identity determine
593		pattern of spontaneous invasion in experimental grasslands. J. Ecol. 97, 32–47.

594		(doi:10.1111/j.1365-2745.2008.01451.x)
595	50.	Harder W, Dijkhuizen L. 1983 Physiological Responses. Annu. Rev. Microbiol.
596		<b>37</b> , 1–23.
597	51.	Magasanik B. 1993 The regulation of nitrogen utilization in enteric bacteria. J.
598		Cell. Biochem. 51, 34-40. (doi:10.1002/jcb.240510108)
599	52.	Commichau FM, Forchhammer K, Stülke J. 2006 Regulatory links between
600		carbon and nitrogen metabolism. Curr. Opin. Microbiol. 9, 167-172.
601		(doi:10.1016/j.mib.2006.01.001)
602	53.	Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW,
603		Schlesinger WH, Tilman DG. 1997 Human alteration of the global nitrogen
604		cycle: sources and consequences. Ecol. Appl. 5, 85.
605		(doi:10.1016/S1240-1307(97)87738-2)
606	54.	Hautier Y, Niklaus PA, Hector A. 2009 Competition for Light Causes Plant
607		Biodiversity Loss After Eutrophication. Science (80 ). 184513, 2-5.
608	55.	Scherer-Lorenzen M, Palmborg C, Prinz A, Schulze E-D. 2003 The Role of
609		Plant Diversity and Composition for Nitrate Leaching in Grasslands. Ecology
610		84, 1539–1552. (doi:10.1890/0012-9658(2003)084[1539:TROPDA]2.0.CO;2)
611		
612		
613		
614		
615		
616		
617		
618		
619		
620		
621		
622		
623		

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	0.00	16:1
1	1.5	0.07	(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

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

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

Model 2 ("Identity-ratio")

QL-A3 Not retained				
QL-A6 1 524.41 < <b>0.</b>	01↓			
QL-117 Not retained				
QL-140 1 25.27 < <b>0.</b>	01↓			
N:P ratio (Ratio) 1 33.74 < <b>0.0</b>	01↓			
QL-A2 * Ratio Not retained				
QL-A3 * Ratio Not retained				
QL-A6 * Ratio Not retained	Not retained			
QL-117 * Ratio Not retained	Not retained			
QL-140 * Ratio Not retained				
182				
Residuals				
Model summary AIC: 136.72, $R^2 = 0.76$				

631

Figure 1. The effects of species richness and N:P ratio on invasion success (relative invader density).
The relative density of the invader was defined as log-10 transformed relative mCherry fluorescence
unit (RFU, mCherry/OD<sub>600</sub>) after 72 h incubation.

635

**Figure 2.** The effect of *R. pickettii* QL-A6 and QL-140 species on the relative density of the invader (panel a and b, respectively). Relative density of the invader was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD<sub>600</sub>) after 72 h incubation. The 0 and 1 on X-axes denote for the presence and absence of *R. pickettii* QL-A6 and QL-140 species in the bacterial community and bars show ±1 standard error (n=186). Asterisks indicate significant differences (\*, P < 0.05; \*\*, P <0.01; \*\*\*, P < 0.001). 642

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

649

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<sub>600</sub>) after 72 h incubation. Invasion 653 success was defined as log-10 transformed relative mCherry fluorescence unit (RFU, mCherry/OD<sub>600</sub>) 654 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 655 total variance explained and asterisks indicate significant effects (\*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 656 657 0.001).

658

Page 27 of 35





http://mc.manuscriptcentral.com/prsb

Submitted to Proceedings of the Royal Society B: For Review Only

Page 28 of 35







X<sup>2</sup> = 2.221, P-value = 0.994, Df = 10, n = 186

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)



Antibiotics addition / prsb



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)







Ammonium concentration in the medium (mM)

(A) (B) Phosphorus concentration in the medium (mM) Phosphorus concentration in the medium (mM) P=0.9 mM P=0.09 mM 0.15 1.2а ab а а а bc а 0.10 0.8 а b b b 0.05 0.4 0.0 0.00 Control QL-A2 QL-A3 QL-A6 QL-117 QL-140 Invader Control QL-A2 QL-A3 QL-A6 QL-117 QL-140 Invader Strain Strain http://mc.manuscriptcentral.com/prsb

Submitted to Proceedings of the Royal Society B: For Review Only





Strain

http://mc.manuscriptcentral.com/prsb



http://mc.manuscriptcentral.com/prsb