



## Stochastic processes govern invasion success in microbial communities when the invader is phylogenetically close to resident bacteria

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1 Stochastic processes govern invasion success in microbial communities when the  
2 invader is phylogenetically close to resident bacteria

3

4 **Short title:** Microbial invasion in naturally enriched NOB guilds

5

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16

17 **Abstract**

18 Despite recent efforts in determining the determinants of invasion in microbial communities, experimental  
19 observations across different ecosystems are inconclusive. While relationships between resident  
20 community diversity and invasion success are often noted, community diversity says little about community  
21 assembly processes. Community assembly processes may provide a more inclusive framework to explain –  
22 and potentially prevent or facilitate- invasion. Here, we let replicate nitrite-oxidizing bacterial guilds  
23 assemble under different conditions from a natural source community and study their compositional  
24 patterns to infer the relative importance of the assembly processes. Then, an invader strain from that same  
25 guild was introduced at one of three propagule pressures. We found no significant correlation between  
26 community diversity and invasion success. Instead, we observed that the effect of selection on invasion  
27 success was surpassed by the effect of drift, as inferred from the substantial influence of propagule  
28 pressure on invasion success. This dominance of drift can probably be generalized to other invasion cases  
29 with high phylogenetic similarity between invader and resident community members. In these situations,  
30 our results suggest that attempting to modulate the invasibility of a community by altering its diversity is  
31 futile because stochastic processes determine the invasion outcome. Increasing or reducing propagule  
32 pressure is then deemed the most efficient avenue to enhance or limit invasion success.

33 **Introduction**

34 Biological invasions can impact resident communities and ecosystems by facilitating fluctuations in  
35 biodiversity and in this way alter community function and productivity. For macro-organisms many factors  
36 enabling successful invasions have been identified and considerable scientific effort has been devoted to  
37 elucidate the determinants of invasion in microbial communities in order to prevent or promote the  
38 establishment of new community members (Mallon *et al.*, 2015a; Amalfitano *et al.*, 2014; De Schryver and  
39 Vadstein, 2014).

40 Competition with resident community members has primarily been suggested to determine invasion  
41 success, and strong competition decreases invasion success (Fargione and Tilman, 2005; Mallon *et al.*,  
42 2015b; Emery and Gross, 2007). The level of competition is usually inferred from resident community  
43 diversity (Elton, 1958) or from the phylogenetic distance between the invader and resident community  
44 members (Darwin, 1859). It is suggested that with small phylogenetic distance between invader and  
45 resident community members, resident community members impose strong competition on the invader  
46 type because phylogenetic similarity implies ecological similarity (Darwin, 1859) , which would reduce  
47 invasion success (Procheş *et al.*, 2008; Jiang *et al.*, 2010; Thuiller *et al.*, 2010; Tan *et al.*, 2015). In a similar  
48 vein, it has been theoretically (Mallon *et al.*, 2015a) and experimentally (van Elsas *et al.*, 2012; Bonanomi *et*  
49 *al.*, 2014; Dillon *et al.*, 2005) suggested that biologically diverse communities are more resistant towards  
50 invasion, as originally proposed by Elton (1958). The most commonly cited reason is that more diverse  
51 communities are able to utilize resources more efficiently, thus leaving little resource space for invaders,  
52 and have higher probability of hosting a type capable of out-competing an invader. However, when  
53 community diversity is examined as a single factor determining invasion success without considering the  
54 specific context for interpretation, false conclusions are likely (Shade, 2017), because other community  
55 assembly processes (i.e., selection, drift, dispersal or speciation) contributing to resident community  
56 diversity are often neglected (Kinnunen *et al.*, 2016).

57 Competition between invader and resident community members is mainly investigated using synthetically  
58 assembled microbial communities (De Roy *et al.*, 2013; van Elsas *et al.*, 2012) with limited similarity to  
59 natural communities. Synthetically assembled microbial communities allow testing invasion success at  
60 different (controlled) diversity levels as well as carefully chosen phylogenetic distances between  
61 community members. However, this approach does not allow testing how all community assembly  
62 processes affect invasion because oftentimes only one or two processes (selection and/or drift) govern  
63 community assembly when establishing synthetic communities with no history of interaction. It is thus

64 unclear if resident community diversity and phylogenetic distance between invader and resident  
65 community members can serve as general predictors of invasion success, beyond synthetic communities.  
66 On the other hand, recent studies have suggested that microbial community assembly is more stochastic  
67 (Daleo *et al.*, 2009) than recognized in the studies focusing on competition, and that invasion success would  
68 primarily depend on propagule pressure (Acosta *et al.*, 2015; Ketola *et al.*, 2017) (the relative abundance of  
69 the invader to the resident community), as postulated for communities of macro-organisms (Lockwood *et*  
70 *al.*, 2005; Simberloff, 2009; Von Holle and Simberloff, 2005). While resident community diversity has  
71 predicted invasion in several cases (van Elsas *et al.*, 2012; Ketola *et al.*, 2017; Dillon *et al.*, 2005), a similar  
72 amount of evidence supports propagule pressure as determinant of invasion (Ketola *et al.*, 2017; Acosta *et*  
73 *al.*, 2015). The lack of consensus across studies may be because the investigations are often limited to only  
74 one determinant of invasion. For example, sometimes communities with different diversities are subject to  
75 invasion at single propagule pressure (Chapelle *et al.*, 2015; Eisenhauer *et al.*, 2013; van Elsas *et al.*, 2012;  
76 Dillon *et al.*, 2005; Jiang *et al.*, 2010), or the phylogenetic distance between resident community members  
77 and invader is so large that it is highly improbable that it accurately represents competition for an  
78 ecological niche (Bonanomi *et al.*, 2014).

79 Hence, here we subject guilds of nitrite-oxidizing bacteria (NOB) to invasion by a NOB strain and thus  
80 investigate invasion outcome in communities where competition is expected, and where phylogenetic  
81 distance between invader and resident community members is low. We hypothesize that with low  
82 phylogenetic distance to the resident community members, invasion success is influenced by propagule  
83 pressure. Since low phylogenetic distance can confer ecological similarity, neither the resident community  
84 members nor the invader would have a competitive advantage and the effect of drift would govern  
85 invasion success.

## 86 **Materials and methods**

### 87 *Invader cultivation*

88 A culture of *Nitrotoga* HW29 was used as the invader, grown according to its enrichment conditions  
89 (Hüpeden *et al.*, 2016) in 250-mL cell culture flasks over a three-month period. After three months, NOB  
90 mineral medium was replaced with sterilized non-chlorinated tap water for one month to adjust the  
91 invader to the conditions of the resident community. No changes in nitrite removal dynamics were  
92 observed in response to this change in the medium. Before the onset of the invasion, all batch cultures  
93 were combined and the cell density was determined using a Thoma cell-counting chamber. Then, dilutions  
94 of the culture in tap water were spiked with either 0.3 mM or 0.03 mM nitrite to introduce the invader to  
95 resident communities with high and low nitrite loading, respectively.

#### 96 *Invasion in flow-through microcosms*

97 The experimental set-up consisted of 40 parallel flow-through microcosms. Biofilms developed on Filtralite  
98 NC 0.8-1.6 filter material (Saint-Gobain Byggevarer A/S, Oslo) fed with tap water spiked with nitrite at a  
99 constant flow rate of 0.43 L/day under ambient temperatures (23 to 25°C). One set of 20 replicates was fed  
100 with tap water with 0.3 mM  $\text{NO}_2^-$ -N addition while another set of 20 replicates received 10-fold lower  
101 nitrogen concentration, 0.03 mM  $\text{NO}_2^-$ -N. Resident community biofilms were allowed to develop for 60  
102 days, after which 4 random columns were destructively sampled and used as before invasion reference  
103 (called 'initial' in results and discussion) and as inocula for batch microcosms (see below) while the  
104 remaining columns were subjected to invasion. Three different propagule pressures were applied; such as  
105 the total invader cells after a 24 hours of continuous invasion were estimated to represent on average 1%,  
106 10% and 100% of resident NOB cells. The absolute abundance of the resident NOB cells before invasion was  
107 estimated from the nitrite removal dynamics and average NOB growth kinetics according to Rittmann and  
108 Mccarty (1980). We observed complete nitrite removal from day 30 onwards, resulting in total of 0.4mg  
109  $\text{NO}_2^-$ -N consumed by the resident bacteria at low nitrite loading, and 4mg  $\text{NO}_2^-$ -N at high nitrite loading,  
110 yielding approximately  $10^7$  and  $10^8$  cells per microcosm at low and high nitrite loading, respectively. Each  
111 propagule pressure treatment consisted of 4 replicates whereas 4 replicates at both nitrogen-loadings were  
112 maintained as controls without invader (referred to as 'none'). The flow-through columns were operated

113 for another 14 days following the invasion after which all material was harvested ('Final after invasion' – or  
114 'Final') and DNA extracted.

#### 115 *Invasion in batch microcosms*

116 Batch microcosms were established in 250-mL cell-culture flasks with the same nitrite concentrations as in  
117 the flow-through microcosms. Nitrite-spiked sterile tap water was used as medium and 0.5 g of wet filter  
118 material from the initial community from either high or low nitrite loading flow-through columns was  
119 added as inoculum. The flasks were subject to rigorous shaking to detach the cells from the filter material  
120 and promote growth in suspension. We assumed the absolute abundance of inoculated resident NOB cells  
121 to correspond to the abundance estimated for the flow-through microcosms, but corrected for filter  
122 material used for inoculation ( $10^3$  cells/ml and  $10^4$  cells/ml in low and high nitrite loading batch  
123 microcosms, respectively). We used this to determine the correct propagule pressure with similar ratios as  
124 in the flow-through microcosms: 1%, 10% and 100% of average resident NOB cells. In batch microcosms,  
125 the invader cells were added at the same time as the inoculum filter material. The absolute abundance of  
126 resident NOB cells differed in flow-through and batch microcosms. Therefore, our experiments included 6  
127 different conditions of absolute propagule pressure, and 3 of relative propagule pressure.

128 The nitrite removal was measured regularly and when depleted, half of the medium was replaced. After 5  
129 transfer events, the cells were recovered by filtering the total microcosm volume and the retentate was  
130 subjected to DNA extraction.

#### 131 *DNA extraction*

132 DNA from was isolated using the FastDNA™ SPIN Kit for Soil and the FastPrep® Instrument (MP Biomedicals,  
133 Santa Ana, CA) according to the manufacturer's instruction at room temperature. DNA from liquid  
134 microcosms (batch and invader culture) was isolated after filtering (100 mL of invader cell culture and all  
135 250 mL of the batch microcosms) through sterile 0.1 um filters using DNeasy® PowerWater® kit (QIAGEN,  
136 Hilden, Germany) according to manufacturer's instructions at room temperature. The concentration and

137 purity of extracted DNA were checked using NanoDrop™ 2000 Spectrophotometer (Thermo Fisher  
138 Scientific, Wilmington, DE). DNA was then stored at -20°C for subsequent molecular analyses.

139 *qPCR*

140 Real-time qPCR assays were performed with a Roche LightCycler® 96 Instrument (Basil, Switzerland).  
141 Reaction mixtures (25 µl) contained 12.5 µl SYBR® Green qPCR Mastermix (iQ™ SYBR® Green Supermix; Bio-  
142 Rad, Hercules, CA) 1 µl forward and reverse primers (20 µM), 5 µl of template DNA (adjusted to 2 ngDNA  
143 µl<sup>-1</sup>) and 5.5 µl PCR-grade water. Total bacteria were quantified based on 16S rRNA gene copy numbers  
144 using the Eubacterial primer set 1055f-1392r as described in Terada *et al.*, (2010). On average 2.5 copies of  
145 16S rRNA gene was estimated per cell, according to *rrnDB* (Stoddard *et al.*, 2015), with the assumption that  
146 majority of the community belongs to *Gallionellaceae* and *Nitrospiraceae*. *Nitrospira* cells were quantified  
147 using *Nitrospira*-specific qPCR with primer set NTS232f (Lim *et al.*, 2008) and Nsr1264r (Dionisi *et al.*, 2002)  
148 targeting the 16S rRNA genes. Cell numbers were calculated assuming a single 16S operon per cell (*rrnDB*).  
149 New primer set Ntoga118F (5'-CTTTCAGCCGAAAGAAAACGCA) and Ntoga840R (5'-  
150 CTAAGGAAGTCTCCTCCC) was developed for this study to target the 16S rRNA gene of *Nitrotoga* cells. The  
151 primers were designed based on *Nitrotoga* amplicon sequences retrieved from previous experiment where  
152 *Nitrotoga* was enriched from tap water spiked with nitrite (Kinnunen *et al.*, 2017). The designed primers  
153 cover 27% of known *Nitrotoga* in the SILVA rRNA database (including the 16S rRNA of *Nitrotoga* HW29) and  
154 100% of the tap water enriched *Nitrotoga* from previous experiment (Kinnunen *et al.*, 2017). These primers  
155 target a 175 bp product that was verified by constructing a clone library of 180 clones, all of which were  
156 determined to belong to *Nitrotoga* genus. The 35 cycles of amplification at 94°C for 30s; 63°C for 30s; 72°C  
157 for 60s was performed. Followed by the melting curve analysis.

#### 158 *Sequencing and amplicon library*

159 Extracted DNA from all samples was PCR-amplified using primer set PRK341F (5'- CCTAYGGGRBGCASCAG-  
160 3') and PRK806R (5'-GGACTACNNGGTATCTAAT-3') for 35 cycles, to amplify the V3-V4 hypervariable



161 regions (Yu *et al.*, 2005). Purified PCR products were sequenced on the Illumina MiSeq platform at the DTU  
162 Multi Assay Core Center (Lyngby, DK).

163 All raw 16S rRNA gene amplicons were processed following the DADA2 (version 1.0.3) pipeline with default  
164 settings (Callahan *et al.*, 2016). These sequence variants were classified based on the SILVA prokaryotic  
165 reference database version 123. Invader sequence was determined from the 100% similarity to 16S  
166 sequence of HW29 found in NCBI database by phylogenetic analysis of all *Nitrotoga* sequence variants  
167 using given reference (Figure S5). All sequences have been submitted to NCBI Sequence Read Archive under  
168 accession number SRP116646.

#### 169 *Statistical analysis*

170 All statistical tests were performed in R. The relative abundance of invader sequence variant of all NOB as  
171 well as the similarity between biological replicates was determined using phyloseq package (McMurdie and  
172 Holmes, 2013). Phylogenetic distances and Bray-Curtis distances were calculated and plotted as NMDS  
173 using phyloseq package. Phylogenetic diversity was calculated using PhyloMeasures package (Tsirogiannis  
174 and Sandel, 2016). The statistical difference of the phylogenetic diversity between treatments was  
175 determined using a Wilcoxon signed-rank test, comparing the non-invaded control groups at different  
176 nitrite loadings. The absolute cell numbers obtained by qPCR were compared using two-way ANOVA test,  
177 with factors corresponding to nitrite loading rate and propagule pressure. Correlations between descriptive  
178 indices and invader relative abundance were determined using linear regression model and the significance  
179 of the difference in correlation between treatments was determined using also a two-way ANOVA test.

## 180 **Results and discussion**

### 181 *Ecological processes governing resident community assembly*

182 We enriched 40 resident communities from a tap water source community in flow-through microcosms  
183 subjected to two nitrite loading regimes to support the coexistence of competing NOB genera (Kinnunen *et*  
184 *al.*, 2017). We described the resident community composition after 60 days of operation (further referred

185 to as initial community) and 14 days after the invasion event (referred to as final community). The flow-  
186 through microcosms are expected to facilitate selection, drift and dispersal. We also inoculated batch  
187 microcosms with the initial community from the flow-through microcosms to establish a set of microcosms  
188 where community assembly processes were simplified by elimination of dispersal. The composition of the  
189 batch microcosms was characterized after the inoculation of the 'resident' community together with the  
190 invader, representing the starting community after the inoculation (initial community), and after five  
191 subsequent transfer events (final community). While adding invader simultaneously with the resident  
192 community can be viewed as co-assembly, and not invasion, here, we emphasize that the inoculum  
193 material originating from the flow-through microcosms already had 60-days of co-evolution and therefore  
194 can be considered as resident community, even when introduced at the same time with the invader cells.

195 Faith's phylogenetic diversity of the resident NOB guild was significantly lower at high vs low nitrite loading  
196 (Table 1) in the flow-through microcosms (Wilcoxon test  $p=0.02$ ) but not in batch microcosms (Wilcoxon  
197 test  $p=0.15$ ). This low phylogenetic diversity in flow-through microcosms corresponded to resident  
198 communities where *Nitrotoga* dominated over *Nitrospira* at high nitrite loading, and was consistent with  
199 known differences in nitrite affinity and specific growth rates of these two genera (Nowka *et al.*, 2015). As  
200 pointed out above, diversity indices without context do not say much regarding the ecological processes  
201 shaping the resident NOB guilds. Therefore, in this study, we elaborated on the relative contribution of the  
202 four processes (i.e. selection, drift, dispersal and speciation) that govern community assembly (Vellend,  
203 2010), and subsequently determine invasion outcome.

204 In Table 1 we provide an overview of the evaluation of the importance of selection, drift and dispersal in  
205 the resident communities. We can neglect speciation, as it is unlikely in the short timeframe of the  
206 experiment that new types arise and achieve significant abundance. Our interpretation of the strength of  
207 processes acting on the resident communities is based on the dynamics and consistency across replicates of  
208 the composition of the non-invaded control communities (Figure 1, Figure S1 and Figure S2) and a  
209 conceptual synthesis of community ecology (Vellend, 2010). We measure stochastic effects as within-group

210 distances of replicate communities, such that large dissimilarities represent strong effect of stochastic  
211 community assembly processes. Similarly, small dissimilarities between replicate communities point  
212 towards strong effect of selection, as suggested in Evans *et al.* (2017).

213 Dispersal was relevant only in flow-through microcosms since they were open to the environment, in  
214 contrast to the batch microcosms, which were fed sterile tap water spiked with nitrite. Dispersal can  
215 influence the diversity, composition, as well as functioning of a community and the effect of dispersal  
216 seems to be enhanced in smaller communities (Zha *et al.*, 2016). For NOB guilds newly assembled from tap  
217 water the contribution of dispersal is low, compared to the contribution of selection and drift (Kinnunen *et al.*,  
218 2017). Hence, we focus on the relative importance of selection and drift from here on.

219 The similarity between the resident communities independently assembled from the same source  
220 community was highest in resident communities assembled under flow-through conditions (Figure 2),  
221 which indicates that selection was most important. The direction of selection was affected by the nitrite  
222 loading, as seen from the difference in the ratio of *Nitrotoga* to *Nitrospira* at different nitrite loadings. At  
223 the time of invasion *Nitrospira* abundance had not reached steady state (Figure 1) since it increased  
224 significantly during the 14 days after the invasion event as seen by comparing the 'Initial Resident' and  
225 'Final' community fractions (ANOVA low nitrite  $p=0.01$ ; high nitrite  $p=0.05$ ). In low nitrite loading flow-  
226 through microcosms, selection pressure was positive towards *Nitrospira*, as *Nitrospira* increased in  
227 abundance relative to *Nitrotoga*. Even though *Nitrospira* also increased significantly in abundance in high  
228 loading flow-through microcosms, the *Nitrotoga*-to-*Nitrospira* ratio was higher than in the low nitrite  
229 loading, indicating strongest selection towards resident *Nitrotoga*. While one *Nitrotoga* type has been  
230 found to be one of the key nitrite-oxidizers in wastewater treatment (Lücker *et al.*, 2014), indicating its  
231 adaptability at higher nitrite concentrations, little is known about the nitrite affinity of different *Nitrotoga*  
232 strains in drinking water communities. Previous studies on competition between *Nitrospira* and *Nitrotoga*  
233 in drinking water treatment, however, have also observed the dominance of *Nitrospira* at low nitrite

234 loading conditions that is outcompeted by *Nitrotoga* at higher nitrite loading conditions (Albers *et al.*, 2018;  
235 Kinnunen *et al.*, 2017).

236 Interestingly, the selection in the batch microcosms favored *Nitrospira* under both loading conditions (see  
237 final community on Figure 1 and Figure S2). This can be due to the dynamic nitrite-loading in these  
238 microcosms, causing nitrite concentration changes over time, providing niches for NOB with a range of  
239 affinities for nitrite. In flow-through microcosms, the nitrite concentration attains steady-state (Figure S1),  
240 likely selecting for NOB with a narrower range in substrate affinity. Based on this, we expect the invader  
241 *Nitrotoga* strain to be less competitive at low nitrite loading than high nitrite loading, in resident  
242 communities dominated by competition.

243 Next, we estimated the relative contribution of drift to the assembly of the resident communities. In both  
244 flow-through and batch microcosms, significantly lower guild abundance was observed at low nitrite than  
245 at high nitrite loading, as expected (ANOVA flow-through  $p < 0.0001$ ; batch  $p = 0.04$ ). Communities with low  
246 abundance are theoretically more affected by drift than communities with more members (Nemergut *et al.*  
247 *et al.*, 2013). The higher dissimilarities between replicate communities after 60 days of low vs high nitrite  
248 loading also support this (Figure 2). The contribution of selection over drift was inferred to be highest in  
249 high nitrite loading flow-through microcosms based on the high similarity in composition of communities  
250 independently assembled from the same source community (Figure 2). In contrast, the contribution of  
251 selection over drift was inferred to be lowest in batch microcosms (Figure 2). In these microcosms, half of  
252 the community was regularly removed, promoting higher turnover in replacement of removed community  
253 members and amplifying the effect of drift compared to the flow-through system.

254 We can now explain the underlying causes for the differences in the observed phylogenetic diversity of  
255 resident NOB guilds (Table 1) based on the community assembly processes discussed above: we saw no  
256 significant difference in NOB phylogenetic diversity in batch microcosms, supporting our interpretation that  
257 similar processes dominate the community assembly in batch microcosms irrespective of the nitrite loading

258 regime. In flow-through microcosms, however, the influence of selection over drift varied between the two  
259 nitrite loading treatments: in the high nitrite loading microcosms higher selection to drift ratio resulted in  
260 significantly lower phylogenetic diversity (Wilcoxon test  $p=0.02$ ) and the dominance of few community  
261 members with high relative fitness.

#### 262 *Successful establishment of the invader*

263 In the flow-through microcosms The resident NOB guild was subject to continuous invasion during a 24  
264 hour period by a culture of *Nitrotoga* HW29 (Hüpeden *et al.*, 2016) while the invader was introduced  
265 simultaneously with the resident community in the batches. In flow-through microcosms, the invader  
266 strain was subjected to competition with 2 other *Nitrotoga* and 6 *Nitrospira* types at low loading conditions  
267 and 3 *Nitrotoga* and 3 *Nitrospira* types at high loading conditions (Figure S2). We used three defined  
268 concentrations of invader cells (see invader qPCR data on Figure 1) to achieve low, medium and high  
269 relative propagule pressure conditions (estimated to be equivalent to 1%, 10% and 100% of the total  
270 resident NOB population), with the aim to test the effect of drift on invasion success. Following the  
271 introduction of the invader strain, we allowed another five biomass turnover times (approximately 14 days,  
272 estimated from the resident community cell numbers and nitrite loading rates) before sampling the follow-  
273 through microcosms. This time for establishment ensured that, if observed, invader persistence would  
274 indicate an active population rather than residual invader cells.

275 Figure 1 shows that the invader cell addition did not significantly change the total NOB cell numbers after 5  
276 biomass turnover times, except in the low nitrite loading batch microcosms (Wilcoxon test  $p=0.02$ ). The  
277 resident community displayed complete nitrite removal during 30 days before the invasion event (Figure  
278 S1), suggesting that the resident community had reached its carrying capacity by the time of the invasion  
279 event. Hence, if established, the invader *Nitrotoga* displaced some of the resident NOB types or established  
280 at low relative abundance.

281 Based on amplicon sequencing, we could monitor the establishment of the invader strain – as its sequence  
282 was not present in the original resident community. The invader *Nitrotoga* strain was only established in  
283 the flow-through microcosms at high propagule pressure, whereas in batch microcosms, it was established  
284 at almost all propagule pressures, although at different relative abundance (Table 1). The frequency of  
285 establishment increased in both batch and flow-through microcosms with increasing propagule pressure  
286 (Figure 3).

### 287 *Descriptive indices of community composition fail to predict invasion success*

288 First, we tested the diversity-invasibility hypothesis in NOB guilds. We determined correlations between the  
289 relative abundance of invader (relative to total NOB) and the phylogenetic diversity of the resident  
290 community (Figure S6) as well as invader relative abundance and nearest (Figure S7) and mean  
291 phylogenetic distance to the resident community members (Figure S8). We need to emphasize that  
292 comparing the guild diversity and phylogenetic distance between invader and resident community is only  
293 appropriate for replicate microcosms assembled by similar processes, because different assembly  
294 processes contribute differently to community diversity as well as invasion success. In flow-through  
295 microcosms, the communities assembled at high and low nitrite loading were governed by different  
296 processes; hence, combining the replicates from different treatments would encourage false conclusions of  
297 what governs invasion success. Hence, we determined correlations separately for invasion in communities  
298 from high nitrite loading and low nitrite loading flow-through microcosms. Because we inferred no  
299 difference in dominating assembly process in batch microcosms, we combined the replicate communities at  
300 different nitrite loading regimes. We reject the common hypothesis that invader establishment is  
301 negatively correlated with resident community diversity (Figure S6). While we observed a negative trend  
302 between the resident community diversity and the relative abundance of the invader after establishment in  
303 flow-through microcosms, we failed to observe any significant correlation in flow-through microcosms,  
304 contrary to batch microcosms. We observed a significant positive correlation between resident community

305 diversity and the relative abundance of the invader in batch microcosms. Clearly, phylogenetic diversity of  
306 the resident community is not a universal predictor for invasion resistance in a functional guild.  
307 Another metric used to predict invasion success – the nearest and/or average phylogenetic distance to the  
308 resident community (Gallien *et al.*, 2014) – is assumed to be positively correlated with invasion. However,  
309 because selection acts similarly on community members that are phylogenetically similar (Darwin, 1859),  
310 we hypothesized here that drift would, therefore, determine invasion success when phylogenetic distance  
311 between invader and the resident community is low. We neither saw significant correlation between the  
312 nearest (Figure S7) nor the average phylogenetic distance and relative abundance of the invader (Figure  
313 S8). Our observations indicate that when the relative importance of selection over drift in communities is  
314 low, mean phylogenetic distance to the resident community correlates negatively with invader relative  
315 abundance. Failing to see consistent correlations between invasion success and resident community  
316 diversity as well as phylogenetic distance between invader and resident community members, we  
317 evaluated, for the different microcosms, the prevailing community assembly processes and related them to  
318 the subsequent invasion outcome.

#### 319 *Stochastic processes determine invasion success in NOB guilds*

320 Resident communities, assembled with different dominating processes, were subject to invasion at  
321 different propagule pressures. We did not see a consistent correlation between the average distance from  
322 the resident community and invader relative abundance, hence our observations indicated that selection  
323 did not govern invasion outcome. Although, when drift dominates invasion, incidence and relative  
324 abundance of the invader would increase with propagule pressure. Based on this, we observed support for  
325 drift as governing process of invasion.

326 First, in batch microcosms, where the selection to drift ratio was lowest, we observed a clear effect of  
327 propagule pressure on invasion outcome (Table 1). Both frequency of invader establishment, as well as  
328 relative abundance of invader increased in response to higher concentrations of added invader cells,  
329 supporting drift as the process governing invasion in batch microcosms.

330 Second, in flow-through microcosms, successful establishment was only observed at high propagule  
331 pressure (Table 1). Failure to establish at lower propagule pressures could be a result of drift supported by  
332 the characteristics of a flow-through microcosm, where the actual propagule pressure in the system is  
333 lower than the theoretical propagule pressure because invader cells could easily flow through the system  
334 without attaching to the biofilm surface. Fewer invader cells are more affected by drift and the probability  
335 of extinction is increased, compared to larger populations.

336 Interestingly, drift explained invasion success also in resident communities where the relative importance  
337 of selection was high. This is somewhat unexpected, since in communities governed by selection, the  
338 competition caused by the fitness difference between invader and resident community members was  
339 expected to govern invasion success. One explanation could be that the high phylogenetic similarity of  
340 invader and resident community members reduced competition due to absence of large fitness differences.

341 Our observations were made using natural communities where phylogenetic distances between community  
342 members and the invader are very low compared to many other invasion experiments with synthetic  
343 communities where phylogenetic distances are up to 10-fold higher (Naughton *et al.*, 2015; Tan *et al.*,  
344 2015). However, when similarly low phylogenetic diversity and low phylogenetic distance between invader  
345 and resident were investigated, a similar conclusion was reached: propagule pressure increased invasion  
346 success and phylogenetic diversity had no effect on invasion success (Ketola *et al.*, 2017).

347 In conclusion, our results suggest that for functional guilds invaded by a guild member, where phylogenetic  
348 distance between resident and invader is typically low, stochastic processes govern invasion success, even  
349 when the relative importance of selection in the resident community is high. Our results also imply that  
350 predicting invasion of functional guilds by a member of the same guild from compositional information is  
351 nearly impossible, making futile the precise characterization of the composition of resident communities  
352 for this purpose. While regular measurements targeting the relative abundance of possible invader can be  
353 used to estimate the probability of establishment, the stochastic nature of drift does not allow predictions  
354 with high certainty. These observations need to be verified for functional guilds that have opportunities for



355 larger ecological differences, to test if we can generalize our findings across any type of invasions in  
356 microbial communities.

357

358 Supplementary information is available at ISME Journal's website.

359

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447

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#### 453 **Author contributions**

454 M.K, A.D, and B.F.S designed the experiments. M.K performed the experiments and carried out all  
455 molecular analyses; the data analysis was performed by M.K supported by A.D. All co-authors assisted in  
456 interpreting the results; M.K initiated the manuscript writing, which was finalized with contributions from  
457 A.D, H-J.A and B.F.S.

#### 458 **Competing interests**

459 The authors declare no competing financial interests.

460

461 **Table and figure headings:**

462

463 Table 1 – Effect of community assembly processes and propagule pressure on frequency of invader  
464 establishment

	Flow-through microcosms						Batch microcosms					
	Low nitrite loading			High nitrite loading			Low nitrite loading			High nitrite loading		
<b>SELECTION (direction)</b>	+			+++			++ ( <i>Nitrospira</i> )			++ ( <i>Nitrospira</i> )		
<b>DRIFT</b>	++			+			++++			+++		
<b>Selection to drift ratio<sup>1</sup></b>	++			++++			+			+		
<b>DISPERSAL</b>	++			+			0			0		
Faith's phylogenetic diversity <sup>2</sup>	0.38±0.05			0.31±0.006			0.39±0.04			0.34±0.03		
Mean phylogenetic distance from invader <sup>3</sup>	0.02±0.002			0.009±0.004			0.07±0.04			0.08±0.04		
Nearest phylogenetic distance from invader <sup>4</sup>	0.011			0.011			0.011			0.011		
Relative propagule pressure	+	++	++++	+	++	+++	+	++	++++	+	++	+++
Invader establishment frequency <sup>5</sup>	0	0	1	0	0	0.5	0.5	0.5	1	0	0.5	0.5
Rel. abundance of invader (%) <sup>6</sup>	0	0	13.9±7.6	0	0	8.1±3.2	16.4±0	10.1±0	24.5±23.7	0	0.9±0	9.8±0

465 <sup>1</sup>Strength of the processes is rated with 0 for no effect to ++++ for highest effect inferred from the community  
466 composition and the dissimilarity between biological replicates

467 <sup>2</sup>Phylogenetic diversity of the NOB guild calculated using the *PhyloMeasures* package

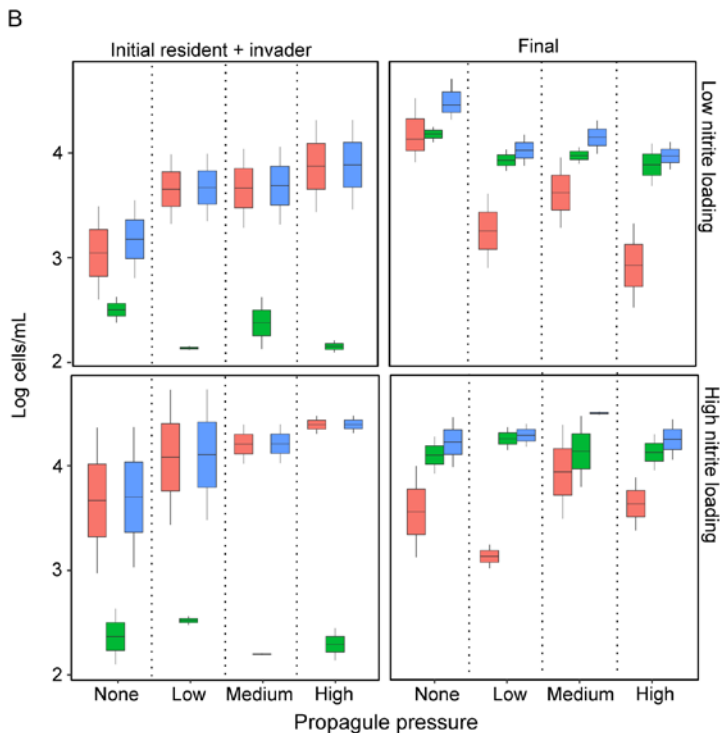
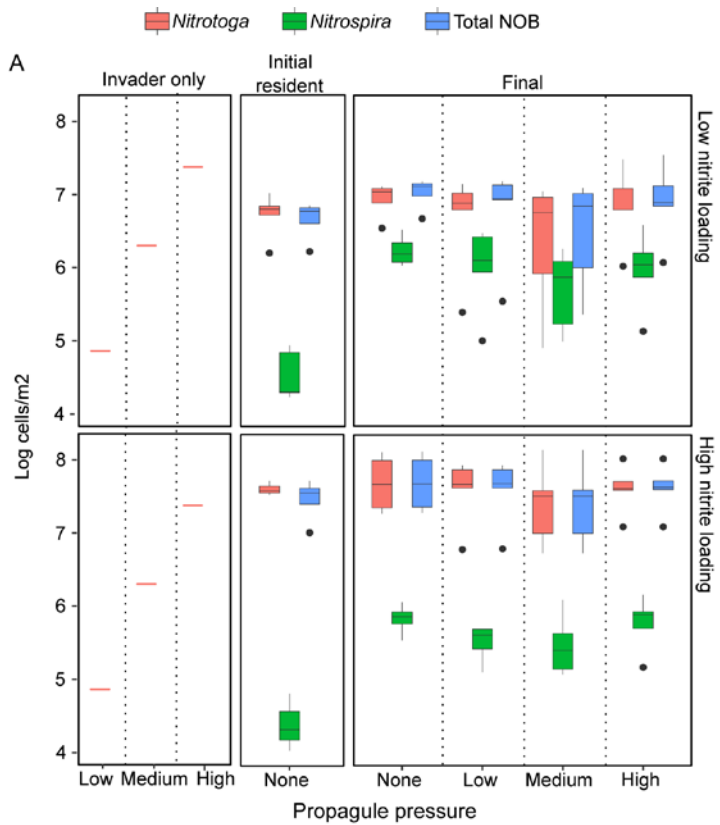
468 <sup>3</sup>phylogenetic Mean Pairwise Distance

469 <sup>4</sup>Mean Nearest Taxon Distance

470 <sup>5</sup>Invader establishment frequency detected in four replicates

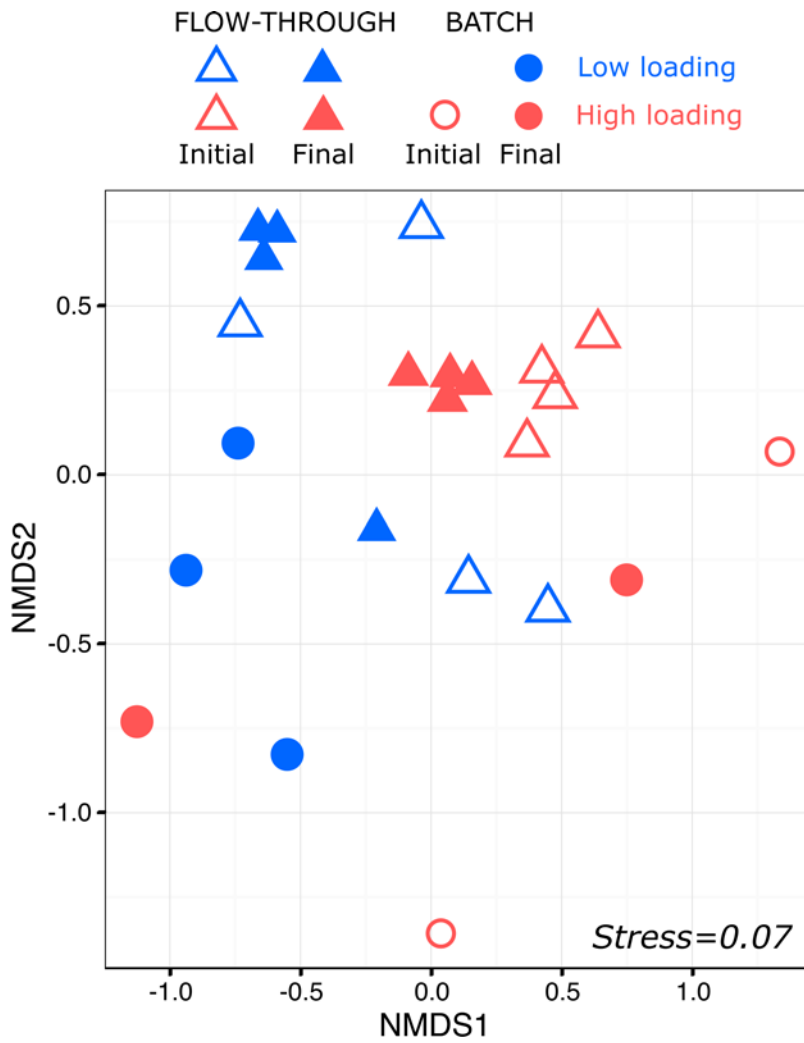
471 <sup>6</sup>Relative abundance of the invader sequence variant out of total NOB sequence variants ± standard deviation within  
472 four replicate communities

473



474

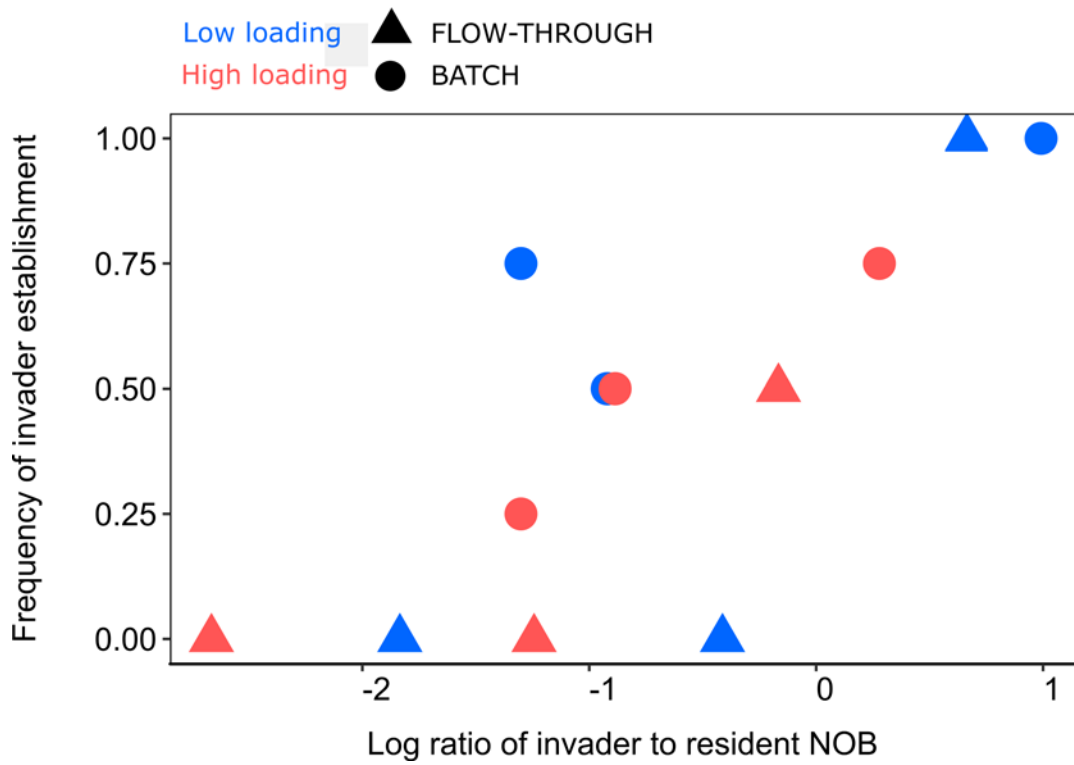
475 Figure 1 – Box-and-whisker plot representing the density of NOB in (A) flow-through and (B) batch  
 476 microcosms before (initial) and after invasion (final) determined by targeted qPCR. The initial community  
 477 composition was measured before invasion for flow-through microcosms and after first transfer for batch  
 478 microcosms. Propagule pressure *none* refers to the non-invaded control microcosms operated in parallel  
 479 with invaded microcosms



480

481 Figure 2 – Similarities between non-invaded resident communities independently assembled from the same  
 482 source community in flow-through and batch microcosms under two nitrite loading, based on nonmetric  
 483 multidimensional scaling ordinations of Bray-Curtis distances across community structures inferred from  
 484 total community 16S rRNA amplicon libraries

485



486

487 Figure 3 – Frequency of invader establishment out of 4 replicate microcosms at different propagule  
 488 pressure (ratio of invader to resident NOB) and in different experimental microcosms (circles for batch  
 489 microcosms and triangles for flow-through microcosms)