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# Plant and Soil

## Assembly of seed-associated microbial communities within and across successive plant generations --Manuscript Draft--

<b>Manuscript Number:</b>	PLSO-D-17-00819
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<b>Funding Information:</b>	
<b>Abstract:</b>	<p><b>Background and Aims</b> Seeds are involved in the transmission of microorganisms from one plant generation to another and consequently may act as the initial inoculum source for the plant microbiota. In this work, we assessed the structure and composition of the seed microbiota of radish (<i>Raphanus sativus</i>) across three successive plant generations.</p> <p><b>Methods</b> According to the bacterial (<i>gyrB</i>) and fungal (ITS1) markers employed in this work, the seed microbiota composition and structure were highly variable between seed samples. The relative contribution of dispersal and ecological drift in inter-individual fluctuations were estimated with a neutral community model.</p> <p><b>Results</b> Fluctuations in microbial community profiles were related to changes in community membership and composition across plant generation but also to variation between individual plants.</p> <p>Overall we found that seed-associated microbial communities are dominated by few bacterial and fungal taxa whose distributions cannot be explained by neutral processes. However, neutral processes are probably responsible for variation in community composition of the vast majority of rare taxa associated to radish seeds.</p> <p><b>Conclusions</b> Altogether, these results provide a first glimpse of the governing processes driving the assembly of the seed microbiota and could be helpful for designing biocontrol-based strategies.</p>
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Dear Editor,

We wish to submit our manuscript entitled “*Assembly of seed-associated microbial communities within and across successive plant generations*” for review to Plant and Soil’s special issue: Seeds and Microbes. This manuscript is a revised version of manuscript PLSO-D-16-01789 that has been submitted in the special issue Seeds and Microbes of Plant and Soil. We tried to address all the comments made by the reviewers.

The present work assessed the structure and composition of the seed microbiota of radish (*Raphanus sativus*) across three successive plant generations. The relative importance of niche and neutral processes (ecological drift and dispersal) in the assembly of the seed microbiota is investigated at the individual plant level. Overall we found that seed-associated microbial communities are dominated by few bacterial and fungal taxa whose distributions cannot be explained by neutral processes. However, neutral processes are probably responsible for variation in community composition of the vast majority of rare taxa associated to radish seeds.

We confirmed that all the material presented in the current manuscript is original research and has neither been published nor been submitted for publication elsewhere. We would sincerely appreciate if you would consider this article for review and would welcome any queries you might have regarding our submission.

Authors of this manuscript confirm that all the work presented in the manuscript is original and report no conflicts of interest.

Yours faithfully,  
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1 **Title: Assembly of seed-associated microbial communities within and across successive**  
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21 **Keywords:** seed-associated microbial community; heritability; community assembly;  
22 dispersion; diversification

23

24 **Abstract**

25 Background and Aims

26 Seeds are involved in the transmission of microorganisms from one plant generation to another  
27 and consequently may act as the initial inoculum source for the plant microbiota. In this work,  
28 we assessed the structure and composition of the seed microbiota of radish (*Raphanus sativus*)  
29 across three successive plant generations.

30 Methods

31 According to the bacterial (*gyrB*) and fungal (ITS1) markers employed in this work, the seed  
32 microbiota composition and structure were highly variable between seed samples. The relative  
33 contribution of dispersal and ecological drift in inter-individual fluctuations were estimated  
34 with a neutral community model.

35 Results

36 Fluctuations in microbial community profiles were related to changes in community  
37 membership and composition across plant generation but also to variation between individual  
38 plants.

39 Overall we found that seed-associated microbial communities are dominated by few bacterial  
40 and fungal taxa whose distributions cannot be explained by neutral processes. However, neutral  
41 processes are probably responsible for variation in community composition of the vast majority  
42 of rare taxa associated to radish seeds.

43 Conclusions

44 Altogether, these results provide a first glimpse of the governing processes driving the assembly  
45 of the seed microbiota and could be helpful for designing biocontrol-based strategies.

46

## 47 **Introduction**

48 Plants have co-evolved with complex microbial communities which are known collectively as  
49 the plant microbiota. The plant microbiota can promote plant growth and plant health (Muller  
50 et al., 2016). For instance, plant-associated microbial communities can modulate biomass  
51 accumulation (Sugiyama et al., 2013), metabolites production (Badri et al., 2013), drought  
52 tolerance (Lau and Lennon, 2012; Rolli et al., 2015) and flowering time (Wagner et al., 2014;  
53 Panke-Buisse et al., 2015, Dombrowski et al., 2017). Moreover, the plant microbiota can also  
54 increase host resistance to foliar or root pathogenic agents (Busby et al., 2016; Ritpitakphong  
55 et al., 2016; Mendes et al., 2011; Santhanam et al., 2015).

56 Owing to the beneficial effects of the microbiota on plant fitness, the relative importance  
57 of different community assembly processes in determining its structure over space and time is  
58 of great interest (Paredes & Lebeis, 2016). Community assembly processes could be divided  
59 into four main forces, namely dispersal, diversification, drift and selection (Vellend 2010,  
60 Nemergut et al., 2013). Over recent years many research groups have investigated the  
61 importance of selection in determining plant-associated microbial assemblage structure. For  
62 instance, it is now widely accepted that rhizosphere bacterial assemblages are partly determined  
63 by selection (Bulgarelli et al., 2013). Indeed, environmental-filtering such as soil  
64 physiochemical properties and to a lesser extend host-filtering are involved in variation of  
65 bacterial root microbiota profiles (Bulgarelli et al., 2012; Lundberg et al., 2012; Peiffer et al.,  
66 2013; Edwards et al., 2015; Dombrowski et al., 2017). Moreover environmental- and host-  
67 filtering are also important drivers of leaf-associated microbiota composition (Bodenhausen et  
68 al., 2013, Horton et al., 2014; Wagner et al., 2016). In comparison to selection, the relative  
69 importance of dispersion, diversification and ecological drift in driving assembly of plant-  
70 associated microbial communities has not been well investigated. To our best knowledge, only  
71 one study has investigated the relative importance of selection, dispersion and ecological drift



72 in the assembly of the leaf microbiota (Maignien et al., 2014). According to the results presented  
73 in this work, community membership of the phyllosphere microbiota is shaped by selection,  
74 while community composition is driven by diversification and ecological drift (Maignien et al.,  
75 2014).

76         Microbial entities of the plant microbiota can be either acquired from the surrounding  
77 environment (horizontal transmission) or directly from the parent (vertical transmission). To  
78 date, the relative contribution of horizontal and vertical transmission in the ultimate  
79 composition of the plant microbiota is unclear (Shade et al., 2017). On the one hand, recruitment  
80 of microbes from the surrounding bulk soil in the rhizosphere strongly support the role of  
81 horizontal transmission (Bulgarelli et al., 2012; Edwards et al., 2015). On the other hand, the  
82 presence of conserved seed-associated micro-organisms across multiple plant generations  
83 (Johnson-Monje & Raizada 2011; Hardoim et al., 2012) suggest that some members of the plant  
84 microbiota are vertically transmitted. Although the contribution of the seed-associated micro-  
85 organisms in shaping the ultimate composition of the plant microbiota is under debate, studying  
86 the biological and ecological processes involved in the assembly of seed-associated microbial  
87 community can have important implication for plant growth and health. Indeed, the composition  
88 of the seed microbiota can contribute to key seed traits such as seed preservation (Chee-Sanford,  
89 2006) or release of seed dormancy (Goggin et al., 2015). Occasionally, seed transmissions of  
90 Shiga toxin producers could dramatically impact the sanitary quality of seed samples (King et  
91 al., 2011). From an epidemiological angle, seed transmission of plant pathogens serves as a  
92 major method of dispersal and is therefore important for predicting disease emergence and  
93 spread. Very low degrees of seed contamination by bacterial pathogens can lead to efficient  
94 plant colonization (Darrasse et al., 2007). Furthermore, seed transmission of phytopathogens  
95 can take place on non-host plants (Darrasse et al., 2010; Darsonval et al., 2008), which can then  
96 serve as a reservoir of plant pathogens. Finally, the seed microbiota can have early or late

97 consequences on plant growth and health with decrease of germination rate (Nelson, 2004;  
98 Munkvold, 2009) or enhancement of ear emergence time (Mitter et al., 2017).

99         Recent diversity surveys of the seed microbiota showed a high level of variation in  
100 microbial assemblages composition (Lopez-Velasco et al., 2013; Links et al., 2014; Barret et  
101 al., 2015; Klaedtke et al., 2016; Rezki et al., 2016). Based on these previous studies, it seems  
102 that selection by the host plant is not a major driver of community assembly (Barret et al., 2015;  
103 Klaedtke et al., 2016). In contrast, environmental-filtering is shaping the structure of seed-  
104 associated fungal assemblages (Klaedtke et al., 2016). Since the high variation in community  
105 composition observed across seed samples remains difficult to explain solely by environmental-  
106 filtering, other ecological forces such as dispersal, diversification and ecological drift  
107 (Nemergut et al., 2013) may play an important role in assembly of the seed microbiota.

108         The objective of the present work was to assess the relative importance of these  
109 ecological processes in the assembly of the seed-associated microbial communities. To do so,  
110 we employed a theoretical framework based on neutral theory that has been successfully  
111 adapted to interpret the composition of host-associated microbial communities (Venkataraman  
112 et al., 2015; Burns et al., 2016; Li et al., 2016). Instead of considering differences of ecological  
113 fitness between community members as the main driver of community assembly (i.e. niche-  
114 based theory), neutrality hypothesis assumes that community members are ecologically  
115 equivalents. Under this assumption, the extinction of a local individual creates an empty site  
116 that is either fill by the offspring of a local individual or by immigration of a new community  
117 member. We used radish (*Raphanus sativus* var. Flamboyant5) as a model system since this  
118 plant has a high multiplication rate (one plant produces 500 seeds on average), allowing analysis  
119 of the seed microbiota for individual plants. The structure and diversity of seed-associated  
120 microbial communities were analysed on seed samples collected on individual plants during  
121 three successive generations through community profiling approaches. Based on this profiling,

122 we observed the frequency of taxa occurrence in local communities (seed samples collected on  
123 individual plant) and their abundances across the metacommunity (all seed samples collected  
124 on the different plants of the same generation) and compared the observed OTU distribution to  
125 an estimated OTU distribution calculated with a neutral community model. According to the  
126 OTUs distribution, it seems that seed-associated microbial communities are composed of few  
127 dominant taxa that are selected by the host or the environment and multiple rare taxa whose  
128 distributions can be explained by neutral processes.

129

## 130 **Material & Methods**

### 131 **Seed collection**

132 Radish seeds (*Raphanus sativus* var. Flamboyant5, Vilmorin) were sown in March 2013 at the  
133 FNAMS experimental station (47°28'12.42" N, 0°23'44.30" W, Brain-sur-l'Authion, France).  
134 Mature seeds of the first plant generation were collected manually on individual plant and  
135 immediately submitted to DNA extraction. To limit border effect, plants located at the border  
136 of the experimental plot were not sampled. The remaining seeds in the experimental plot were  
137 harvested mechanically with a thresher. Seed samples collected mechanically were stored at  
138 9°C at a relative humidity of 60% for 6 months. These mechanically harvested seeds were used  
139 for sowing in the following season. The same procedure was repeated for seeds of the second  
140 (2014) and third generation (2015).

141 DNA extraction was performed on 73 seed samples collected manually from individual  
142 plants in 2013 (9 plants), 2014 (32 plants) and 2015 (32 plants). In addition, DNA extraction  
143 was performed on 3 subsamples of 1,000 seeds for each seed lot harvested mechanically in  
144 2013, 2014 and 2015 (9 subsamples in total). Seeds were initially soaked in 25 mL of phosphate  
145 buffer saline (PBS) supplemented with 0.05% (v/v) of Tween® 20 during 2 hours and 30  
146 minutes at room temperature under constant agitation (140 rpm). Suspensions were centrifuged  
147 (6000 x g, 10 min, 4°C) and pellets were resuspended in approximately 2 ml of supernatant and  
148 transferred in microtubes. Total DNA was extracted with the Power Soil DNA Kit (MoBio  
149 Laboratories) following procedure described earlier (Barret et al., 2015).

150

### 151 **Libraries construction and sequencing**

152 Community profiling approaches were performed on 82 environmental DNA samples  
153 corresponding to seed samples harvested manually (73) or mechanically (9) from three  
154 consecutive plant generations. Amplifications of one bacterial marker (*gyrB*) and one fungal

155 marker (ITS1) were performed with the primer sets *gyrB*\_aF64/*gyrB*\_aR353 (Barret et al.,  
156 2015) and ITS1F/ITS2 (Buée et al., 2009) following procedures described earlier (Barret et al.,  
157 2015). Amplicon libraries were mixed with 5% PhiX control according to Illumina's protocols.  
158 A total of three sequencing runs was performed for this study with MiSeq Reagent Kits v3 (600  
159 cycles).

160

## 161 **Data analysis**

162 The choice of *gyrB* as a bacterial molecular marker is dictated by its lowest taxonomic  
163 resolution (species-level) in comparison to classic molecular markers based on the  
164 hypervariable regions of the 16S rRNA gene (Barret et al., 2015). In addition, *gyrB* is a single-  
165 copy gene that encodes the  $\beta$  subunit of the bacterial gyrase, which then limits the  
166 overestimation of taxa possessing multiple copies of *rrn* operons. The occurrence of *gyrB* was  
167 investigated in a set of 32,062 bacterial genomic sequences available in the IMG database v4  
168 (Markowitz et al., 2012) at the time of analysis (10<sup>th</sup> December 2015). Coding sequences that  
169 belong to the protein family TIGR01059 and KO2470 were defined as GyrB orthologs and  
170 retrieved for further analysis (30,627 hits found in 30,175 genomic sequences). The  
171 corresponding nucleotidic sequences were aligned against a reference *gyrB* alignment (Barret  
172 et al., 2015) with the align.seqs command of mothur 1.36.1 (Schloss et al., 2009) using the  
173 following parameters : align=gotoh, match=3, mismatch=-2, gapopen=-50, gapextend=-50,  
174 ksize=8. Sequences that did not aligned (102) were discarded and only unique sequences  
175 (haplotypes) were conserved in the reference alignment (10,427 haplotypes). According to the  
176 gANI cliques defined previously by Varghese and collaborators (Varghese et al., 2015), the  
177 *gyrB* marker has the best precision (0.964) and sensitivity (0.955) at a genetic distance of 0.02  
178 (98% identity cutoff).

179           Sequence analyses were performed as described earlier by Barret et al., 2015 using a  
180 sequence curation pipeline (Kozich et al., 2013) within mothur 1.36.1 (Schloss et al., 2009).  
181 Briefly, *gyrB* sequences were aligned against a *gyrB* reference alignment composed of 10,427  
182 haplotypes. Chimeric sequences were detected and removed from the dataset using the  
183 command chimera.uchime (Edgar et al., 2011). Taxonomic affiliation of *gyrB* sequences was  
184 performed with a Bayesian classifier (Wang et al., 2007) implemented in the classify.seqs  
185 command against an in-house *gyrB* database containing 30,525 representative sequences at a  
186 80% bootstrap confidence score. All sequences not affiliated at the phylum level and containing  
187 a predicted stop codon were discarded from the dataset. A *de novo* clustering method (i.e.  
188 average-linkage clustering at a 98% identity cut-off) was performed with the cluster.split  
189 command for assigning *gyrB* sequences to operational taxonomic units (OTUs).

190           The variable ITS1 regions of the fungal internal transcribed spacer were extracted with  
191 the Perl-based software ITSx 1.0.4 (Bengtsson-Palme et al., 2013). Reference-based clustering  
192 was performed with UCLUST 1.2.22 algorithm (Edgar, 2010) at a 97% identity cut-off against  
193 the UNITE 7.1 database (Abarenkov et al., 2010).

194           Observed richness (number of OTUs) and evenness (estimated with Simpson's  
195 reciprocal index) were calculated with the R package phyloseq (McMurdie & Holmes, 2013)  
196 on OTU tables rarefied to 5,000 sequences per sample. Differences in richness and evenness  
197 between variables were assessed by one-way ANOVA with post-hoc Tukey's HSD test.

198           Differences in community membership and composition between plant generations  
199 were assessed with Jaccard and Bray-Curtis indices, respectively. Jaccard and Bray-Curtis  
200 indices were calculated on OTU tables transformed to even sampling depth (OTU count divided  
201 by the number of read per sample and multiply by 1,000,000). Principal coordinate analysis  
202 (PCoA) was used for ordination of Jaccard and Bray-Curtis distances. Permutational  
203 multivariate analysis of variance (PERMANOVA; Anderson, 2001) was carried out to assess

204 the importance of the plant generation on seed-associated microbial community profiles. To  
205 quantify the contribution of the plant generation in microbial community profiles, canonical  
206 analysis of principal coordinates was performed with the function `capscale` of the R package  
207 `vegan` 2.4.2 (Oksanen, 2017) followed with PERMANOVA with the function `adonis`. Changes  
208 in relative abundance of OTUs between the different plant generations (2013, 2014 and 2015)  
209 were assessed with likelihood ratio test (LRT) with the R package `DESeq2` 1.14.1 (Anders &  
210 Huber, 2010). OTU with a corrected p-value  $< 0.01$  and a  $\log_2FC > |2|$  were considered as  
211 differentially abundant between the different plant generations.

212

### 213 **Testing the importance of neutral processes in community assembly**

214 The role of neutral processes in assembly of seed-associated microbial communities was  
215 estimated by fitting the Sloan neutral community model for prokaryotes (Sloan et al., 2007) to  
216 the observed OTU distribution. The conceptual framework on which is based this model is as  
217 follow: a large community (a.k.a metacommunity), which is composed of all micro-organisms  
218 associated to seed samples collected on all plants located in the same experimental plot, serves  
219 as a source of immigrants to a local community that is composed of micro-organisms associated  
220 to one seed sample collected from one individual plant. Since neutrality hypothesis assumes  
221 that community members are ecologically equivalents, changes in structure of the local  
222 community is due to the death of an individual that is either replaced by an immigrant or by  
223 local reproduction.

224 We assessed the capability of the Sloan neutral community model for prokaryotes to  
225 estimate the distribution of seed-associated micro-organisms. The Sloan model estimates the  
226 rate of immigration ( $m$ ) into the local community by comparing OTU frequency across multiple  
227 samples (Sloan et al., 2007). The Sloan model predicts that abundant entities in the large  
228 community are widespread across samples since their dispersions could occur by chance, while

229 rare members are more likely to be lost by drift in the local community. We used a custom R  
230 script written by Burns and collaborators for fitting the Sloan neutral community model to OTU  
231 distribution (Burns *et al.*, 2016). The fit of Sloan model was compared with the fit of a binomial  
232 distribution model using the Akaike information criterion of each model (Burns *et al.*, 2016).  
233 Binomial distribution model is used for assessing the importance of random sampling on  
234 community structure in absence of drift and dispersal limitation (Sloan *et al.*, 2007).

235         The validity of prediction of neutral community models with barcoding datasets has  
236 been recently assessed by Sommeria-Klein and collaborators (2016). According to this study,  
237 neutral-model inference is affected by the number of reads per sample, which should be smaller  
238 than the number of sampled individuals (Sommeria-Klein *et al.*, 2016). This limitation could be  
239 easily tested by subsampling the number of reads and assessing that the estimators of  
240 immigration are unchanged. The number of sequences per sample was then rarefy to 1,000 and  
241 5,000 counts before fitting both neutral community models.

242



## 243 **Results**

244           The assembly of radish seed-associated microbial communities was monitored across  
245 three plant generations (2013, 2014 and 2015) through community profiling approach. A total  
246 of 73 seed samples were collected manually from individual plant in 2013 (9 plants), 2014 (32  
247 plants) and 2015 (32 plants). Seeds of the remaining plants in the experimental plot were  
248 harvested with a threshing machine and used for sowing the following year. Since manual and  
249 mechanical harvesting can result in differences in composition of seed-associated microbial  
250 community, three subsamples of 1,000 mechanically-harvested seeds per year (9 subsamples in  
251 total) were also subjected to community profiling, therefore resulting in 82 seed samples in  
252 total.

253           Bacterial and fungal assemblage profiles were estimated after assignment of *gyrB* and  
254 ITS1 sequences to operational taxonomic units (OTUs). Overall, 43,102 bacterial OTUs and  
255 25,618 fungal OTUs were detected across all seed samples. While the number of observed  
256 bacterial OTUs was not significantly different between manual- and mechanically-harvested  
257 seed samples, fungal richness was significantly higher in mechanically-harvested seed samples  
258 ( $P < 0.01$ , one-way ANOVA with post hoc Tukey's HSD test, **Fig. S1**). However, this increase  
259 in fungal richness did not impact evenness (as assessed with Simpson's reciprocal index, **Fig.**  
260 **S1**). As the level of alpha-diversity associated to seed samples used for sowing (mechanically  
261 harvest) was comparable to the alpha-diversity of seed samples collected from individual plants,  
262 the harvesting method is not involved in variation of microbial community composition across  
263 years. Therefore, to investigate variation in seed-associated microbial assemblages between  
264 individual plants, only seed samples collected manually were considered in the subsequent  
265 analyses.

266

## 267 **Assembly of the seed microbiota over successive plant generations**

268           The assembly patterns of radish seed microbiota was monitored among samples  
269 collected manually in 2013, 2014 and 2015. First, we compared microbial richness and  
270 evenness over the three plant generations (**Fig. 1**). A significant increase in bacterial and fungal  
271 richness (**Fig. 1A** and **Fig. 1B**) was observed for samples collected in 2015 vs. those collected  
272 the previous years ( $P < 0.01$ , one-way ANOVA with post hoc Tukey's HSD test). However,  
273 this increase in microbial richness was not associated with significant differences in microbial  
274 evenness over the successive plant generations (**Fig. 1C** and **Fig. 1D**).

275           Similarity in community membership and community composition was estimated  
276 through calculation of Jaccard and Bray-Curtis similarity indices, respectively. Ordination of  
277 Jaccard and Bray-Curtis similarity indices with principal coordinate analysis (PCoA) revealed  
278 a significant clustering (PERMANOVA,  $P < 0.001$ ) of seed-associated fungal communities  
279 according to the plant generation (**Fig. 2A** and **Fig. 2B**). In contrast, seed-associated bacterial  
280 communities were only significantly clustered for the third plant generation ( $P < 0.001$ ; **Fig. 2**  
281 **C** and **Fig. 2D**). Jaccard ordinations had higher explanatory value and more discrete clustering  
282 by year than Bray-Curtis, suggesting that inter-annual differences in community structure can  
283 be attributed to patterns in the presence and absence of taxa (e.g. many taxa observed  
284 exclusively in one year). The relative contribution of the plant generation in community profiles  
285 was further investigated through canonical analysis of principal coordinates (CAP) followed by  
286 PERMANOVA. According to CAP analyses, the plant generation explained 22% and 32% of  
287 variation in bacterial and fungal community membership and 27% and 39% of bacterial and  
288 fungal community composition (PERMANOVA,  $P < 0.001$ ).

289           To determine if some OTUs were specifically associated with one particular generation,  
290 we performed likelihood ratio tests (LRT) with DESeq2 on OTU table. LRT revealed that the  
291 relative abundances of 25 bacterial OTUs were significantly different ( $pvalue \leq 0.01$  and  
292  $\log_2FC \geq |2|$ ) between the first and second plant generations (**Fig. S2A**). The number of

293 significant bacterial OTUs with differences in relative abundant increases to 62 between the  
294 second and third plant generation (**Fig. S2E**). Significant changes in relative abundances of 69  
295 and 125 fungal OTUs were also detected between 2013 vs 2014 and 2014 vs 2015, respectively  
296 (**Fig. S2B and Fig. S2F**). In conclusion, differences in richness, diversity, community  
297 membership, community composition and OTUs relative abundance indicate a significant  
298 contribution of plant generation and/or harvest year on the structure of seed-associated  
299 microbial communities.

300

### 301 **Assembly of seed-associated microbial communities among individual plants within the** 302 **same harvest year**

303 Results obtained in the previous subsection have shown that plant generation and/or harvest  
304 year are important drivers of the structure of the seed microbiota. However, variation in richness  
305 (**Fig. 1**) and community composition (**Fig. 3**) of the radish seed microbiota were observed  
306 between seed samples collected the same year on distinct individual plants. Overall, the radish  
307 seed microbiota is mainly composed of 2 bacterial genera, namely *Pantoea* and *Pseudomonas*  
308 (**Fig. 3A**), and 2 fungal genera: *Alternaria* and *Cladosporium* (**Fig. 3B**). While the presence of  
309 these microbial genera in seed samples was relatively conserved across individual, their relative  
310 abundances were variables (**Fig. 3A and Fig. 3B**).

311 To further evaluate variation in community membership and composition of the radish  
312 seed microbiota, we counted the number of OTUs that were conserved among all individual  
313 plants (core microbiome, Shade & Handelsman 2012). Among the 43,102 bacterial OTUs  
314 detected in the *gyrB* dataset, only three were observed in all seed communities. These three  
315 bacterial OTUs are affiliated to *Pantoea agglomerans*, *Pseudomonas viridiflava* and *Erwinia*  
316 *tasmaniensis* (**Table 1**). Regarding fungal communities, nineteen fungal OTUs among 25,618

317 OTUs detected were conserved in all seed samples. Among these 19 fungal OTUs, 14 were  
318 affiliated to the *Alternaria* genus (**Table 1**).

319 Although, the core fraction of the radish seed microbiota was relatively restricted in  
320 OTU number, it was composed of highly abundant taxa, representing on average 70% and 87%  
321 of all bacterial and fungal reads, respectively. Despite their strong conservations across  
322 individuals, the relative abundance of the core members of the radish seed microbiota varies  
323 greatly between seed samples (**Fig. S3**). For instance the relative abundance of bacterial  
324 OTU00001, which is affiliated to *P. agglomerans*, ranges from 7% to 82% within seed samples  
325 collected in 2014 (**Fig. S3A**). Shift in relative abundance of fungal OTUs was also important  
326 between seed samples, with variation from 4% to 43% for OTU00002 (*Cladosporium*) in 2014  
327 (**Fig. S3B**). This variability of community composition in seed samples collected from the same  
328 plant genotype grown on the same experimental plot, suggest that non-selective processes such  
329 as dispersal, drift and diversification may be involved in community assembly of the seed  
330 microbiota.

331

### 332 **Neutral processes are involved in intra-annual community assembly of the seed** 333 **microbiota**

334 In order to investigate the relative role of dispersal and ecological drift in shaping seed-  
335 associated microbial community, we used a conceptual framework based on neutral theory. In  
336 this conceptual framework, every seed sample harvested from one individual plant represents a  
337 local community that is part of a larger metacommunity corresponding to all seed samples  
338 collected on the different plants of the same generation. Based on the microbial community  
339 profiles obtained with *gyrB* and ITS1 sequences, we observed the frequency of OTU occurrence  
340 in local communities and their abundance across the metacommunity.

341 First, the fit of the Sloan model to the observed OTU distribution was used for assessing  
342 the relative importance of dispersal limitation and ecological drift. Since neutral-model  
343 inference is affected by the number of reads per sample (Sommeria-Klein et al., 2016), the  
344 number of sequences per sample was either rarefy to 1,000 or 5,000 counts before fitting the  
345 Sloan neutral community model (Sloan et al., 2007). Both rarefaction procedures did not  
346 significantly impact the estimated migration rate (**Table 2**). Hence the largest dataset (5,000  
347 counts per sample) was used in subsequent analyses. Overall, the estimated migration rate was  
348 more important for seed-associated fungal communities in comparison to seed-associated  
349 bacterial communities (**Table 2**). The goodness of fit of Sloan model was estimated for each  
350 plant generation by root-mean square error (RMSE, **Fig. 4**). Frequency of bacterial and fungal  
351 OTUs occurrence within local community was adequately described the neutral model for seed  
352 collected in 2014 and 2015 with  $RMSE < 0.1$  (**Fig. 4**). Larger differences between observed  
353 OTUs occurrence and predicted OTUs occurrence were reported for microbial communities  
354 associated to seed harvested in 2013 ( $RMSE = 0.12$  and  $0.14$  for bacterial and fungal OTU  
355 datasets, respectively). This increase in RMSE values could be explained by the limited number  
356 of samples employed ( $n = 9$ ) in 2013. In order to estimate the effect of dispersal and drift on  
357 local microbial community structure, we compared the fit of the Sloan model to the fit of a  
358 binomial distribution model. Binomial distribution model is used for assessing the importance  
359 of random sampling on community structure in absence of drift and dispersal limitation (Sloan  
360 *et al.*, 2007). According to Akaike information criterion (AIC), both models performed equally  
361 well (**Table 2**), therefore suggesting that dispersal, ecological drift and random sampling are  
362 involved in local assembly of seed-associated bacterial communities.

363 To identify microbial taxa that are potentially subjected to deterministic processes, we  
364 compared the observed OTU frequency to the predicted OTU frequency by Sloan model. OTUs  
365 that are found more frequently than estimated could represent microbial taxa that are positively

366 selected by the host or the environment, whereas OTUs that are less frequent than expected  
367 could represent taxa negatively selected. Using the 95% confidence interval of the Sloan neutral  
368 model we detected bacterial and fungal taxa that depart from the estimated neutral distributions  
369 (**Fig. 4**). Interestingly only a subset of microbial taxa consistently diverged from neutral  
370 distribution across the three plant generation (**Table 3**). A total of three bacterial OTUs related  
371 to the *Pseudomonas fluorescens* group were less frequent than expected by Sloan neutral model  
372 (**Table 3**), and could represent taxa that under negative selection. In contrast, one bacterial and  
373 five fungal OTUs possessed a higher observed frequency for each plant generation. These  
374 putative positively-selected taxa correspond to one bacterial endophyte affiliated to  
375 *Plantibacter flavus* and 5 OTUs related to the *Alternaria* genus (**Table 3**) that are not part of  
376 the radish seed core microbiota.

377 **Discussion**

378 Seeds are involved in the transmission of microorganisms from one plant generation to another  
379 and consequently may act as the initial inoculum source for the plant microbiota (Truyens et  
380 al., 2015; Barret et al., 2016). From an epidemiological point of view, seed transmission of  
381 phytopathogenic microorganisms represents an important mean of pathogen dispersion and is  
382 therefore significant in emergence of disease (Nelson et al., 2004). For these reasons, exploring  
383 (i) the transmission of seed-microbial communities across multiple generations and (ii) the  
384 processes involved in community assembly is of interest to control plant diseases (Shade et al.,  
385 2017).

386 In the present study, we investigated the structure of microbial communities associated  
387 to several seed samples collected on different plant individuals during three successive plant  
388 generations. Based on the molecular markers employed in this work, seed microbiota  
389 composition and structure were distinct between plant generations. These differences in  
390 community structure across successive generations may solely be due to annual fluctuations in  
391 local weather conditions. Indeed, it is well accepted that climatic changes influence the  
392 composition of soil and plant-associated microbial communities (Compant et al., 2010; Classen  
393 et al., 2015). Other variables such as the type of cultivated plants that surrounded the  
394 experimental plots could also be involved in the observed variation in community composition.  
395 Recent work indicates that emigration of epiphytic bacteria and fungi from plants surfaces are  
396 important local sources of airborne micro-organisms and therefore of the surrounding plants  
397 (Lympelopoulou et al., 2016). Although the same experimental plot was employed during the  
398 course of this study, cultivated plant species in the immediate vicinity of the radish plants were  
399 not controlled between the different years and could account for the observed diversity.

400 While generation-specific variation was observed within seed-associated microbial  
401 communities, highest variation was found between seed samples collected the same year on

402 different individual plants. As most diversity surveys of seed-associated microbial communities  
403 are performed on seed samples consisting of multiple plant entities (Barret et al., 2015; Links  
404 et al., 2014; Klaedtke et al., 2016), this source of variation is usually ignored. Overall only three  
405 bacterial OTUs and 19 fungal OTUs were detected in all seed samples collected in this work.  
406 These core OTUs are affiliated to bacterial (e.g. *Pantoea agglomerans* or *Pseudomonas*  
407 *viridiflava*) and fungal taxa (*Alternaria* or *Cladosporium*) frequently associated to seed of  
408 various plant species (Barret et al., 2015; Hodgson et al., 2014; Links et al., 2014; Rezki et al.,  
409 2016; Truyens et al., 2015).

410         The fluctuations in microbial community composition observed between seed samples  
411 collected on different plant individuals could be explained by neutral-based processes. The  
412 Sloan neutral model employed in this work was able to predict the distribution of microbial taxa  
413 across seed-associated microbial communities, suggesting that ecological drift and dispersal  
414 generate a significant amount of diversity. Given the small value of estimated migration rate  
415 for seed-associated bacterial communities ( $m = 0.04$ ), it seems that ecological drift is an  
416 important driver of bacterial community assembly. The importance of ecological drift in  
417 community assembly has been previously reported in communities (i) under weak host selection  
418 and (ii) where the vast majority of taxa are in low relative abundances (Nemergut *et al.*, 2013).  
419 These two features are frequently encountered in seed-associated bacterial communities.  
420 Indeed, the overall composition of seed-associated bacterial communities is not shaped by the  
421 host genotype (Barret et al., 2015; Klaedtke et al., 2016). Moreover, seed-associated microbial  
422 communities are composed of few abundant entities and a multitude of low-abundance  
423 members (Rezki et al., 2016) that might be prone to extinction at the local level.

424         Regarding fungal taxa, it seems that the estimated migration rate is responsible for the  
425 observed variation in structure of seed-associated fungal communities. This result is not in  
426 accordance with the biogeographical diversity observed for fungal communities, which has



427 been primarily related to dispersal limitation of these micro-organisms (Peay et al., 2016).  
428 However, at a finer spatial scale, such as the experimental plot employed over the course of this  
429 study, dispersal limitation should be rather limited and passive dispersion of spore-forming  
430 organisms by water or wind facilitated, therefore resulting in stochastic distribution. Owing to  
431 the observed composition of seed-associated fungal communities, the influence of ecological  
432 drift in community assembly is probably less plausible for fungal taxa. Indeed, seed-associated  
433 fungal communities are composed of multiple highly-abundant entities that are partly selected  
434 by the environment (Klaedtke et al., 2016).

435         Although the observed distribution of most microbial OTUs followed the estimated  
436 distribution of Sloan neutral model, some OTUs deviated clearly from the model. The taxa that  
437 are more widespread than expected could represent microbial entities that are positively  
438 selected by the host or the environment. Among those taxa, one bacterial OTU related to  
439 *Plantibacter flavus*, a bacterial endophyte of several plant species (Lumactud et al., 2016), and  
440 5 fungal OTUs related to the *Alternaria* genus were consistently overrepresented in every plant  
441 generations. In addition to these taxa, the core members of the radish seed microbiota detected  
442 in every seed samples over the three plant generations metacommunity are probably indicative  
443 of non-neutral processes. This is especially true for highly abundant OTUs such as *Pantoea*  
444 *agglomerans* that represents on average more than 50% of all reads or to a lesser extent for  
445 fungal OTUs related to *Cladosporium* and *Alternaria*.

446         In conclusion, the present work highlighted a low heritability of the seed microbiota  
447 during successive plant generations. This low level of heritability could be explained in part by  
448 the importance of neutral based processes in community assembly. Neutral processes related to  
449 ecological drift might be important for the structure of seed-associated bacterial communities,  
450 while dispersion is potentially involved in assembly of the fungal fraction of the seed  
451 microbiota. These findings are important for developing biocontrol-based strategies. Indeed, if

452 neutral processes are the main drivers of community assembly, then one might expect that early-  
453 arriving taxa would prevent or limit colonization of late-arriving ones, especially if resource  
454 use between these taxa greatly overlaps. Therefore, inoculation of plant at flowering stage with  
455 micro-organism having a high overlap in resource use with the phytopathogenic agent(s) is  
456 likely to restrict seed transmission of the plant pathogen. The efficiency of this strategy will be  
457 of course strongly linked to the competitive potential of the selected bacterial strains.

458

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467

468 **Conflict of interest**

469 Matthieu Barret and Philippe Simoneau are Guest Editors of the special issue. This does not,  
470 however, interfere with the reviewing process.

471

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758 **Table & Figure legends**

759

760 **Figure 1: Diversity of seed-associated microbial communities**

761 Richness (“Observed”, A, B) and diversity (“InvSimpson”, C, D) were estimated for bacterial  
762 (A, C) and fungal communities (B, D) with *gyrB* and ITS1 sequences, respectively. Richness  
763 and evenness were assessed with the number of OTUs rarefied to 5,000 sequences per sample  
764 and Simpson’s reciprocal index. Each dot correspond to a seed sample collected in 2013 (red),  
765 2014 (green) or 2015 (blue). Letters “a” and “b” denote significant changes between conditions  
766 considered at a p-value  $\leq 0.01$  (as assessed by ANOVA with post hoc Tukey’s HSD test).

767

768

769 **Figure 2: Microbial community similarities between seed samples harvested from three**  
770 **different generations**

771 Principal coordinate analysis (PCoA) ordination of Jaccard (A and C) and Bray-Curtis (B and  
772 D) indices calculated with ITS1 (A and B) and *gyrB* sequences (C and D). Each dot represents  
773 a microbial community associated with seeds harvested in 2013 (red), 2014 (green) and 2015  
774 (blue).

775

776 **Figure 3: Variation in relative abundance of microbial communities**

777 Relative abundance of the most prevalent 5 bacterial and fungal genera within seed samples  
778 collected from individual plants across 3 successive generations (2013, 2014 and 2015)  
779 according to *gyrB* (A) and ITS1 (B) sequences. Taxonomic affiliation of bacterial and fungal  
780 OTUs was performed with an in-house *gyrB* database and the UNITE database, respectively.  
781 Unknown taxa represent OUT that are not affiliated at the genus level.

782

783 **Figure 4: Fit of the Sloan neutral model**

784 Frequency and abundance of bacterial (A, B, C) and fungal (D, E, F) OTUs in seed samples  
785 collected in 2013 (A, D), 2014 (B, E) and 2015 (C, F). Dashed lines represent 95% confidence  
786 intervals around the neutral community model prediction. OTUs that occurred more and less  
787 frequently than predicted by the neutral model are displayed in red and blue, respectively.

788

789 **Table 1: Frequency and relative abundance of the core members of the seed microbiota**

OTU	Median relative abundance (%)	Taxonomic affiliation
<b>Bacterial OTUs</b>		
Otu000001	57.88	<i>Pantoea agglomerans</i>
Otu000002	8.71	<i>Pseudomonas viridiflava</i>
Otu000004	4.10	<i>Erwinia tasmaniensis</i>
<b>Fungal OTUs</b>		
OTU00001	29.35	<i>Alternaria</i>
OTU00002	24.98	<i>Cladosporium</i>
OTU00003	13.49	<i>Alternaria</i>
OTU00004	2.77	<i>Alternaria</i>
OTU00005	2.28	<i>Mycosphaerella</i>
OTU00006	2.03	<i>Filobasidiales</i>
OTU00007	1.95	<i>Alternaria</i>
OTU00008	1.91	<i>Alternaria</i>
OTU00012	1.34	<i>Alternaria</i>
OTU00009	1.16	<i>Alternaria</i>
OTU00010	1.11	<i>Stemphylium</i>
OTU00015	1.06	<i>Alternaria</i>
OTU00013	1.05	<i>Alternaria</i>
OTU00014	0.83	<i>Alternaria</i>
OTU00019	0.47	<i>Alternaria</i>
OTU00021	0.41	<i>Filobasidium</i>
OTU00023	0.34	<i>Alternaria</i>
OTU00033	0.24	<i>Alternaria</i>
OTU00036	0.21	<i>Alternaria</i>

790

791 The core members of the seed microbiota were assessed with *gyrB* and ITS1 sequences for

792 bacterial and fungal entities, respectively.

793

794

**Table 2: Fitting the Sloan neutral model to the radish seed microbiota**

Datasets	Samples	N	OTUs	m	RMSE Sloan	RMSE binomial	AIC Sloan	AIC binomial
ITS1_2013_1000	9	1000	236	$0.83 \pm 0.17$	0.13	0.08	-292	-508
ITS1_2013_5000	9	5000	712	$0.78 + 0.10$	0.12	0.07	-977	-1840
ITS1_2014_1000	32	1000	501	$0.47 + 0.08$	0.09	0.09	-954	-974
ITS1_2014_5000	32	5000	1434	$0.41 + 0.05$	0.08	0.09	-3136	-3071
ITS1_2015_1000	32	1000	650	$0.79 + 0.09$	0.07	0.05	-1696	-1982
ITS1_2015_5000	32	5000	2169	$0.75 + 0.07$	0.06	0.05	-5801	-6942
gyrB_2013_1000	9	1000	232	$0.18 + 0.07$	0.17	0.20	-169	-141
gyrB_2013_5000	9	5000	640	$0.12 + 0.02$	0.14	0.20	-666	-384
gyrB_2014_1000	32	1000	633	$0.03 + 0.01$	0.08	0.14	-1342	-780
gyrB_2014_5000	31	5000	1906	$0.04 + 0.01$	0.07	0.13	-4440	-2575
gyrB_2015_1000	32	1000	1044	$0.02 + 0.01$	0.07	0.12	-2664	-1545
gyrB_2015_5000	32	5000	3813	$0.04 + 0.01$	0.06	0.10	-10118	-7147

795

796



797 **Table 3: OTUs that deviated from the Sloan model frequency**

798

OTUs	Kingdom	Taxonomic affiliation	2013-2014- 2015
Otu000015	Bacteria	<i>Pseudomonas trivialis</i>	-1
Otu000018	Bacteria	<i>Pseudomonas fluorescens</i>	-1
Otu000019	Bacteria	<i>Pseudomonas fluorescens</i>	-1
Otu000074	Bacteria	<i>Plantibacter flavus</i>	+1
OTU00112	Fungi	<i>Alternaria alternata</i>	+1
OTU00131	Fungi	<i>Alternaria infectoria</i>	+1
OTU00133	Fungi	<i>Alternaria infectoria</i>	+1
OTU00148	Fungi	<i>Alternaria alternata</i>	+1
OTU00156	Fungi	<i>Alternaria alternata</i>	+1

799

800 OTUs with a lower (-1) and higher (+1) frequency than expected by the Sloan neutral are  
 801 indicated. All reported values were consistent across years.

Figure 1

[Click here to download colour figure Fig1.eps](#)

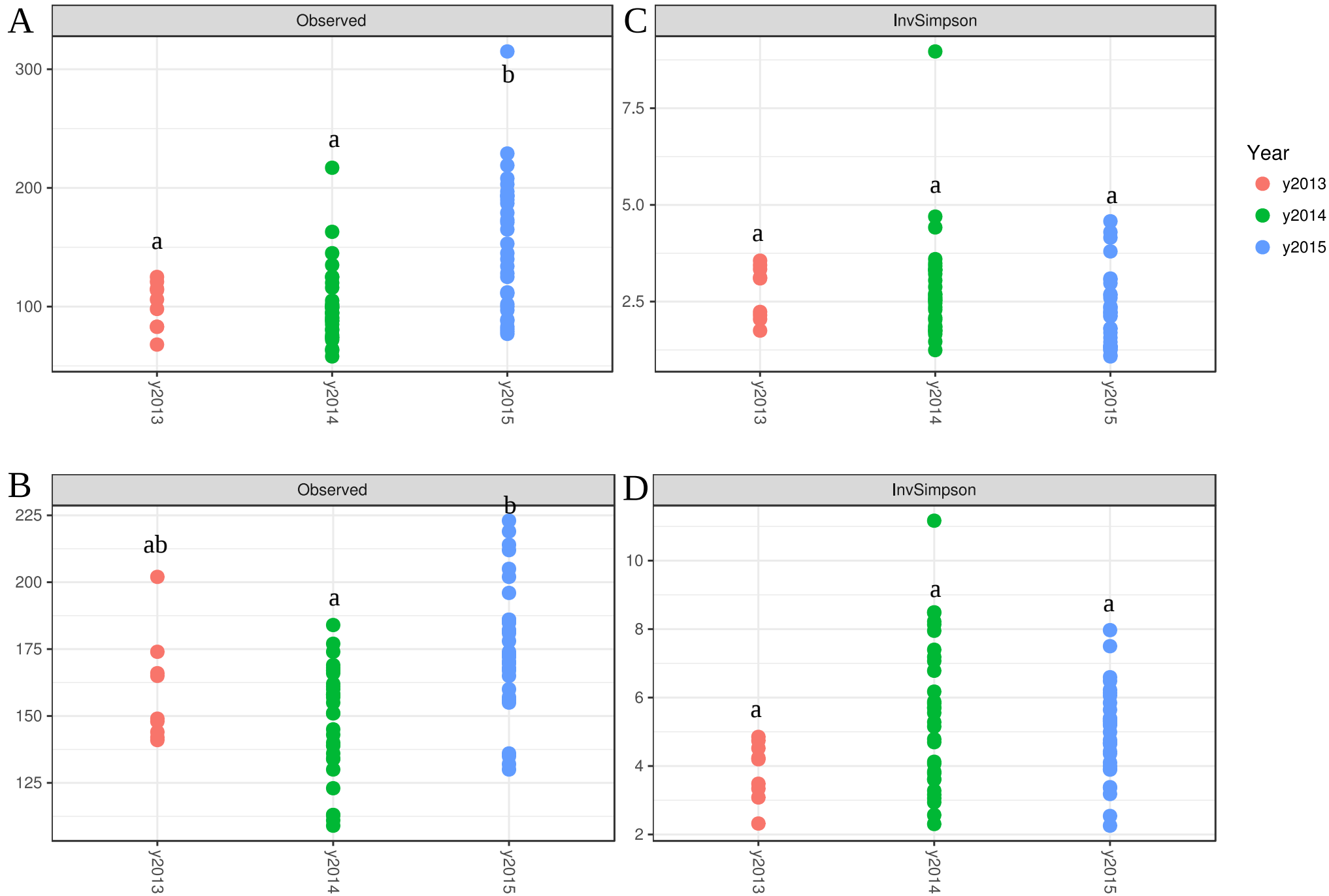


Figure 2

Year  
y2013  
y2014  
y2015

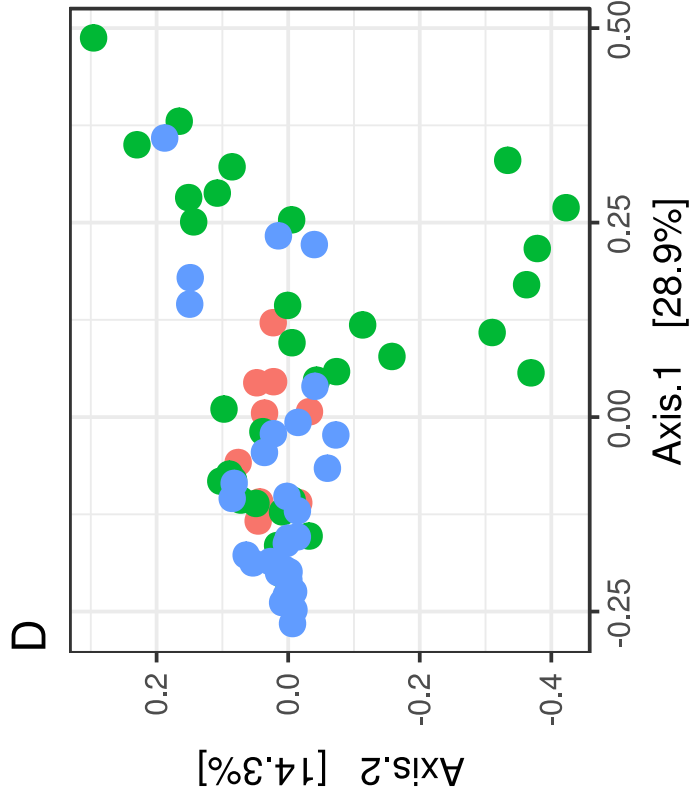
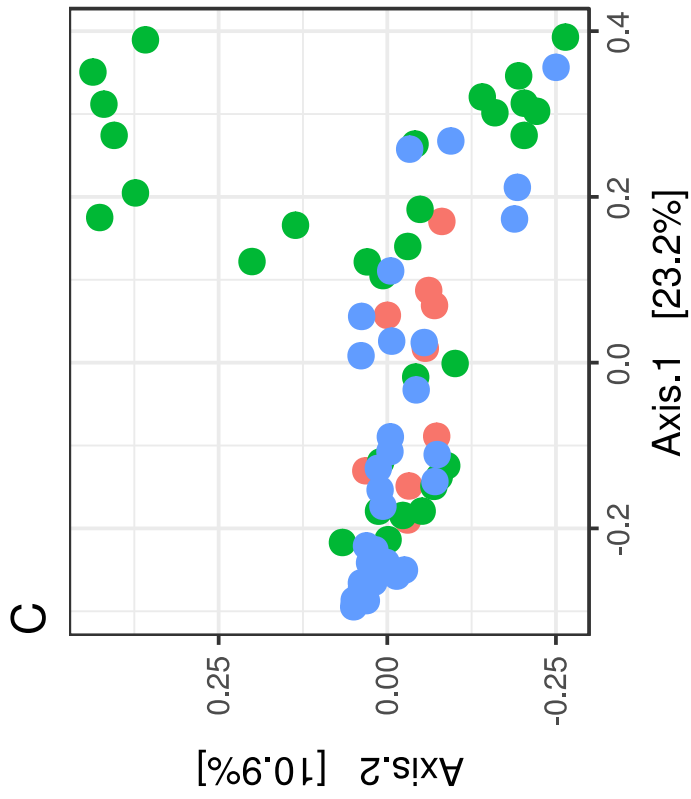
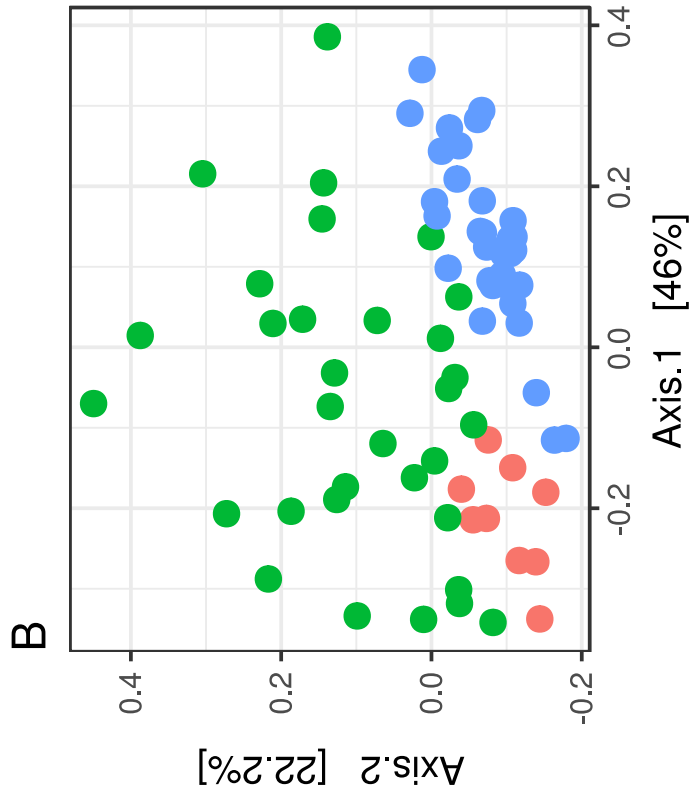
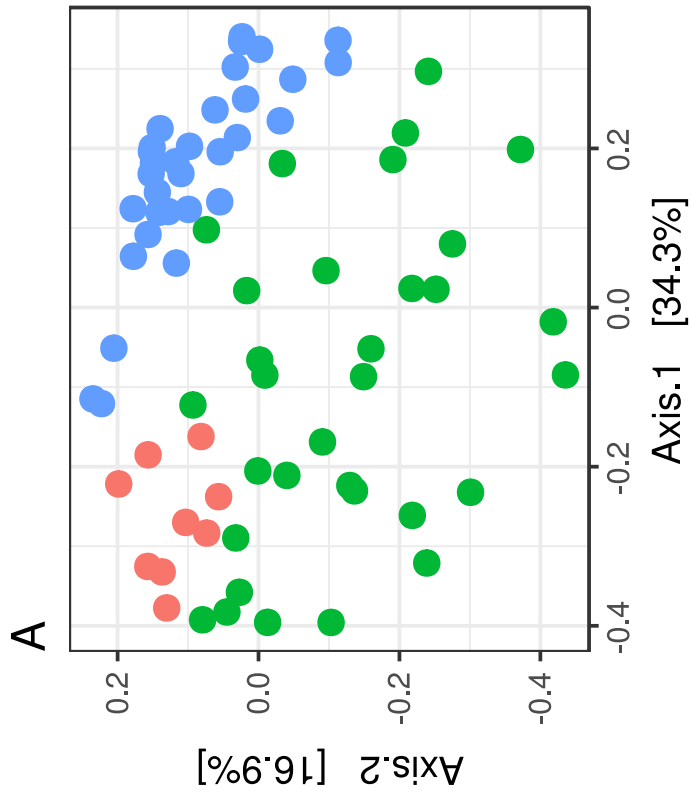
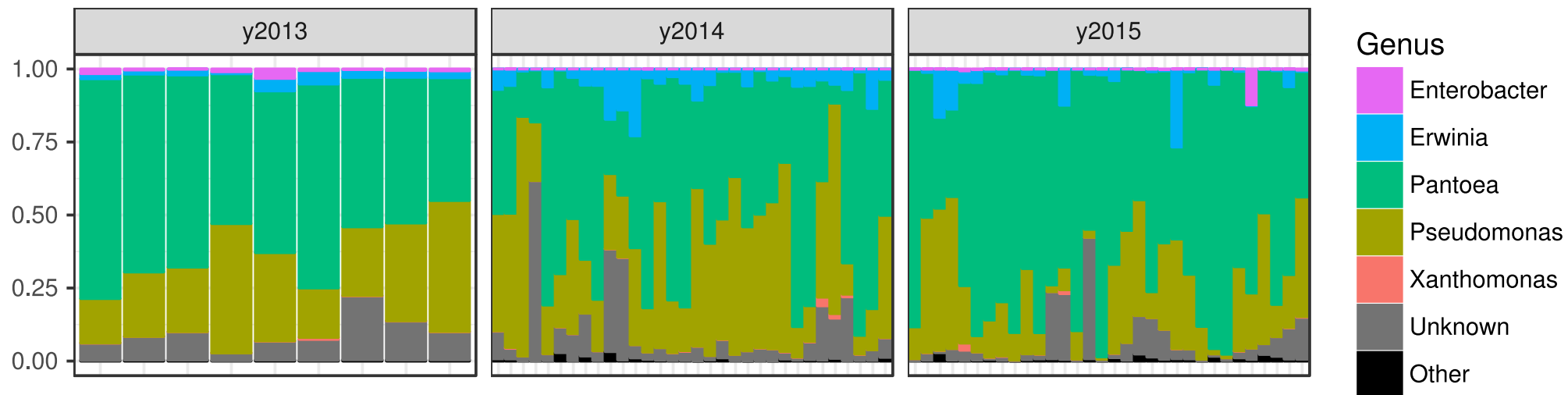


Figure 3

A



B

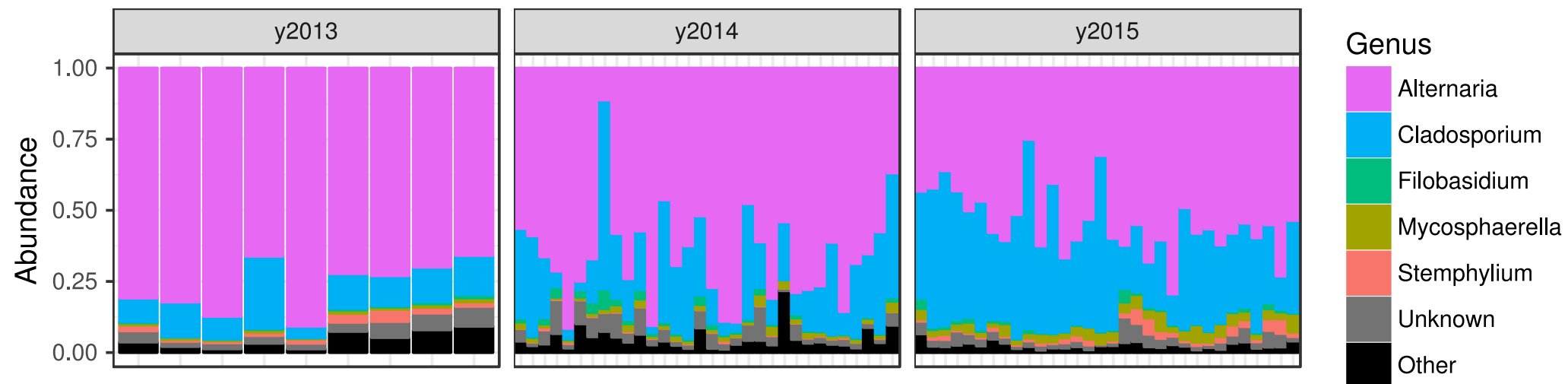
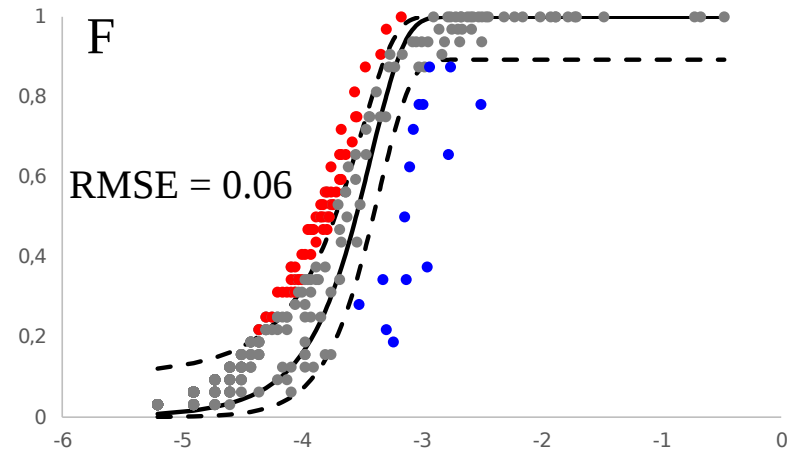
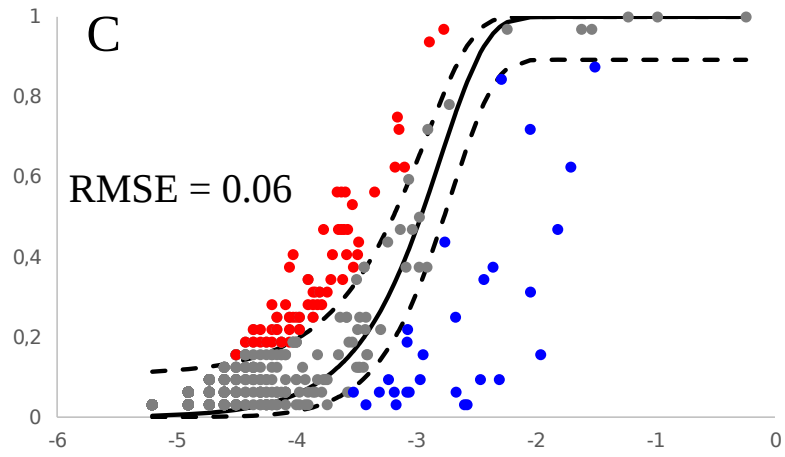
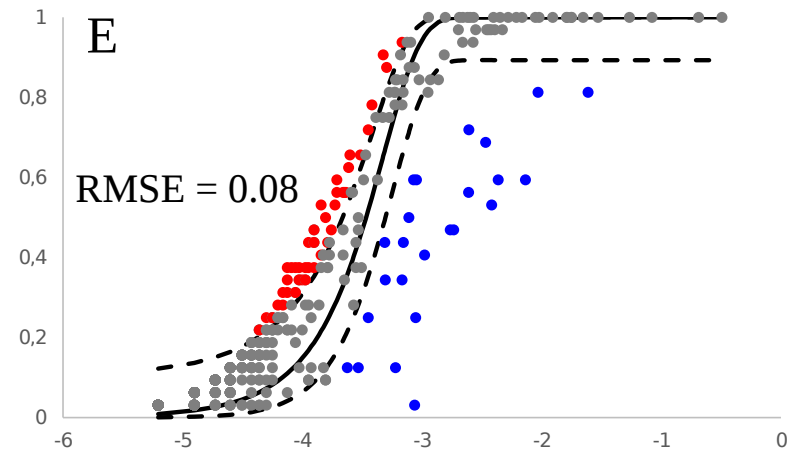
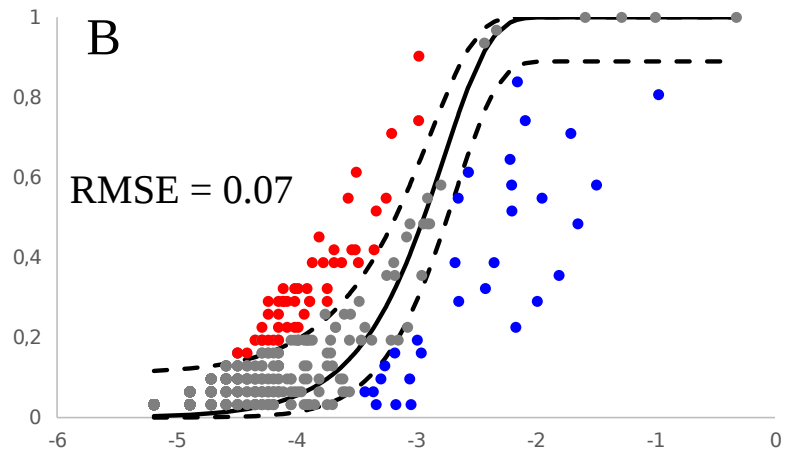
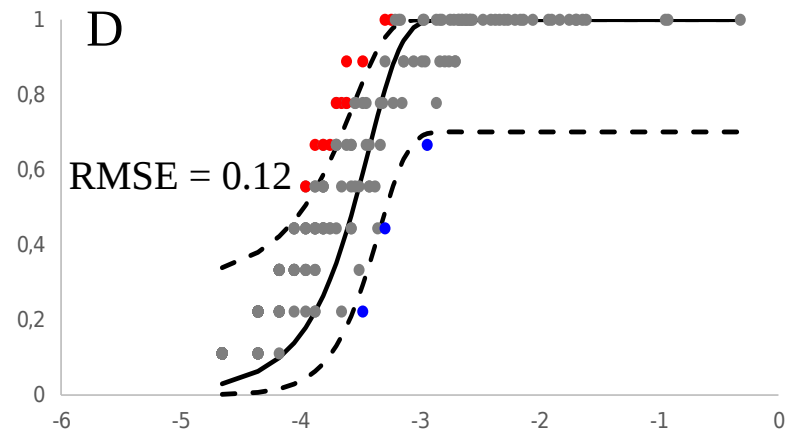
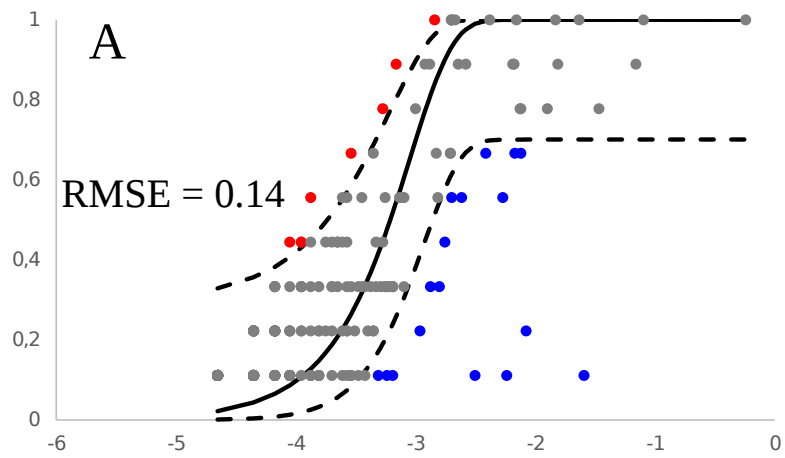


Figure 4

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1 **Supplemental Information**

2 Article title: **Assembly of the seed microbiota within and across successive plant**  
3 **generations**

4 Authors: Samir Rezki, Claire Campion, Philippe Simoneau, Marie-Agnès Jacques, Ashley  
5 Shade and Matthieu Barret

6

7

8 **Figure S1: Diversity of microbial communities associated to seed samples collected**  
9 **manually and mechanically**

10 Richness (A and B) and evenness (C and D) were estimated for bacterial (A and C) and fungal  
11 communities (B and D) with *gyrB* and ITS1 sequences, respectively. Richness and evenness  
12 are assessed with the number of OTUs and Inverse-Simpson index. Each dot correspond to a  
13 seed sample collected in 2013 (red), 2014 (green) or 2015 (blue). The asterisk denotes  
14 significant changes between conditions considered at a p-value  $\leq 0.01$  (as assessed by ANOVA  
15 with post hoc Tukey's HSD test).

16

17 **Figure S2: Differences in OTU relative abundance between plant generations**

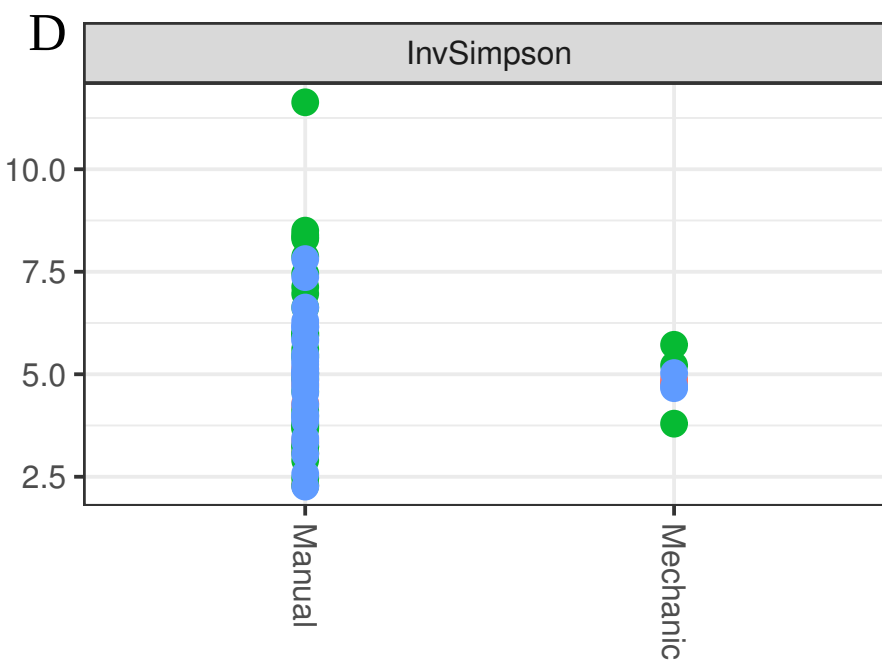
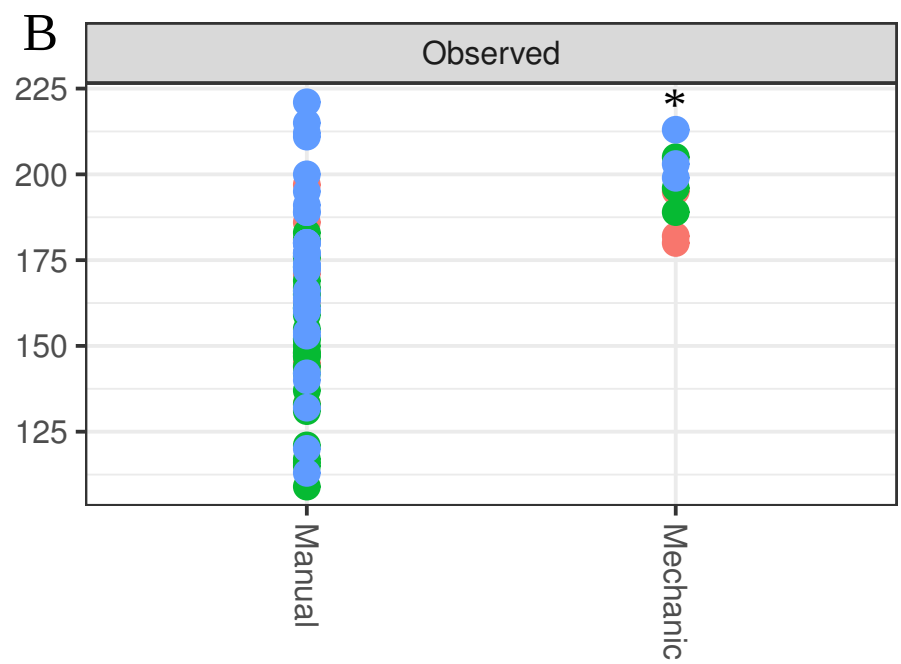
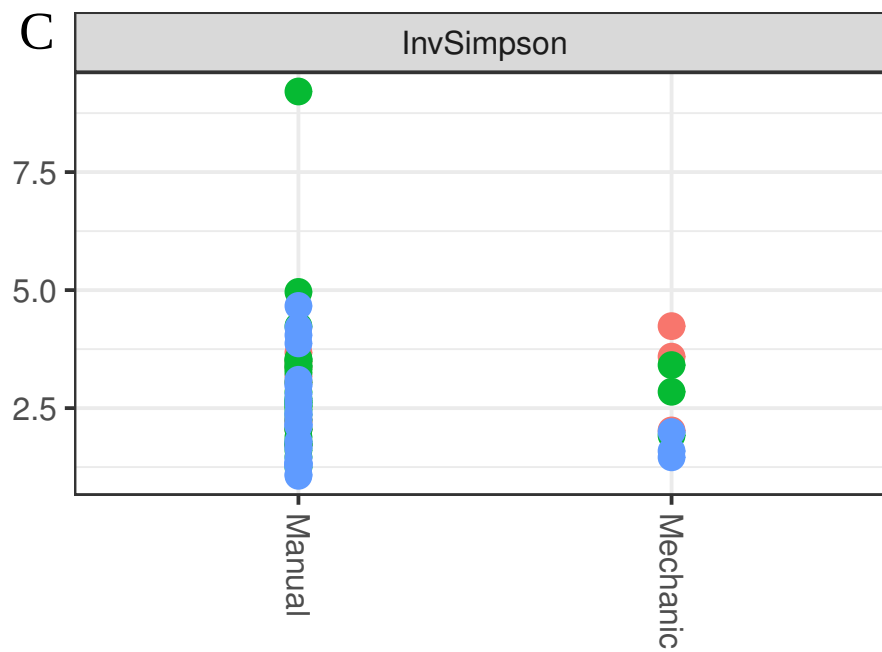
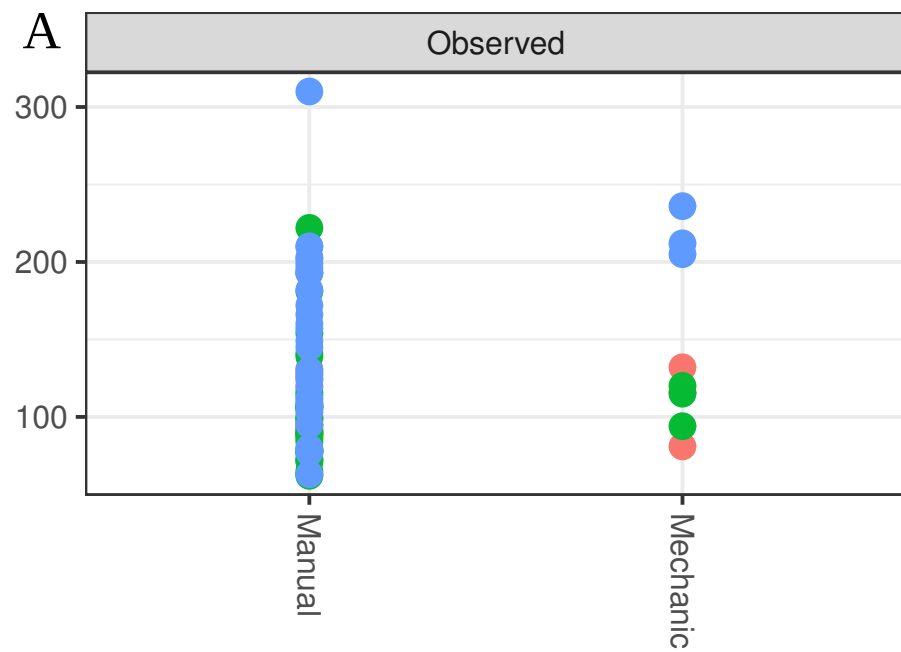
18 Bacterial (A, C and E) and fungal (B, D and F) OTUs differentially enriched in the following  
19 contrasts: 2013 vs 2014 (A and B); 2013 vs 2015 (C and D) and 2014 vs 2015 (E and F). Only  
20 OTUs with  $\log_2FC > |2|$  and corrected pvalue  $< 0.01$  are displayed.

21

22 **Figure S3: Relative abundance of bacterial and fungal OTUs**

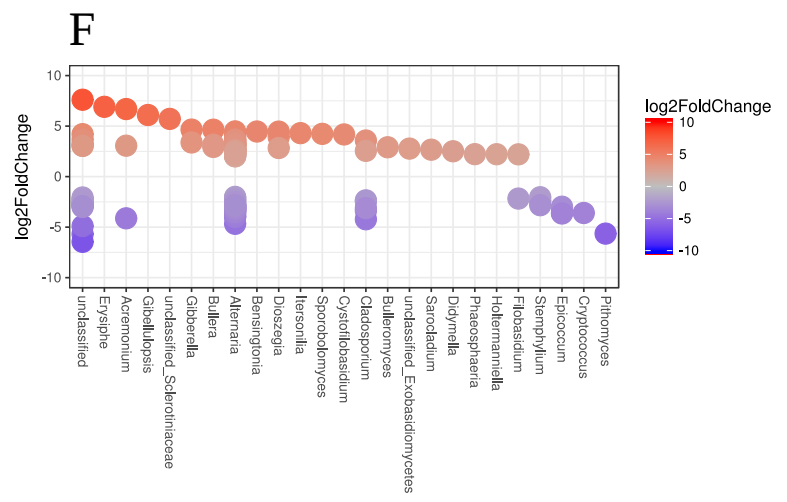
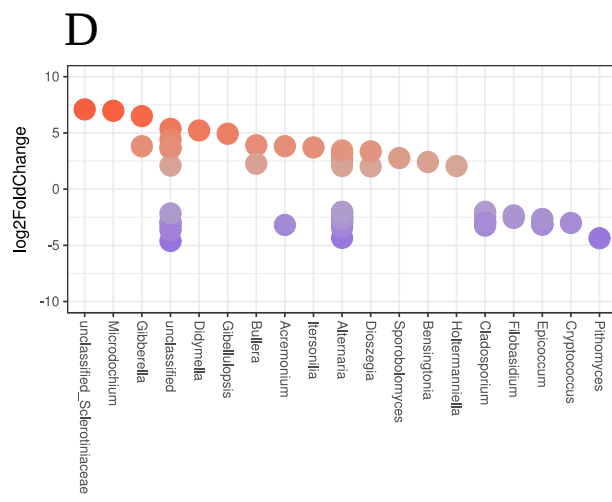
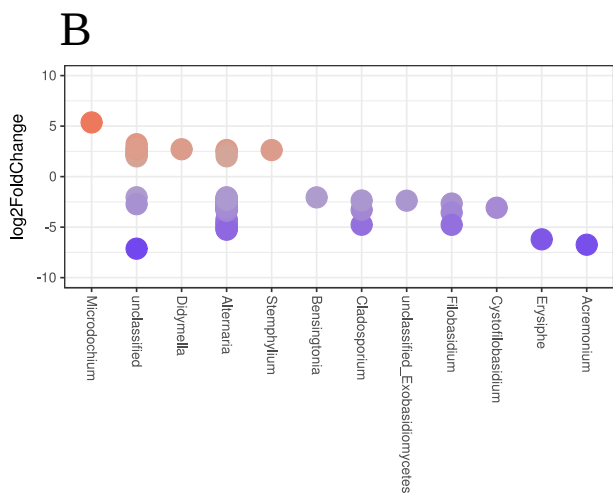
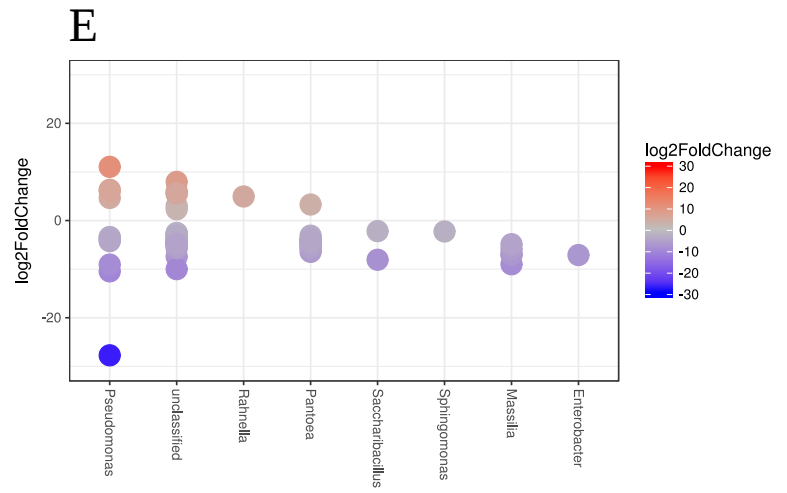
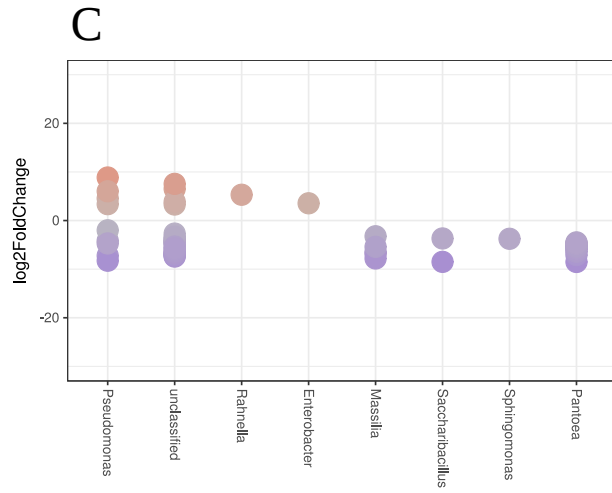
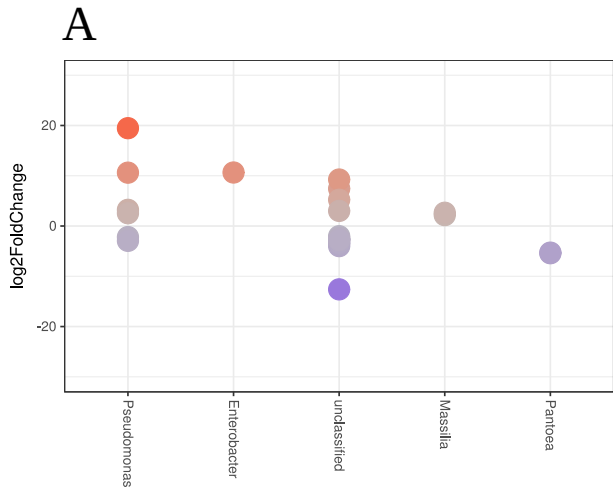
23 Mean relative abundance of the top 5 bacterial (A) and fungal OTUs (B) in seed samples  
24 collected in 2013, 2014 and 2015.

25



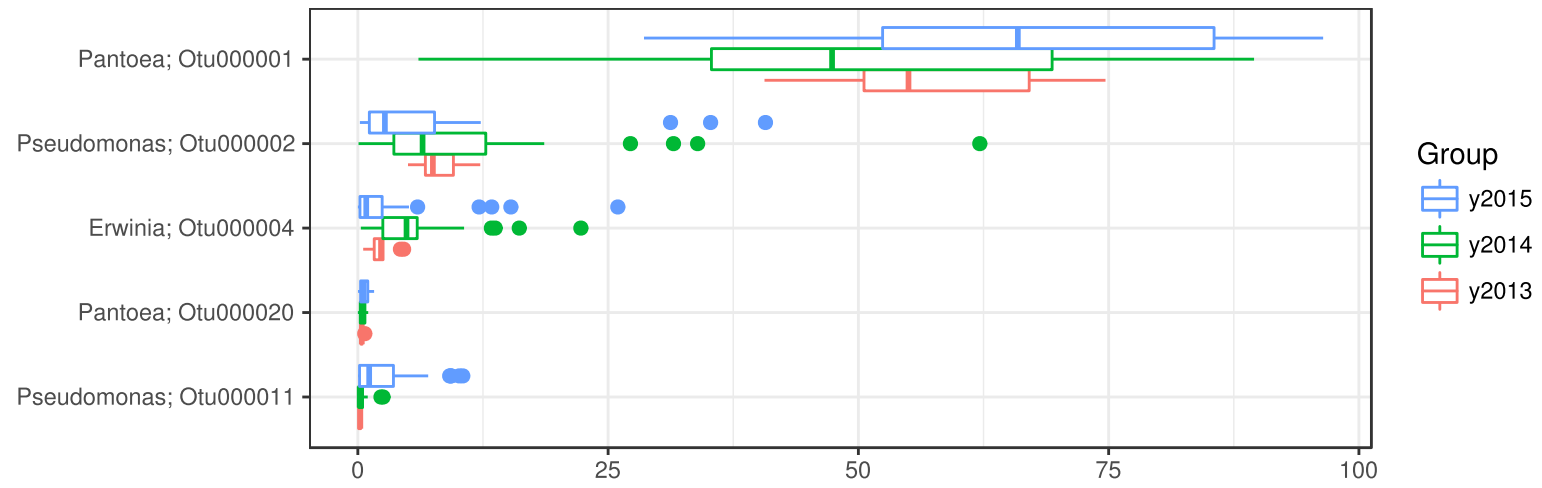
Year

- y2013
- y2014
- y2015





A



B

