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Plant and Soil

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

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| Abstract: | <p>Background and Aims 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>Methods 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>Results 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>Conclusions 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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06/06/2017

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**
2 **plant generations**

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21 **Keywords:** seed-associated microbial community; heritability; community assembly;
22 dispersion; diversification

23

24 **Abstract**

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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.

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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 β 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 (10th 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 align (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,
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471

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756 73: 5261-5267. doi: 10.1128/AEM.00062-07.
757

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 ≤ 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 |
|-----------------------|-------------------------------|--------------------------------|
| Bacterial OTUs | | |
| Otu000001 | 57.88 | <i>Pantoea agglomerans</i> |
| Otu000002 | 8.71 | <i>Pseudomonas viridiflava</i> |
| Otu000004 | 4.10 | <i>Erwinia tasmaniensis</i> |
| Fungal OTUs | | |
| 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 ± 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

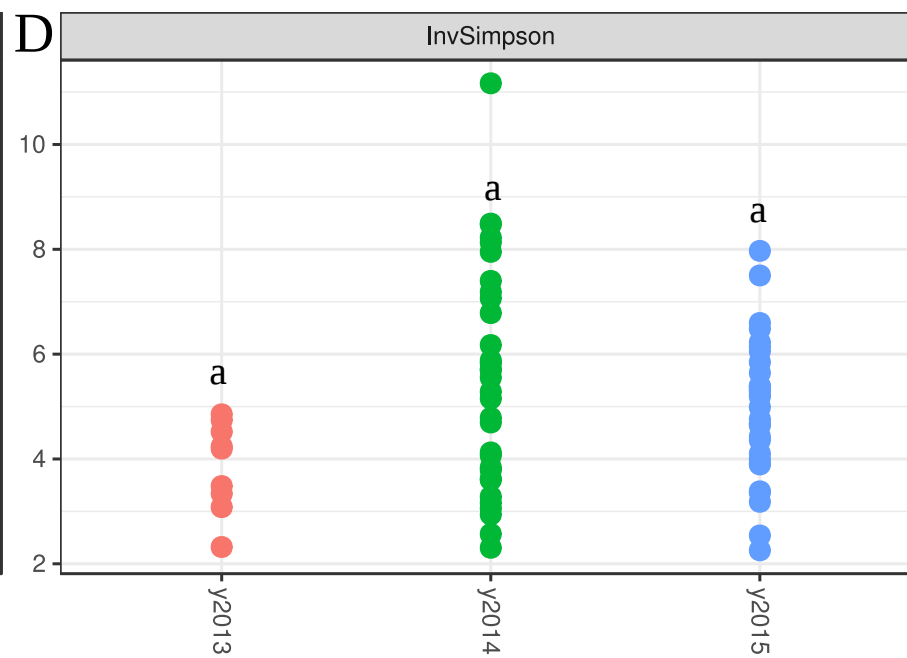
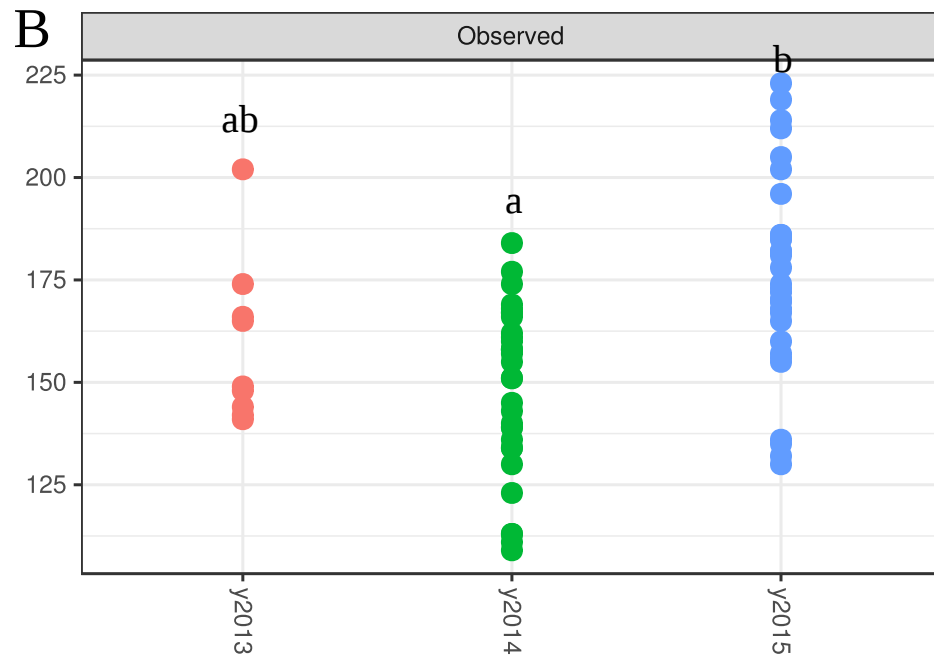
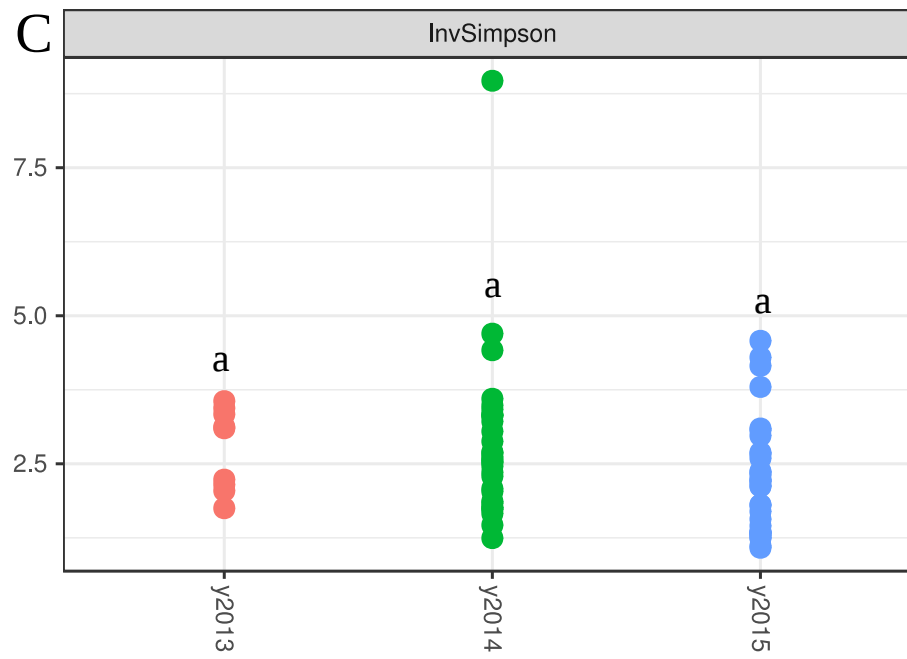
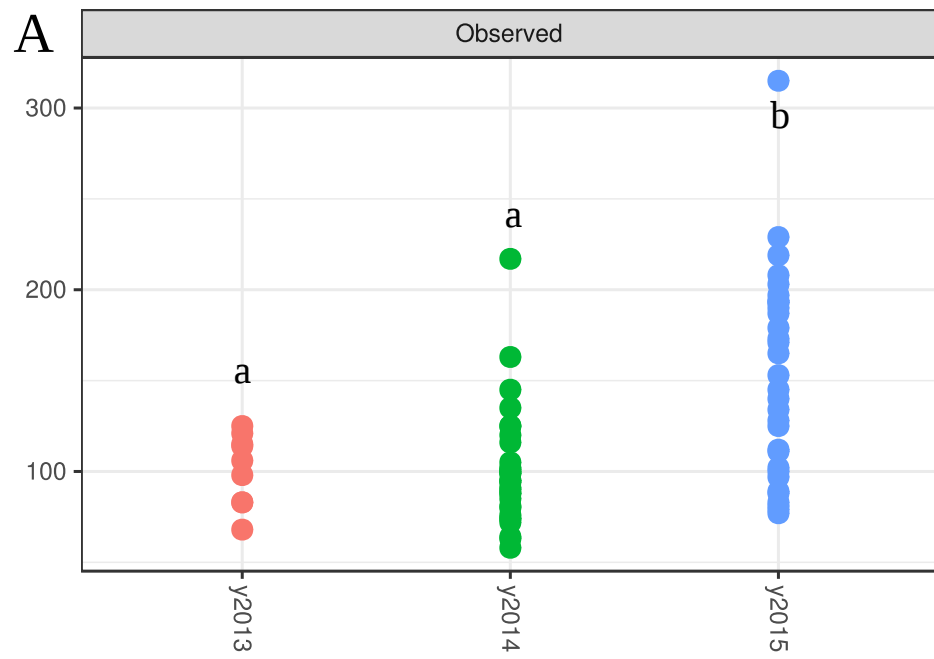


Figure 2

Year
y2013
y2014
y2015

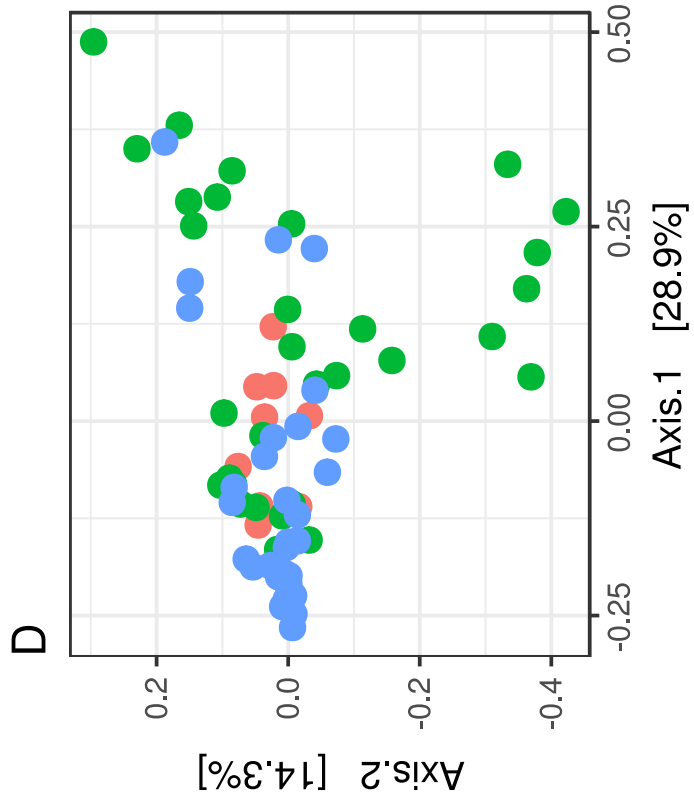
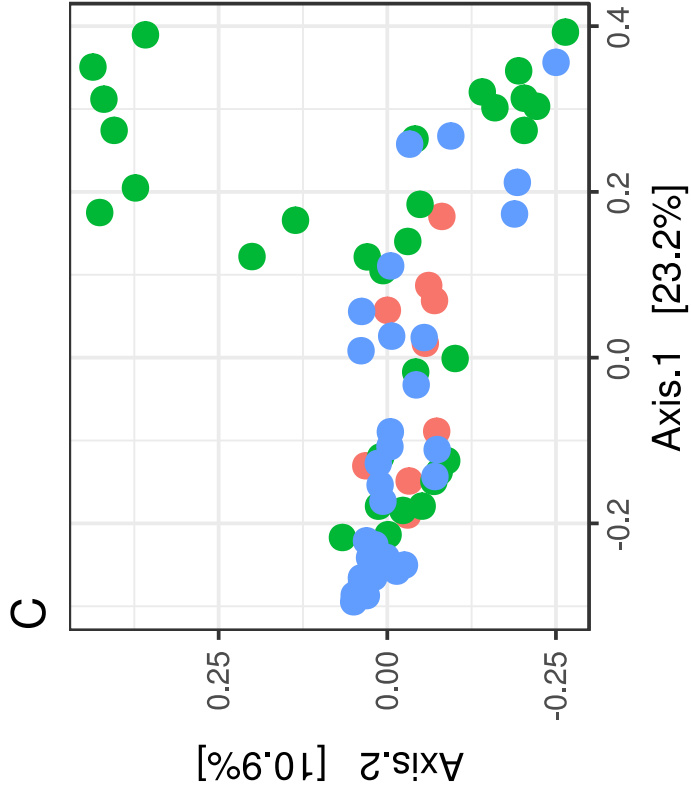
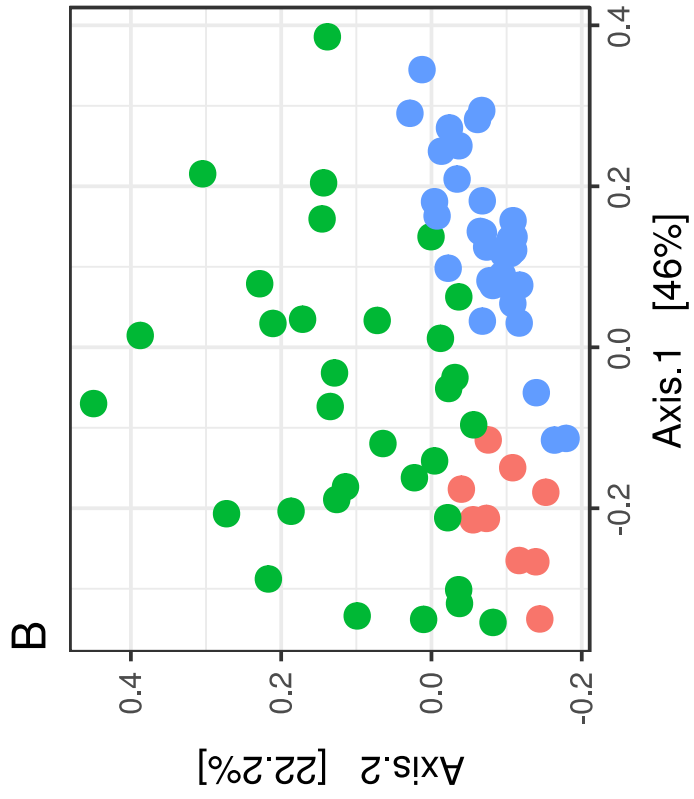
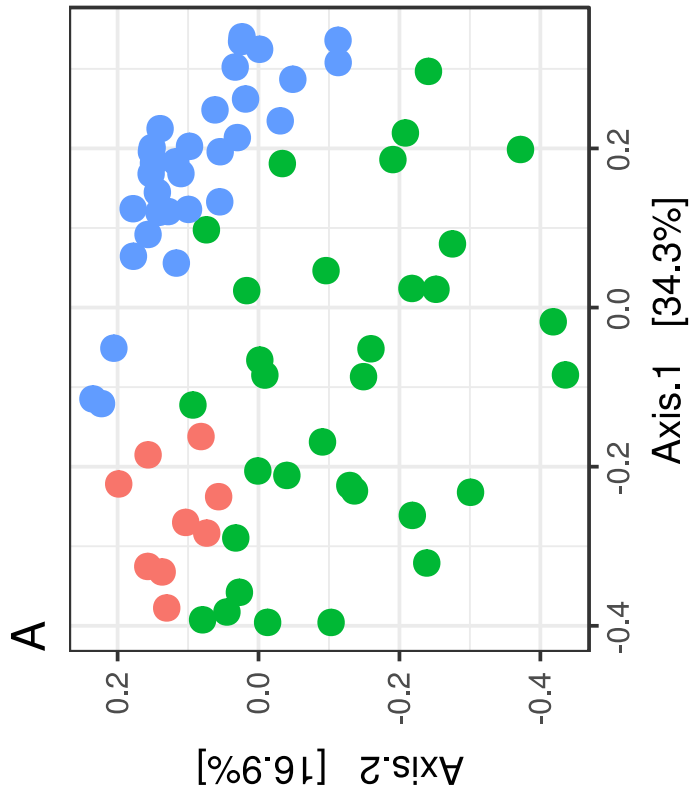
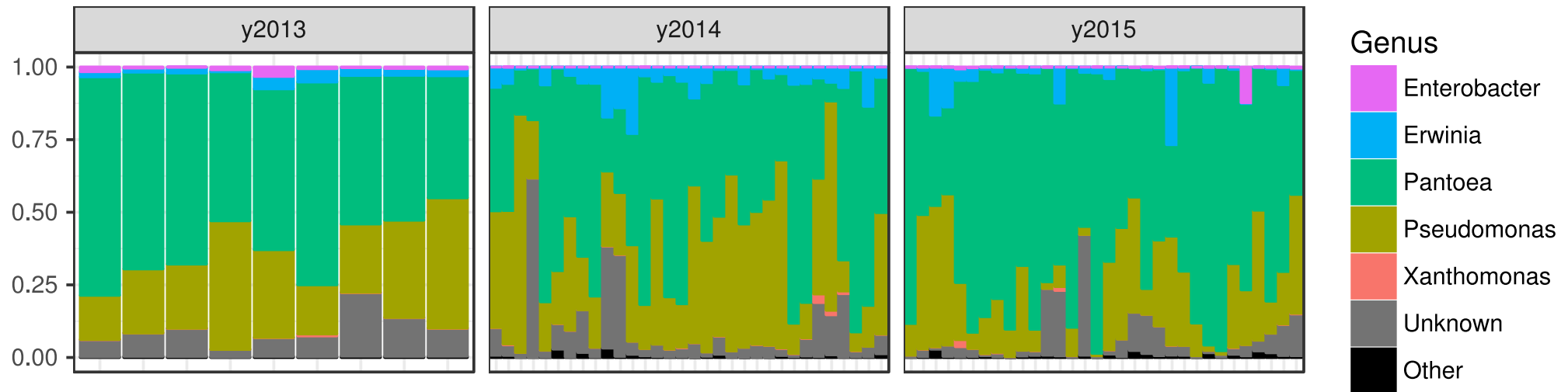


Figure 3

A



B

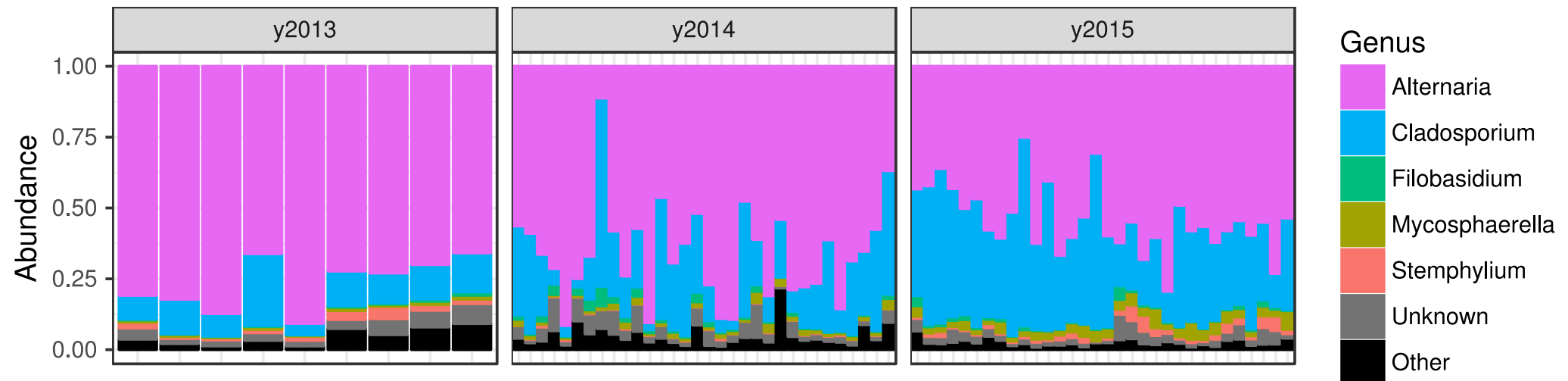
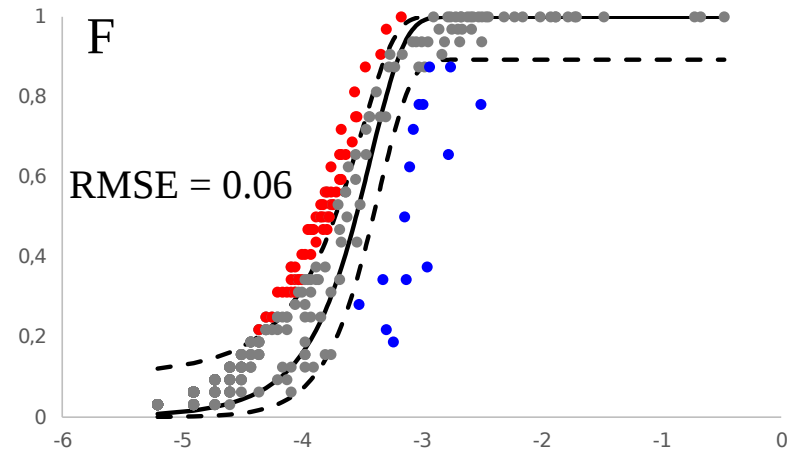
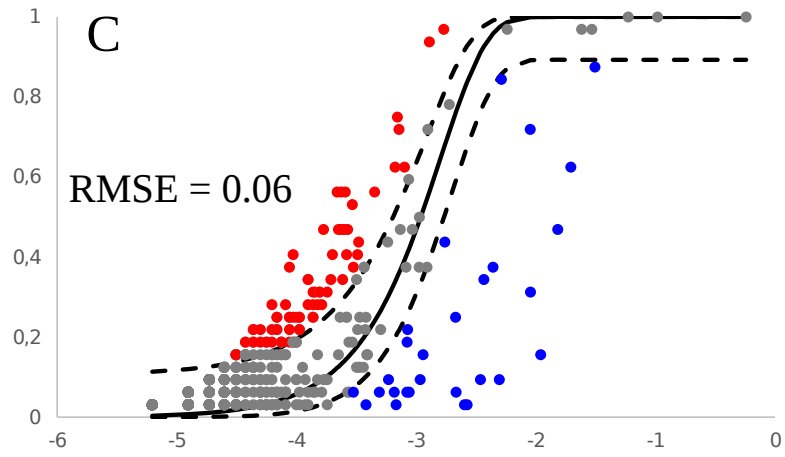
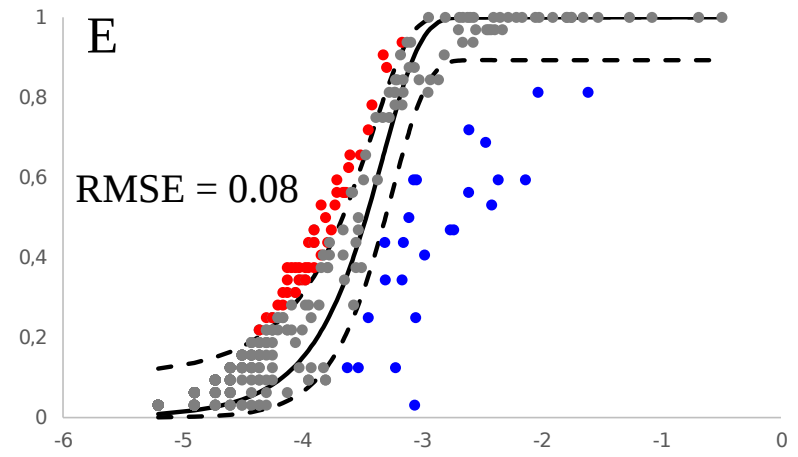
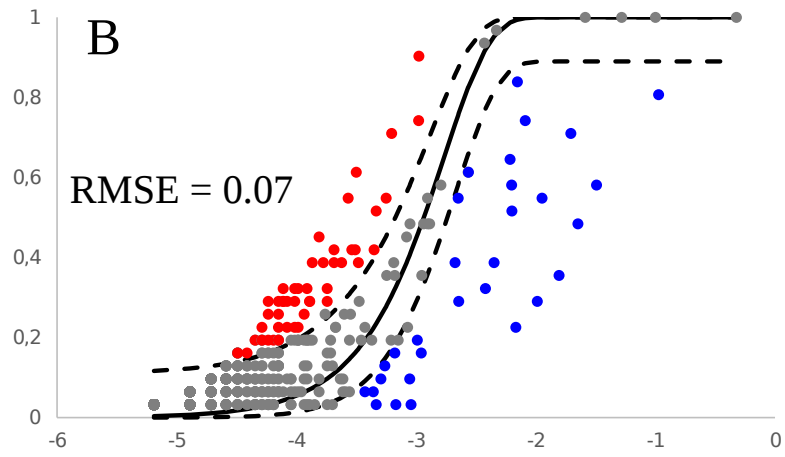
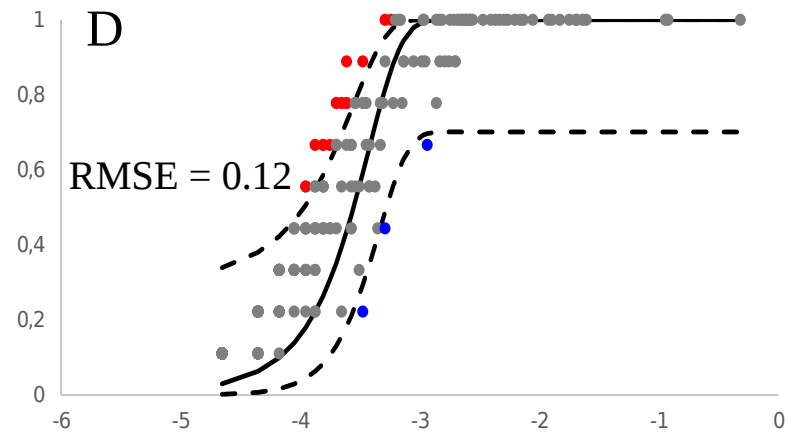
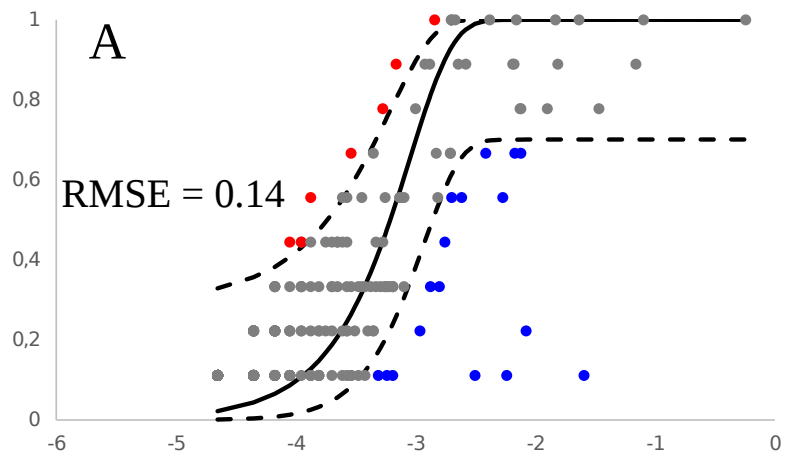


Figure 4

[Click here to download colour figure Fig4.eps](#)



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 ≤ 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**

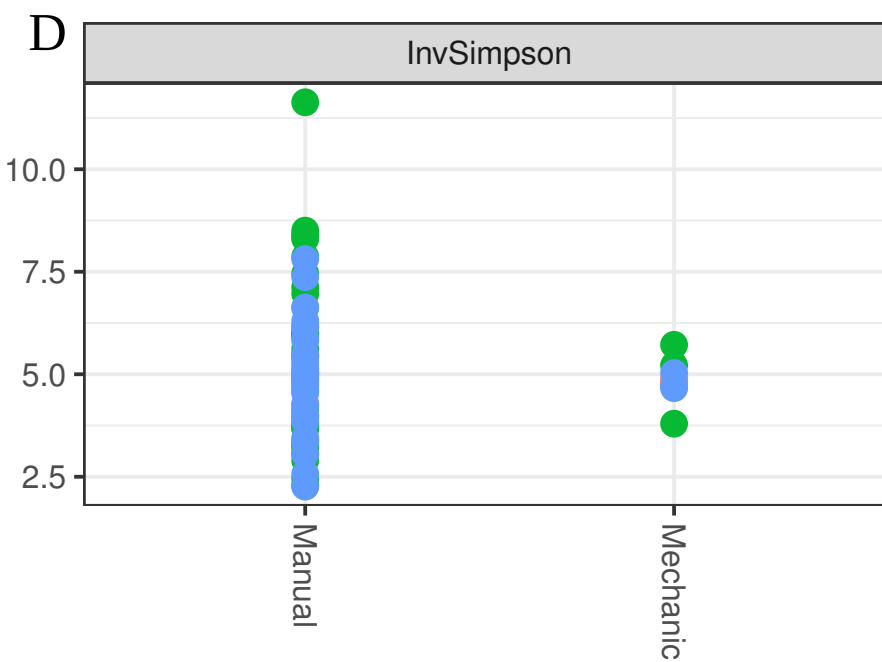
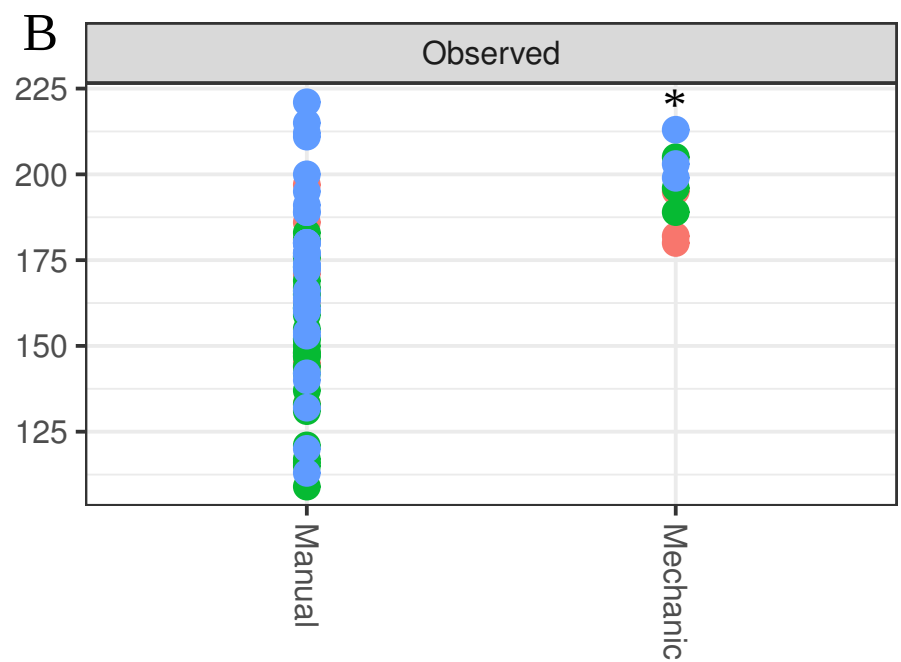
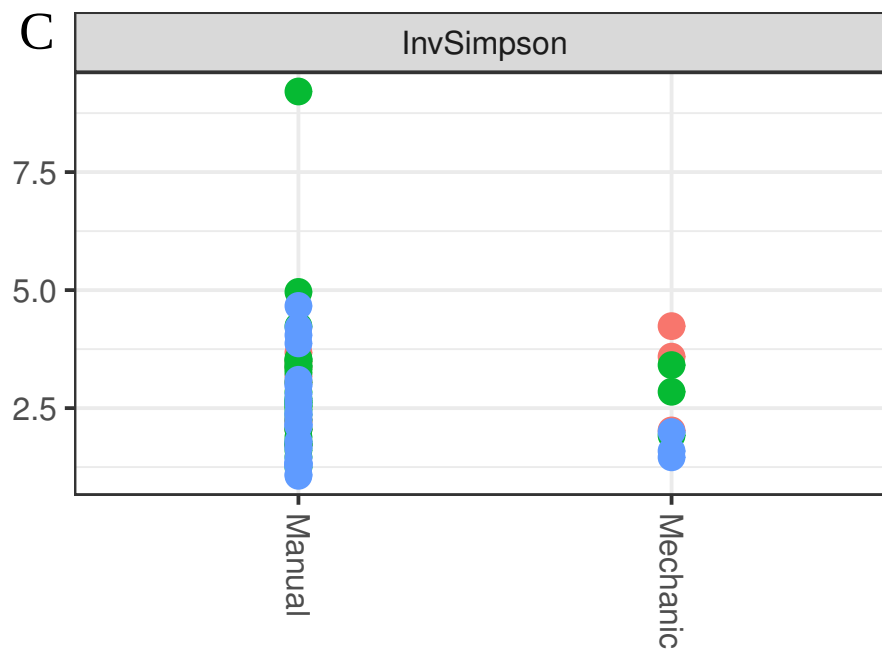
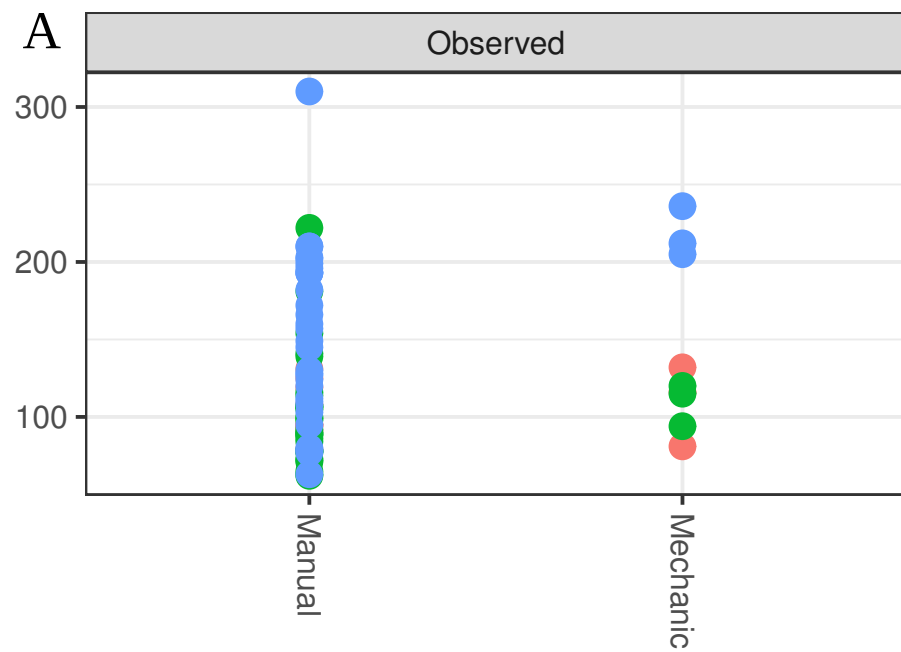
18 Bacterial (A, C and E) and fungal (B, D and F) OTUs differentially enriched in the following
19 contrasts: 2013 vs 2014 (A and B); 2013 vs 2015 (C and D) and 2014 vs 2015 (E and F). Only
20 OTUs with $\log_2FC > |2|$ and corrected pvalue < 0.01 are displayed.

21

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

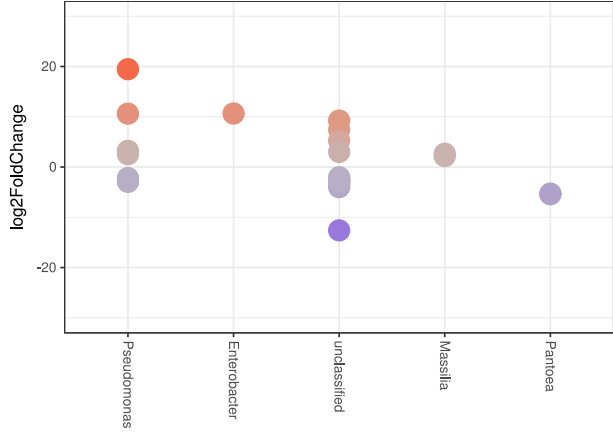
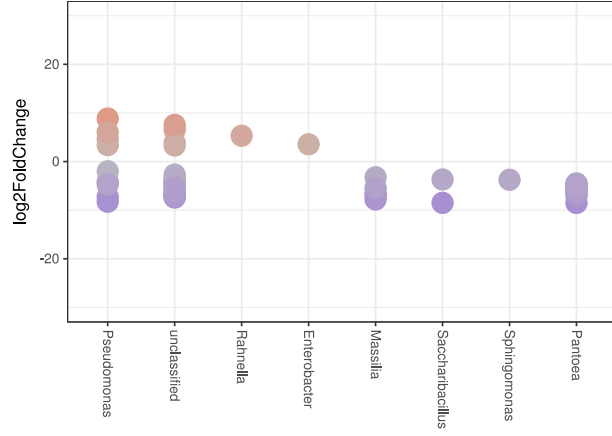
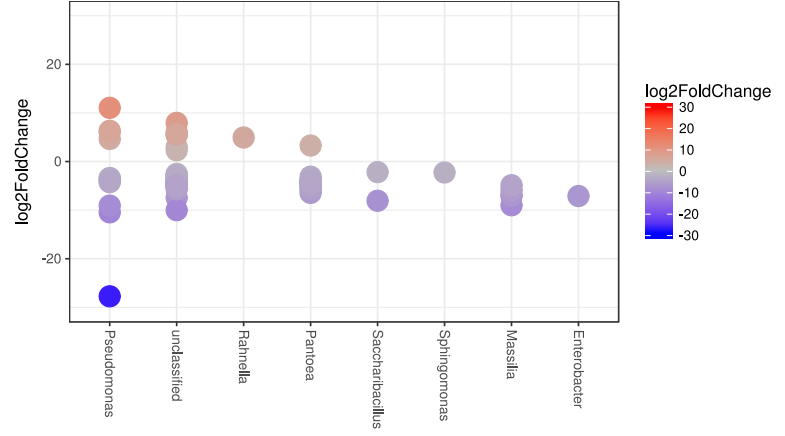
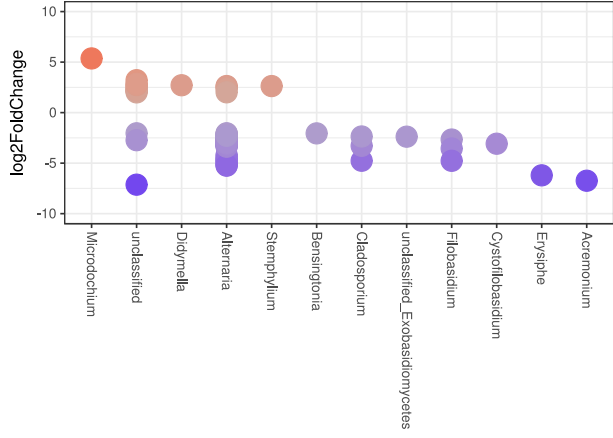
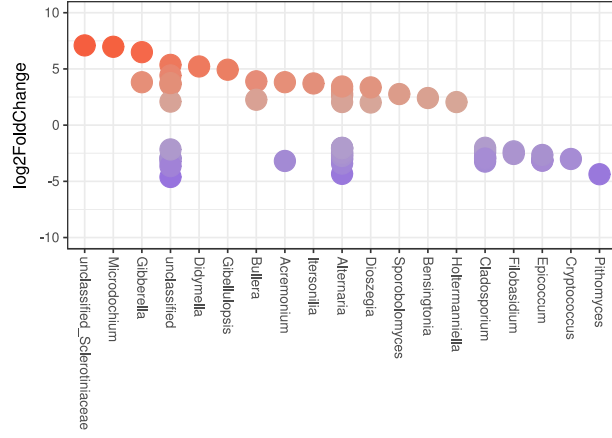
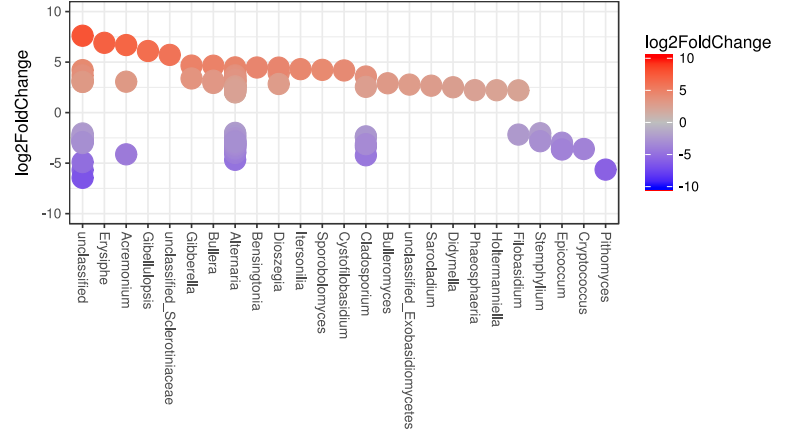
23 Mean relative abundance of the top 5 bacterial (A) and fungal OTUs (B) in seed samples
24 collected in 2013, 2014 and 2015.

25

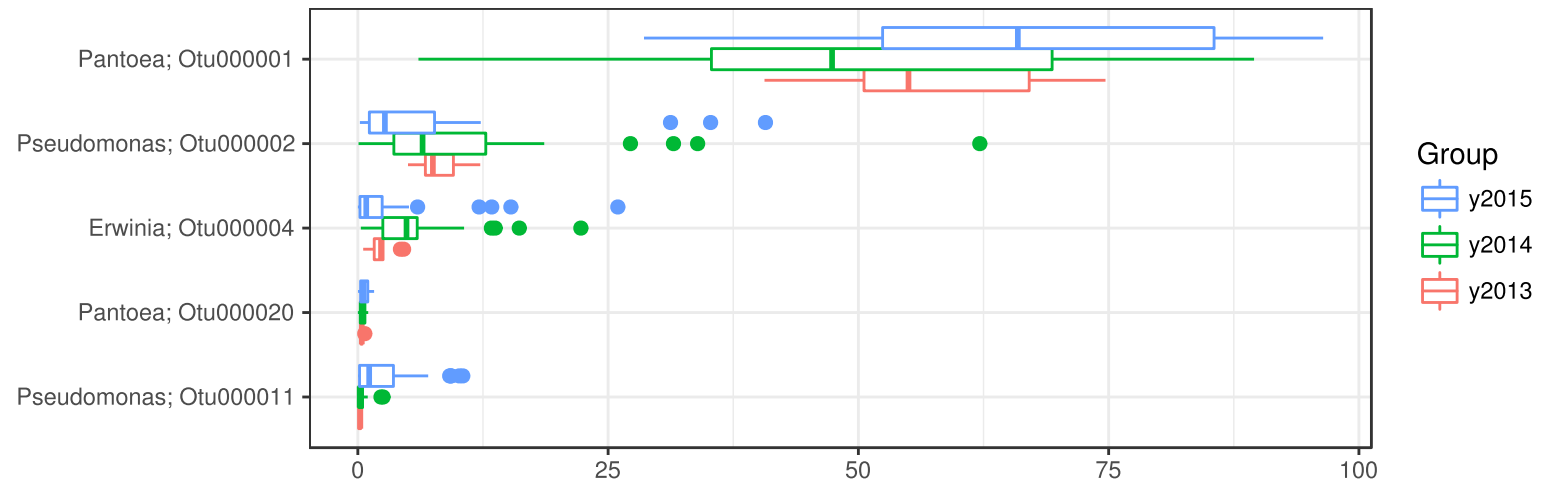


Year

- y2013
- y2014
- y2015

A**C****E****B****D****F**

A



B

