



**HAL**  
open science

# Influence of environment and host plant genotype on the structure and diversity of the *Brassica napus* seed microbiota

Aude Rochefort, Martial Briand, Coralie Marais, Marie-Hélène Wagner, Anne Laperche, Patrick Vallée, Matthieu Barret, Alain Sarniguet

## ► To cite this version:

Aude Rochefort, Martial Briand, Coralie Marais, Marie-Hélène Wagner, Anne Laperche, et al.. Influence of environment and host plant genotype on the structure and diversity of the *Brassica napus* seed microbiota. *Phytobiomes Journal*, 2019, 3 (4), pp.326-336. 10.1094/PBIOMES-06-19-0031-R. hal-02275205

**HAL Id: hal-02275205**

<https://institut-agro-rennes-angers.hal.science/hal-02275205v1>

Submitted on 30 Aug 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

1 **Influence of environment and host plant genotype on the structure and**  
2 **diversity of the *Brassica napus* seed microbiota**

3

4 **Authors and affiliations:** Aude ROCHEFORT<sup>1</sup>, Martial BRIAND<sup>2</sup>, Coralie MARAIS<sup>2</sup>, Marie-  
5 Hélène WAGNER<sup>3</sup>, Anne LAPERCHE<sup>1</sup>, Patrick VALLEE<sup>1</sup>, Matthieu BARRET<sup>2</sup>, Alain SARNIGUET<sup>1\*</sup>

6

7 <sup>1</sup>INRA, Agrocampus-Ouest, Université de Rennes 1, UMR 1349 IGEPP (Institute of Genetics,  
8 Environment and Plant Protection) – 35653 Le Rheu, France.

9 <sup>2</sup>INRA, Agrocampus-Ouest, Université d'Angers, UMR 1345 IRHS (Research Institute on  
10 Horticulture and Seeds), SFR4207 QuaSaV, 49071 Beaucouzé, France.

11 <sup>3</sup>Groupe d'Etude et de contrôle des Variétés Et des Semences, Beaucouzé, France.

12 \*Corresponding author: A. Sarniguet; Email address: alain.sarniguet@inra.fr

13

14 **Keywords:** *Brassica napus*, environment, germination, host genotype, seed microbiota, self-  
15 pollination

16 **ABSTRACT**

17 Seeds are involved in the transmission of microorganisms from one plant generation to the  
18 next and consequently act as reservoirs for the plant microbiota. The driving processes  
19 influencing seed microbiota assemblage have not been yet deciphered because of  
20 confounding factors related to environmental location, agricultural practices and host  
21 genotype selection. Nine genotypes were chosen among a large panel of genetically diverse  
22 *Brassica napus* accessions. The taxonomic structure of the seed microbiota was monitored  
23 by amplification and subsequent high-throughput sequencing of *gyrB* and ITS1 markers for  
24 two successive years on seed lots collected from self-pollinated plants. Seed germination  
25 capacities were compared between all seed lots. Although harvesting year was the main  
26 driver of seed microbiota composition, the host genotype also significantly altered the  
27 structure of seed microbial assemblages. The core microbiota of *B. napus* included nine  
28 fungal taxa shared between all the genotypes and years, while no bacterial taxa were  
29 conserved across all genotypes and years. The harvesting year had the major effect on seed  
30 germination but with some differences between genotypes. The study demonstrated the  
31 relative contribution of host- and environmental-filtering on the assemblage of the seed  
32 microbiota. It suggested some influence of these assemblages on seed germination.

33

34 Plants live in association with a diverse and complex set of microorganisms also known as  
35 microbiota. The plant microbiota can modify several host traits, such as plant nutrition  
36 (Paredes *et al.*, 2018), flowering kinetics (Panke-Buisse *et al.*, 2015) and resistance against  
37 plant pathogens and herbivores (Haney *et al.*, 2018; Kwak *et al.*, 2018). Owing to such  
38 impacts on plant growth and health, it is of interest to decipher the biological and ecological  
39 processes involved in the assembly of the plant microbiota. Several studies have notably  
40 investigated the impact of environmental factors and host genetic variation on the  
41 composition of plant microbiota (*e.g.* Bulgarelli *et al.*, 2012; Peiffer *et al.*, 2013; Horton *et al.*,  
42 2014; Edwards *et al.*, 2015; Pérez-Jaramillo *et al.*, 2017; Fitzpatrick *et al.*, 2018). These  
43 studies have revealed a major impact of environment on the taxonomic structure of plant  
44 microbiota and a limited but significant contribution of the host genotype. Accordingly, it  
45 has been recently highlighted that community heritability ( $H^2_C$ , Opstal & Bordenstein, 2015)  
46 of the maize rhizosphere microbiota is low, even if the abundance of some operational  
47 taxonomic units (OTUs) is significantly affected by host genetics (Walters *et al.*, 2018). This  
48 broad-sense heritability ( $H^2$ ) of some bacterial taxa is probably linked to host genes involved  
49 in the recruitment of its microbiota, for instance genes related to specific plant metabolites  
50 and to plant immunity (Hacquard *et al.*, 2017; Sasse *et al.*, 2018).

51 In contrast to other plant habitats, diversity of seed microbial assemblages is restrained  
52 (Barret *et al.*, 2015; Klaedtke *et al.*, 2016; Leff *et al.*, 2017; Rybakova *et al.*, 2017; Rodríguez  
53 *et al.*, 2018; Adam *et al.*, 2018; Bergna *et al.*, 2018; Rezki *et al.*, 2018), perhaps as a  
54 consequence of a seed bottleneck for microbial transmission (Paredes & Lebeis, 2016;  
55 Newcombe *et al.*, 2018). In fact considering that rhizosphere and phyllosphere are plant  
56 habitats with a highly diversified and abundant microbiota, seeds act as a filter and select

57 smaller sized and less diversified microbial assemblages. Therefore, the relative importance  
58 of vertical transmission from maternal plant to its progeny over horizontal transmission from  
59 the environment is probably limited in plants (Leff *et al.*, 2017; Newcombe *et al.*, 2018).  
60 Nevertheless, seeds represent the initial inoculum of the plant microbiota and can thus  
61 potentially impact plant fitness especially during its early development stages.

62 Rapidity and uniformity of seed germination and seedling growth together with  
63 emergence ability under adverse environmental conditions and performance after storage  
64 are collectively referred to as seed vigor (Finch-Savage & Bassel, 2016). Seed vigor is  
65 determined by seed dormancy (Bentsink & Koornneef, 2008) and seed longevity (Leprince *et al.*,  
66 2017); both traits being influenced by genetic and environmental perturbations. For  
67 instance, seed dormancy in *Brassica napus* is affected by the genotype and pre-harvest  
68 environmental conditions (Gulden *et al.*, 2004; Gruber *et al.*, 2009). In contrast to  
69 physiological and environmental factors, the impact of seed microbiota composition on seed  
70 vigor has not been yet explored. However, experimental evidences indicated that some  
71 plant-associated bacteria can repress germination through the production of oxyviniglycines  
72 (McPhail *et al.*, 2010; Chahtane *et al.*, 2018) or decrease dormancy through free cytokinins  
73 production (Rodrigues Pereira *et al.*, 1972; Goggin *et al.*, 2015). Hence, one may argue that  
74 changes in seed microbiota structure could ultimately result in differences of seed vigor.

75 The few studies that have investigated the importance of ecological processes involved  
76 in the assembly of the seed microbiota have reported that the environment significantly  
77 impacts the structure of the seed microbiota, and especially fungal assemblages (Klaedtke *et al.*,  
78 2016). Part of these studies has also highlighted that the host genotype could have a  
79 weaker but significant influence on bacterial endophytes of oilseed rape ; however, it is

80 difficult to eliminate environmental variations that could be confounding (Rybakova *et al.*,  
81 2017). In addition, neutral-based processes such as dispersal and ecological drift are also  
82 involved in the assembly of seed microbial assemblages (Rezki *et al.*, 2018).

83 *Brassica napus* (oilseed rape) is a cropping plant cultivated worldwide for its oilseed and  
84 for protein cattle feed production. *B. napus* is sensitive to a range of soilborne plant  
85 pathogens including *Plasmodiophora brassicae*, *Rhizoctonia solani* and *Verticillium*  
86 *longisporum*, which can cause clubroot, seedling damping-off and Verticillium wilt,  
87 respectively (Hwang *et al.*, 2012; Sturrock *et al.*, 2015; Depotter *et al.*, 2016). In absence of  
88 efficient control methods to restrict the populations of these phytopathogenic  
89 microorganisms, some biocontrol-based methods based on seed inoculation with microbial  
90 consortia have been proposed (Kataria & Verma, 1992; Müller & Berg, 2008). However,  
91 employing these seed inocula requires more basic knowledge on processes involved in  
92 assembly and dynamics of the seed microbiota (Barret *et al.*, 2016). In this study, we  
93 estimated the impact of host genotypes and environment on the structure of the seed  
94 microbiota of nine genotypes representing the diversity of winter oilseed rape (WOSR)  
95 during two consecutive years. In addition, we explored the potential effect of seed  
96 microbiota composition on seed germination of these nine genotypes.

## 97 MATERIALS AND METHODS

### 98 Selection of winter oilseed rape (WOSR) genotypes.

99 A total of 116 cultivars of winter oilseed rape (*Brassica napus*), registered in France between  
100 the 1950's and the 2000's, were gathered from the CRB BraCySol (INRA, IGEPP, France) to  
101 represent the diversity of inbred lines cultivated in Western Europe. All cultivars were  
102 genotyped with the Infinium Brassica 60K SNP array (Clarke *et al.*, 2016). A set of 628  
103 polymorphic SNPs presenting a low level of linkage disequilibrium ( $r^2 < 0.2$ ) were selected to  
104 analyze the structure of the genetic diversity within the accessions.

105 The structure of the collection was studied using admixture (Alexander *et al.*, 2009) and  
106 ten cross-validations. The genetic diversity of the collection was analyzed using Darwin  
107 software (Perrier & Bonnot, 2003). Sokal and Michener genetic distances were estimated  
108 between each couple of cultivars, and then clustering was performed using UPGMA. Nine *B.*  
109 *napus* genotypes, namely Astrid, Aviso, Boston, Colvert, Express, Major, Mohican, Tenor and  
110 Zorro, were chosen among the collection (Supplementary Fig. S1, Table S1) as representative  
111 of *B. napus* diversity.

### 112 Seed production.

113 The selected genotypes were cultivated in 2012-2013 (Y0) in the same experimental field  
114 (INRA, Le Rheu, France) to minimize the effect of any former environmental difference  
115 between genotypes, and under self-pollination cages to ensure autogamy and therefore  
116 produce genetically homogenous lines. The 2013 (Y0) seeds, harvested individually for each  
117 genotype were sown in 2015 in a field located close to the field dedicated to seed 2013  
118 production. Pollination bags were applied over 20 plants per genotype after cutting off the

119 early emerging flowers. The 2015-2016 (Y1) seeds of 20 plants were harvested and pooled  
120 independently for each genotype. In 2016-2017 (Y2), the seeds harvested for each genotype  
121 in the Y1 trial were sown in a field near the previous locations (INRA, Le Rheu, France) with  
122 the same cropping and harvesting process as Y1. GPS coordinates, distances between fields  
123 and cropping history for each year are available on Supplementary Table S2. Climatic data  
124 for the cropping periods Y1 and Y2 are reported in Supplementary Figure S2. All seed lots  
125 harvested in Y1 and Y2 were stored in paper bags in dark dry conditions and at room  
126 temperature.

### 127 **Seed phenotyping.**

128 Seed imbibition, germination, and early radicle growth were monitored *in vitro* with an  
129 automated phenotyping platform (Phenotic, SFR Quasav, Angers, France), which is described  
130 in detail in Ducournau *et al.* (2004, 2005) and Wagner *et al.* (2012). Briefly, four subsamples  
131 of 25 seeds by genotype and year (Y1 and Y2) were incubated at 20°C on germination test  
132 paper (GE Healthcare, type 3644) continuously moistened for 5 days and image acquisition  
133 was performed every two hours. Image acquisition, image analysis and data analysis  
134 methods are described in Demilly *et al.* (2014). The following parameters were determined:  
135 seed area (Area; in mm<sup>2</sup>), volume 8h after initiation of imbibition (Imb\_Vol; in mm<sup>3</sup>), radicle  
136 elongation after 8h (Elon\_germ, in mm), mean germination time (germination, in hours), and  
137 time to reach 50% of germination per seed subsample (T50, in hours). Differences were  
138 assessed with Kruskal-Wallis tests and considered as significant at a *p*-value < 0.01.

### 139 **Microbial DNA sample preparation.**



140 DNA extraction was performed on five subsamples of 1,000 seeds (~4 g) per genotype (45  
141 subsamples in total per year). First, seeds were soaked in 20 mL of phosphate buffered  
142 saline (PBS) supplemented with 0.05% (v/v) of Tween 20 for 2h30 under constant agitation  
143 (140 rpm) at room temperature. After this seed soak that enables the recovery of epiphytic  
144 and endophytic microorganisms (Barret *et al.*, 2015), the suspensions were centrifuged  
145 (4,500 *g*, 10 min) and DNA extraction was performed on the resulting pellets with the  
146 DNeasy PowerSoil HTP 96 Kit (Qiagen), following the manufacturer's procedure. Seed  
147 suspensions in PBS are classically performed in seed pathology (see International Seed  
148 Testing Association –ISTA: <https://www.seedtest.org/en/home.html>) to PCR-amplify  
149 bacterial strains located in the endosperm (e.g. *Acidovorax citrulli*; or *Xanthomonas citri*).  
150 Numerous antimicrobial compounds are associated to seed tissues and are released during  
151 grinding, which therefore can result in underestimation of seed microbial diversity.

## 152 **Libraries construction and sequencing.**

153 An initial PCR amplification was performed with the primer sets *gyrB*\_aF64/*gyrB*\_aR553  
154 (Barret *et al.*, 2015) and ITS1F/ITS2 (Buée *et al.*, 2009), which target a portion of *gyrB* and  
155 the fungal ITS1 region, respectively. The cycling conditions for ITS1F/ITS2, were an initial  
156 denaturation at 94°C for 3 min, followed by 35 cycles of amplification at 94°C (30 s), 50°C (45  
157 s) and 68°C (90 s), and a final elongation step at 68°C for 10 min. The cycling conditions for  
158 *gyrB* were: initial denaturation at 94°C for 3 min, 35 cycles of amplification at 94°C (30 s),  
159 55°C (45 s) and 68°C (90 sec), and final elongation step at 68°C for 10 min. Amplicons were  
160 purified with magnetic beads (Sera-Mag™, Merck). A second PCR amplification was  
161 performed to incorporate Illumina adapters and barcodes. PCR cycling conditions were  
162 identical for the two molecular markers: a first denaturation step at 94°C (1 min), followed

163 by 12 cycles at 94°C (1 min), 55°C (1 min) and 68°C (1 min), and a final elongation at 68°C for  
164 10 min. Amplicons were purified as previously described and quantified with the QuantIT  
165 PicoGreen ds DNA Assay Kit (ThermoFisher Scientific). All the amplicons were pooled in  
166 equimolar concentrations and the concentration of the equimolar pool was monitored with  
167 quantitative PCR (KAPA SYBR<sup>®</sup> FAST, Merck). Amplicon libraries were mixed with 10% PhiX  
168 and sequenced with MiSeq reagent kit v2 500 cycles (one sequencing cartridge per year).

### 169 **Sequence processing.**

170 Fastq files were processed with DADA2 version 1.6.0 (Callahan *et al.*, 2016), using the  
171 parameters described in the workflow for “Big Data: Paired-end”  
172 ([https://benjjneb.github.io/dada2/bigdata\\_paired.html](https://benjjneb.github.io/dada2/bigdata_paired.html)). The only modification made  
173 relative to this protocol was a change in the truncLen argument according to the quality of  
174 the sequencing run. Taxonomic affiliations for amplicon sequence variants (ASV) generated  
175 with DADA2 were assigned with a naive Bayesian classifier (Wang *et al.*, 2007) on an in-  
176 house *gyrB* database (Bartoli *et al.*, 2018) and the UNITE v7.1 fungal database (Abarenkov *et*  
177 *al.*, 2010).

### 178 **Microbial community analyses.**

179 Analyses of diversity were conducted with the R package Phyloseq version 1.22.3 (McMurdie  
180 & Holmes, 2013). Data were normalized based on sequencing depth, using a rarefaction of  
181 1,100 and 15,000 sequences per sample for *gyrB* and ITS1, respectively. Observed richness  
182 (number of ASV per sample), estimated richness (Chao1 index), and inverse Simpson’s index  
183 were calculated with Phyloseq. Faith’s phylogenetic diversity was calculated on *gyrB* dataset  
184 with the R package picante version 1.6-2 (Kembel *et al.*, 2010). Differences in alpha-diversity

185 estimators between years and genotypes were assessed with Kruskal-Wallis tests.  
186 Differences were considered as significant at a  $p$ -value  $< 0.01$ .

187 Changes in microbial assemblage composition were assessed with Bray-Curtis (BC) index  
188 and weighted UniFrac (wUF) distance (Lozupone & Knight, 2005). Principal coordinate  
189 analysis (PCoA) was used for ordination of BC index and wUF distance. To quantify the  
190 relative contribution of plant generation and plant genotype in microbial community  
191 profiles, canonical analysis of principal coordinates (CAP) was performed with the function  
192 `capscale` of the R package `vegan` 2.4.2 (Oksanen *et al.*, 2017) followed with permutational  
193 multivariate analysis of variance (PERMANOVA; Anderson, 2001).

194 A presence/absence matrix of common ASVs was constructed as follows. Common ASV  
195 was recorded as present if detected in all subsamples ( $n=5$ ) of one seed lot. One seed lot  
196 corresponded to seeds collected each year from each plant genotype. Visualization of  
197 genotype-specific ASVs and shared ASVs between plant genotypes was assessed for each  
198 year with the R package `UpSetR` (Lex *et al.*, 2014). The number of common bacterial and  
199 fungal ASV between years for each individual genotype was also assessed using Venn  
200 diagrams. This binary matrix was used for assessing Jaccard and unweighted UniFrac  
201 distance (uUF) between genotypes. Comparisons of Sokal-Michener genetic distance and  
202 Jaccard/uUF distances were monitored with coefficient of determination.

203 Distribution of bacterial and fungal ASVs in other Brassicaceae seeds was assessed by  
204 investigating their prevalence in 479 seed samples associated to *Brassica oleracea*, *Brassica*  
205 *rapa* and *Raphanus sativus* (Barret *et al.*, 2015; Rezki *et al.*, 2016, 2018).

206

207 The datasets supporting the conclusions of this article are available in the SRA database  
208 under the accession number PRJEB31617.

## 209 RESULTS

### 210 Diversity of the seed microbiota of *B. napus*.

211 Overall 4,451 bacterial and 359 fungal ASVs were detected within *gyrB* and ITS1 datasets.  
212 ASV provided a finer resolution than OTU (Callahan *et al.*, 2017), this descriptor was  
213 therefore used for estimating the taxonomic structure of the seed microbiota of *B. napus*.

214 According to coverage estimates, the number of *gyrB* sequences was not sufficient for  
215 reaching the plateau after rarefaction, while saturation was achieved for fungal reads.  
216 Hence, predicted richness (Chao1 index) was assessed for bacterial assemblages, while  
217 observed richness (number of ASVs) was measured for fungal assemblages. Although  
218 bacterial and fungal richness were significantly different between the two harvesting years  
219 ( $P < 0.01$ ), seed samples collected in Y2 contained on average fewer bacterial and more  
220 fungal ASVs than Y1 seed sample (Fig. 1A, D). The increase of fungal richness in Y2 was  
221 associated with a significant decrease in fungal diversity (Fig. 1E). While bacterial diversity  
222 did not differ between years (Fig. 1B), bacterial phylogenetic diversity (Faith's PD), was  
223 significantly higher in Y2 ( $P < 0.01$ ; Fig. 1C). In contrast to the harvesting year, no significant  
224 differences in  $\alpha$ -diversity estimators were observed between plant genotypes ( $P > 0.01$ ).

225 Similarities in composition of bacterial and fungal assemblages were estimated with  
226 weighted UniFrac (wUF) distance and Bray-Curtis (BC) index, respectively. Ordination of wUF  
227 and BC revealed a significant clustering of seed-associated bacterial and fungal assemblages  
228 according to the harvesting year (Fig. 2, Table 1). The relative influence of the harvesting  
229 year and the plant genotype in assemblage composition was further inspected through  
230 canonical analysis of principal coordinates (CAP) followed by PERMANOVA. Based on CAP  
231 analyses, 16.4% and 65.5% of variances in bacterial and fungal assemblage compositions,

232 respectively, were explained by the harvesting year, ( $P < 0.01$ ). Changes in bacterial and  
233 fungal assemblage compositions were also significantly ( $P < 0.01$ ) explained by the plant  
234 genotype, with 19.3% and 11.5% of variances driven by this factor, respectively (Table 1).  
235 Interaction between harvesting year and genotype was significant ( $P < 0.01$ ) and explained  
236 16.6% and 13.3% of variances of bacterial and fungal assemblage compositions respectively.  
237 When each year is analyzed separately, the impact of plant genotype ( $P < 0.01$ ) ranged from  
238 40% to 90% of explained variance (Table 1, Supplementary Fig. S3). However, similarity in  
239 assemblage compositions between genotypes were different in Y1 and Y2.

#### 240 **Core and specific fractions of the *B. napus* microbiota.**

241 Differences in relative abundance of bacterial orders between genotypes were mostly  
242 observed within Y1 (Fig. 3A). Notably we detected a higher abundance of Sphingomonadales  
243 and Pseudomonadales in Astrid, Aviso and Boston in comparison to the other genotypes  
244 (Fig. 3A). However, these differences between genotypes were not detected in Y2, where  
245 principal variations were related to increase in relative abundance of Enterobacteriales in  
246 Express and Zorro. Regarding fungal assemblages, differences in relative abundance of  
247 fungal orders were mostly observed between harvesting years, with a decrease in relative  
248 abundance of Tremellales and Helotiales in Y2 (Fig. 3B).

249 The distribution of ASVs between plant genotypes for each year of production was also  
250 assessed (Fig. 4, Fig. 5). Among the 15 bacterial ASVs systematically detected in at least one  
251 genotype in Y1, none were shared between all genotypes (Fig. 4A). In Y2, six bacterial ASVs  
252 (out of 268) were shared between all genotypes (Fig. 4B). Four of these six ASVs have been  
253 already detected in more than 10% of seed samples ( $n = 479$ ) collected from various  
254 Brassicaceae including *Brassica oleracea*, *Brassica rapa* and *Raphanus sativus* (Table 2). The

255 inheritance of bacterial ASVs between years was quite low, since only three genotypes  
256 (Astrid, Aviso and Boston) possessed four and five ASVs in common in Y1 and Y2,  
257 respectively (Supplementary Fig. S4A). These ASVs shared between both years are related to  
258 *Sphingomonas*, *Pseudomonas* and *Frigoribacterium*.

259 Fungal assemblages shared more ASVs between all genotypes in comparison to bacterial  
260 assemblages. Indeed, 18 (out of 37) and 19 (out of 52) fungal ASVs were shared between all  
261 the genotypes in Y1 and Y2, respectively (Fig. 5). For each genotype, 35% to 40% fungal ASVs  
262 were conserved from Y1 to Y2 (Supplementary Fig. S4B). In addition, nine fungal ASVs were  
263 systematically detected during both years. These fungal ASVs are also highly prevalent in  
264 seed samples of other Brassicaceae species (Table 2). There were very few bacterial ASVs  
265 specifically associated to one genotype in Y1, while there were in general more specific  
266 bacterial ASVs per genotype in Y2. Conversely, very few genotypes harbor specific fungal  
267 ASVs in Y1 as in Y2 (Supplementary Table S3, Table S4).

### 268 **Relationship between *B. napus* genotypes and seed microbiota composition.**

269 To assess if the composition of the seed microbiota could be related to genetic relatedness  
270 of *B. napus*, we compared Sokal-Michener genetic distance to Jaccard and uUF distances  
271 calculated with ITS1 and *gyrB* sequences, respectively (Supplementary Fig. S5). Coefficient of  
272 determination between Jaccard/uUF distances and Sokal-Michener distance were low for  
273 bacterial ( $r^2= 0.026$  in Y1;  $r^2= 0.009$  in Y2) and fungal ( $r^2= 0.005$  in Y1;  $r^2= 0.117$  in Y2)  
274 assemblages. While a significant ( $P = 0.002$ ) positive correlation between genetic distance  
275 and fungal assemblage composition was detected in Y2, the overall genetic similarities  
276 between the *B. napus* genotypes employed in this work is not a robust predictor of seed  
277 microbiota composition.

278 **Impact of production year and host genotype on seed phenotypes.**

279 There were no significant differences between years and genotypes for seed area, radicle  
280 elongation and imbibition speed (Supplementary Fig. S6). However, according to average  
281 germination rate ( $n=100$ ), germination of seed samples harvested in Y1 was significantly ( $P <$   
282  $0.01$ ) slower than germination of seed samples harvested in Y2 (Fig. 6). Significant  
283 differences were also observed between genotypes within specific year. For instance, seeds  
284 from Astrid germinated on average earlier ( $P < 0.01$ ) than the other genotypes in Y1 but not  
285 in Y2 (Fig. 6). Seeds from Tenor had a slower mean germination time ( $P < 0.01$ ) in comparison  
286 to the other genotypes but only for samples collected in Y2 (Fig. 6). Thus, another metric,  
287 T50, varied in the same way as mean germination time (Supplementary Fig. S6D). Of note,  
288 three bacterial ASV were specifically associated with Astrid in Y1 (Supplementary Table S3).  
289 Moreover, 3 bacterial ASVs and 5 fungal ASVs were specifically associated to Tenor in Y2  
290 (Supplementary Table S4).



## 291 **DISCUSSION**

292 The aim of this study was to assess the relative influence of the environment (*i.e.* year) and  
293 the host genotype on the structure of the *B. napus* seed microbiota. To eliminate  
294 environmental variations within a year, we sowed seeds of nine *B. napus* genotypes in the  
295 same field. In addition, to ensure homogeneous genetic material and preserve genotype  
296 specificities, each individual plant was cultivated in self-pollination.

297 Over two years we observed a decrease in bacterial richness for seed samples collected  
298 during the second year. This loss of bacterial richness could be explained by a selection of  
299 bacterial taxa across years, in an analogous process that has been described for continuous  
300 cropping (*e.g.* Zhao *et al.*, 2018). Loss of bacterial richness might be related to the repeated  
301 self-pollination process, which limits dispersal of individuals among local bacterial  
302 assemblages (Vannette & Fukami, 2017). If this assumption was correct, a similar reduction  
303 of richness would be expected within seed fungal assemblages. However, fungal richness  
304 increased in Y2. Therefore, alteration of richness could be solely due to differences in  
305 richness of local microbial assemblages between years. Influence of the harvesting year was  
306 the main driving factor of seed fungal assemblage composition with 65.5% of explained  
307 variance. These results agree with a previous report showing that seed fungal assemblages  
308 of common bean are mostly shaped by crop management practices and geographical  
309 locations (Klaedtke *et al.*, 2016). The high impact of environmental filtering on composition  
310 of the fungal fraction of the seed microbiota could be related to local biogeographical  
311 patterns of fungal assemblages (Peay *et al.*, 2016).

312 Diversity and composition of the *B. napus* seed microbiota was different between years  
313 but also between the host genotypes used in this study. Host-genotype is an important

314 driver in the assembly of the seed microbiota, especially for the bacterial fraction. The  
315 impact of host genotypes on the structure of *B. napus* microbiota was already highlighted for  
316 endophytic bacteria (Rybakova *et al.*, 2017). Since seed lots employed by Rybakova *et al.*  
317 (2017) were collected from different geographical regions, the influence of host genotype  
318 was therefore inseparable from the influence of the environment. Our study confirmed  
319 without any confounding factor that the structure of the *B. napus* seed bacterial assemblage  
320 are partly genotype-dependent. Since interaction between year and genotype was  
321 significantly influencing  $\beta$ -diversity, we assessed the influence of host genotypes on  
322 microbiota compositions for each year. According to these analyses, the plant genotype  
323 explained a major part of variation in seed microbial assemblage composition, therefore  
324 suggesting an important influence of host-filtering in the assembly of the seed microbiota.  
325 However, composition of seed microbial assemblage differed greatly between harvesting  
326 years. In addition, a small part of the *B. napus* seed bacterial assemblage is inherited from  
327 one generation to the next. Overall, our study highlighted a low inheritance of bacterial and  
328 fungal assemblages across *B. napus* generations. Indeed no bacterial ASV were consistently  
329 detected between plant generations, while nine fungal ASVs were conserved between years.  
330 The absence of conserved bacterial ASV between year and genotypes could be explained by  
331 a stronger impact of host-filtering on assembly of the bacterial fraction of the seed  
332 microbiota. Alternatively, the absence of generalist bacterial ASV observed between years  
333 could be partly explained by the weak number of bacterial ASVs detected in Y1. The  
334 inheritance of seed-borne microbial taxa is quite low, therefore confirming previous  
335 observations performed on *R. sativus* over three consecutive generations (Rezki *et al.*, 2018)  
336 and *Solanum lycopersicum* over two generations (Bergna *et al.*, 2018). Hence, the main

337 acceptable conclusion is that few microbial taxa associated with seeds are inherited and that  
338 most of seed-borne taxa represented cases of horizontal transmission (Leff *et al.*, 2017).  
339 Despite these changes in assemblage compositions, the ASVs detected on seed samples  
340 corresponded to microbial taxa frequently associated with other seed samples. For instance,  
341 four of the bacterial generalists detected in Y2, namely *Pantoea agglomerans*, *Pseudomonas*,  
342 *Sphingomonas* and *Frigoribacterium*, were already detected in more than 10% of other  
343 Brassicaceae seed lots (Barret *et al.*, 2015; Rezki *et al.*, 2018). Hence, these taxa might be  
344 adapted to the seed habitat. The case was different for fungi with a significant number of  
345 fungal ASVs recovered through generations. However this does not mean that these taxa are  
346 vertically-transmitted. Assessing the relative importance of vertical and horizontal  
347 transmission will required an in-depth analysis of the seed transmission pathways (internal,  
348 floral and external) employed by these fungal taxa. Therefore, it is tempting to speculate that  
349 these differences in seed microbiota composition between years are the result of  
350 fluctuations of the local microbial reservoir. In this way, specific microbial recruitment and  
351 selection by the genotypes are modulated among years. Consequently, the relative impact of  
352 the host genotype on the structure of seed microbial assemblages is difficult to predict, as it  
353 is dependent of fluctuations of the local reservoir.

354

355 Previous works performed on the rhizosphere and phyllosphere of multiple maize  
356 genotypes have highlighted a relatively low  $H^2$  of microbial taxa (Wallace *et al.*, 2018;  
357 Walters *et al.*, 2018). Although the number of genotypes employed in our study precluded  
358 the measurement of this broad sense heritability, we did not establish any significant link  
359 between the genetic distance of the nine genotypes and their microbial distances. While  
360 considering the variability between subsamples collected from plants of the same genotype

361 and cultivated on the same field, in addition to the relative influence of environment and  
362 host genotype, there is a contribution of other neutral-based processes on the assembly of  
363 the seed microbiota (Rezki *et al.*, 2018). However, some specific plant functions and traits,  
364 and especially genes implicated in plant immune system, shape the structure and diversity of  
365 their own microbiota (Horton *et al.*, 2014; Li *et al.*, 2018). Thus, it would be necessary to use  
366 a larger number of genetically diverse genotypes of WOSR to explore this hypothesis at a  
367 finer resolution (*i.e.* intra-species). To date, some studies failed to establish this link even  
368 when microbial diversification due to genetic diverse plant species was demonstrated  
369 (Bouffaud *et al.*, 2012; Schlaeppi *et al.*, 2014). However, establishing a direct correlation  
370 between genotypes and their microbiota would be of great interest for selecting genotypes  
371 with a specific microbiota in which certain taxa would ensure positive functions for the plant  
372 fitness.

373 Among different observed seed phenotypes, only the mean germination time and T50  
374 (half-mean germination time) varied between years. Seed germination progresses through  
375 three phases (Bewley, 1997). The first phase, known as imbibition, is a physical process  
376 driven by difference in water potential between the inside and the outside of the seed. Based  
377 on seed volume monitored during 8 hours after initiation of imbibition, no significant  
378 differences in imbibition was observed between seed samples. This observation suggested  
379 that seed moisture was quite comparable between seed samples and that the resulting  
380 differences in germination kinetics are related to later phases. Since the environmental factor  
381 (year) more strongly shapes the bacterial and fungal communities, we assessed whether the  
382 change in microbiota composition could explain variation in germination rates. Authors  
383 demonstrated that the environmental conditions, age of seed and genotype (He *et al.*, 2014;  
384 Nagel *et al.*, 2015; Leprince *et al.*, 2017) affect different seed performances like secondary

385 dormancy and seed vigor. The requirement to perform germination assays for all seed  
386 samples simultaneously ultimately results in differences in longevity of seed collected in Y1  
387 and Y2. However, long-term storage (*i.e.* several years) of *B. napus* seeds samples does not  
388 impact germination time but rather germination percentage (Nagel *et al.*, 2011), which was  
389 not different between Y1 and Y2. Moreover, Astrid and Tenor genotypes did not support the  
390 ageing effect hypothesis as their germination rates were either similar or inverted  
391 respectively between Y1 and Y2 compared to the other genotypes. In addition, for Tenor  
392 different bacterial and fungal ASVs were effectively described in seed assemblages. Seed  
393 moisture cannot be involved as there was no significant differences during imbibition phase.  
394 Therefore, because of the most probable assumption of microbiota involvement, further  
395 experiments are required to investigate further the implication of microbial assemblages in  
396 germination or extended seed phenotypes variations.

397 In conclusion, the microbial assemblages of *B. napus* seeds are mainly influenced by the  
398 environment but to a lesser extent by the plant genotype. The inheritance of some abundant  
399 bacterial and fungal ASVs also differs between genotypes. Further studies will explore more  
400 extensively the host genetic determinants driving the microbial assemblages and the impact  
401 of such assemblages on seed vigor.

**402 ACKNOWLEDGEMENTS**

403 We want to apologize to authors whose relevant work was not included in this article due to  
404 space constraints. The authors wish to thank Muriel Bahut from the ANAN platform (SFR  
405 Quasav) for amplicon sequencing, the Phenotic Platform for performing seed germination  
406 experiments and Thomas Baldwin for English proofreading.

407 **LITERATURE CITED**

- 408 Abarenkov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller  
409 R, Larsson E, Pennanen T, *et al.* 2010. The UNITE database for molecular identification of  
410 fungi – recent updates and future perspectives. *New Phytologist* 186: 281–285.
- 411 Adam E, Bernhart M, Müller H, Winkler J, Berg G. 2018. The Cucurbita pepo seed  
412 microbiome: genotype-specific composition and implications for breeding. *Plant and Soil*  
413 422: 35–49.
- 414 Alexander DH, Novembre J, Lange K. 2009. Fast model-based estimation of ancestry in  
415 unrelated individuals. *Genome Research* 19: 1655–1664.
- 416 Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance.  
417 *Austral Ecology* 26: 32–46.
- 418 Barret M, Briand M, Bonneau S, Prévieux A, Valière S, Bouchez O, Hunault G, Simoneau P,  
419 Jacques M-A. 2015. Emergence Shapes the Structure of the Seed Microbiota. *Appl.*  
420 *Environ. Microbiol.* 81: 1257–1266.
- 421 Barret M, Guimbaud J-F, Darrasse A, Jacques M-A. 2016. Plant microbiota affects seed  
422 transmission of phytopathogenic microorganisms: Plant microbiota affects seed  
423 transmission. *Molecular Plant Pathology* 17: 791–795.
- 424 Bartoli C, Frachon L, Barret M, Rigal M, Huard-Chauveau C, Mayjonade B, Zanchetta C,  
425 Bouchez O, Roby D, Carrère S, *et al.* 2018. In situ relationships between microbiota and  
426 potential pathobiota in Arabidopsis thaliana. *The ISME Journal* 12: 2024–2038.

- 427 Bentsink L, Koornneef M. 2008. Seed Dormancy and Germination. *The Arabidopsis Book* /  
428 *American Society of Plant Biologists* 6.
- 429 Bergna A, Cernava T, Rändler M, Grosch R, Zachow C, Berg G. 2018. Tomato Seeds Preferably  
430 Transmit Plant Beneficial Endophytes. *Phytobiomes Journal* 2: 183–193.
- 431 Bewley JD. 1997 Seed Germination and Dormancy. *Plant Cell* 9: 1055-1066.
- 432 Bouffaud M-L, Kyselková M, Gouesnard B, Grundmann G, Muller D, Moëgne-Loccoz Y. 2012.  
433 Is diversification history of maize influencing selection of soil bacteria by roots?  
434 *Molecular Ecology* 21: 195–206.
- 435 Buée M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F. 2009. 454 Pyrosequencing  
436 analyses of forest soils reveal an unexpectedly high fungal diversity. *New Phytologist*  
437 184: 449–456.
- 438 Bulgarelli D, Rott M, Schlaeppi K, Ver Loren van Themaat E, Ahmadinejad N, Assenza F, Rauf  
439 P, Huettel B, Reinhardt R, Schmelzer E, *et al.* 2012. Revealing structure and assembly  
440 cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* 488: 91–95.
- 441 Callahan BJ, McMurdie PJ, Holmes SP. 2017. Exact sequence variants should replace  
442 operational taxonomic units in marker-gene data analysis. *The ISME Journal* 11: 2639–  
443 2643.
- 444 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-  
445 resolution sample inference from Illumina amplicon data. *Nature Methods* 13: 581–583.



- 446 Chahtane H, Nogueira Füller T, Allard P-M, Marcourt L, Ferreira Queiroz E, Shanmugabalaji V,  
447 Falquet J, Wolfender J-L, Lopez-Molina L. 2018. The plant pathogen *Pseudomonas*  
448 *aeruginosa* triggers a DELLA-dependent seed germination arrest in *Arabidopsis* (CS  
449 Hardtke and DJ Kliebenstein, Eds.). *eLife* 7: e37082.
- 450 Clarke WE, Higgins EE, Plieske J, Wieseke R, Sidebottom C, Khedikar Y, Batley J, Edwards D,  
451 Meng J, Li R, *et al.* 2016. A high-density SNP genotyping array for *Brassica napus* and its  
452 ancestral diploid species based on optimised selection of single-locus markers in the  
453 allotetraploid genome. *Theoretical and Applied Genetics* 129: 1887–1899.
- 454 Demilly D, Ducournau S, Wagner M-H, Dürr C. 2014. Digital imaging of seed germination. In:  
455 Gupta S, Ibaraki Y, eds. *Plant Image Analysis*. CRC Press, 147–164.
- 456 Depotter JRL, Deketelaere S, Inderbitzin P, Tiedemann AV, Höfte M, Subbarao KV, Wood TA,  
457 Thomma BPHJ. 2016. *Verticillium longisporum*, the invisible threat to oilseed rape and  
458 other brassicaceous plant hosts: *Verticillium longisporum*, causal agent of stem striping.  
459 *Molecular Plant Pathology* 17: 1004–1016.
- 460 Ducournau S, Feutry A, Plainchault P, Revollon P, Vigouroux B, Wagner MH. 2004. An image  
461 acquisition system for automated monitoring of the germination rate of sunflower  
462 seeds. *Computers and Electronics in Agriculture* 44: 189–202.
- 463 Ducournau S, Feutry A, Plainchault P, Revollon P, Vigouroux B, Wagner MH. 2005. Using  
464 computer vision to monitor germination time course of sunflower (*Helianthus annuus* L.)  
465 seeds. *Seed Science and Technology* 33: 329–340.

- 466 Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA,  
467 Sundaresan V. 2015. Structure, variation, and assembly of the root-associated  
468 microbiomes of rice. *Proceedings of the National Academy of Sciences of the United*  
469 *States of America* 112: E911–E920.
- 470 Finch-Savage WE, Bassel GW. 2016. Seed vigour and crop establishment: extending  
471 performance beyond adaptation. *Journal of Experimental Botany* 67: 567–591.
- 472 Fitzpatrick CR, Copeland J, Wang PW, Guttman DS, Kotanen PM, Johnson MTJ. 2018.  
473 Assembly and ecological function of the root microbiome across angiosperm plant  
474 species. *Proceedings of the National Academy of Sciences* 115: E1157–E1165.
- 475 Goggin DE, Emery RJN, Kurepin LV, Powles SB. 2015. A potential role for endogenous  
476 microflora in dormancy release, cytokinin metabolism and the response to fluridone in  
477 *Lolium rigidum* seeds. *Annals of Botany* 115: 293–301.
- 478 Gruber S, Emrich K, Claupein W. 2009. Classification of canola (*Brassica napus*) winter  
479 cultivars by secondary dormancy. *Canadian Journal of Plant Science* 89: 613–619.
- 480 Gulden RH, Thomas AG, Shirliffe SJ. 2004. Relative contribution of genotype, seed size and  
481 environment to secondary seed dormancy potential in Canadian spring oilseed rape  
482 (*Brassica napus*). *Weed Research* 44: 97–106.
- 483 Hacquard S, Spaepen S, Garrido-Oter R, Schulze-Lefert P. 2017. Interplay Between Innate  
484 Immunity and the Plant Microbiota. *Annual Review of Phytopathology* 55: 565–589.

- 485 Haney CH, Wiesmann CL, Shapiro LR, Melnyk RA, O'Sullivan LR, Khorasani S, Xiao L, Han J,  
486 Bush J, Carrillo J, *et al.* 2018. Rhizosphere-associated *Pseudomonas* induce systemic  
487 resistance to herbivores at the cost of susceptibility to bacterial pathogens. *Molecular*  
488 *Ecology* 27: 1833–1847.
- 489 He H, de Souza Vidigal D, Snoek LB, Schnabel S, Nijveen H, Hilhorst H, Bentsink L. 2014.  
490 Interaction between parental environment and genotype affects plant and seed  
491 performance in *Arabidopsis*. *Journal of Experimental Botany* 65: 6603–6615.
- 492 Horton MW, Bodenhausen N, Beilsmith K, Meng D, Muegge BD, Subramanian S, Vetter MM,  
493 Vilhjálmsson BJ, Nordborg M, Gordon JI, *et al.* 2014. Genome-wide association study of  
494 *Arabidopsis thaliana*'s leaf microbial community. *Nature communications* 5: 5320.
- 495 Hwang S-F, Strelkov SE, Feng J, Gossen BD, Howard RJ. 2012. *Plasmodiophora brassicae*: a  
496 review of an emerging pathogen of the Canadian canola (*Brassica napus*) crop.  
497 *Molecular Plant Pathology* 13: 105–113.
- 498 Kataria HR, Verma PR. 1992. *Rhizoctonia solani* damping-off and root rot in oilseed rape and  
499 canola. *Crop Protection* 11: 8–13.
- 500 Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP,  
501 Webb CO. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*  
502 26: 1463–1464.
- 503 Klaedtke S, Jacques M-A, Raggi L, Préveaux A, Bonneau S, Negri V, Chable V, Barret M. 2016.  
504 Terroir is a key driver of seed-associated microbial assemblages: Terroir shapes the seed  
505 microbiota. *Environmental Microbiology* 18: 1792–1804.

- 506 Kwak M-J, Kong HG, Choi K, Kwon S-K, Song JY, Lee J, Lee PA, Choi SY, Seo M, Lee HJ, *et al.*  
507 2018. Rhizosphere microbiome structure alters to enable wilt resistance in tomato.  
508 *Nature Biotechnology* 36: 1100–1109.
- 509 Leff JW, Lynch RC, Kane NC, Fierer N. 2017. Plant domestication and the assembly of  
510 bacterial and fungal communities associated with strains of the common sunflower,  
511 *Helianthus annuus*. *New Phytologist* 214: 412–423.
- 512 Leprince O, Pellizzaro A, Berriri S, Buitink J. 2017. Late seed maturation: drying without  
513 dying. *Journal of Experimental Botany* 68: 827–841.
- 514 Lex A, Gehlenborg N, Strobel H, Vuillemot R, Pfister H. 2014. UpSet: Visualization of  
515 Intersecting Sets. *IEEE Transactions on Visualization and Computer Graphics* 20: 1983–  
516 1992.
- 517 Li Y, Wu X, Chen T, Wang W, Liu G, Zhang W, Li S, Wang M, Zhao C, Zhou H, *et al.* 2018. Plant  
518 Phenotypic Traits Eventually Shape Its Microbiota: A Common Garden Test. *Frontiers in*  
519 *Microbiology* 9.
- 520 Lozupone C, Knight R. 2005. UniFrac: a New Phylogenetic Method for Comparing Microbial  
521 Communities. *Applied and Environmental Microbiology* 71: 8228–8235.
- 522 McMurdie PJ, Holmes S. 2013. phyloseq: An R Package for Reproducible Interactive Analysis  
523 and Graphics of Microbiome Census Data. *PLoS ONE* 8.

- 524 McPhail KL, Armstrong DJ, Azevedo MD, Banowetz GM, Mills DI. 2010. 4-  
525 Formylaminoxyvinylglycine, an Herbicidal Germination-Arrest Factor from  
526 Pseudomonas Rhizosphere Bacteria. *Journal of Natural Products* 73: 1853–1857.
- 527 Müller H, Berg G. 2008. Impact of formulation procedures on the effect of the biocontrol  
528 agent *Serratia plymuthica* HRO-C48 on *Verticillium* wilt in oilseed rape. *BioControl* 53:  
529 905–916.
- 530 Nagel M, Kranner I, Neumann K, Rolletschek H, Seal CE, Colville L, Fernández-Marín B,  
531 Börner A. 2015. Genome-wide association mapping and biochemical markers reveal  
532 that seed ageing and longevity are intricately affected by genetic background and  
533 developmental and environmental conditions in barley: Genetic and biochemical  
534 markers of seed ageing. *Plant, Cell & Environment* 38: 1011–1022.
- 535 Nagel M, Rosenhauer M, Willner E, Snowdon RJ, Friedt W, Börner A. 2011. Seed longevity in  
536 oilseed rape (*Brassica napus* L.) – genetic variation and QTL mapping. *Plant Genetic  
537 Resources* 9: 260–263.
- 538 Newcombe G, Harding A, Ridout M, Busby PE. 2018. A Hypothetical Bottleneck in the Plant  
539 Microbiome. *Frontiers in Microbiology* 9.
- 540 Oksanen J, Blanchet F, Kindt R, Legendre P, Minchin P, O’Hara R, Simpson G, Solymos P,  
541 Stevens M, Wagner H. 2017. Vegan: community ecology package. R package version 2.4-  
542 2.
- 543 Opstal EJ van, Bordenstein SR. 2015. Rethinking heritability of the microbiome. *Science* 349:  
544 1172–1173.

- 545 Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J. 2015. Selection on soil  
546 microbiomes reveals reproducible impacts on plant function. *The ISME Journal* 9: 980–  
547 989.
- 548 Paredes SH, Gao T, Law TF, Finkel OM, Mucyn T, Teixeira PJPL, González IS, Feltcher ME,  
549 Powers MJ, Shank EA, *et al.* 2018. Design of synthetic bacterial communities for  
550 predictable plant phenotypes. *PLOS Biology* 16: e2003962.
- 551 Paredes SH, Lebeis SL. 2016. Giving back to the community: microbial mechanisms of plant–  
552 soil interactions. *Functional Ecology* 30: 1043–1052.
- 553 Peay KG, Kennedy PG, Talbot JM. 2016. Dimensions of biodiversity in the Earth mycobiome.  
554 *Nature Reviews Microbiology* 14: 434–447.
- 555 Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE. 2013. Diversity and  
556 heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of*  
557 *the National Academy of Sciences* 110: 6548–6553.
- 558 Pérez-Jaramillo JE, Carrión VJ, Bosse M, Ferrão LFV, de Hollander M, Garcia AAF, Ramírez CA,  
559 Mendes R, Raaijmakers JM. 2017. Linking rhizosphere microbiome composition of wild  
560 and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits. *The ISME*  
561 *Journal* 11: 2244–2257.
- 562 Perrier X, Bonnot O. 2003. Methods of data analysis. *Genetic Diversity of Cultivated Tropical*  
563 *Plants*.

- 564 Rezki S, Champion C, Iacomi-Vasilescu B, Preveaux A, Toualbia Y, Bonneau S, Briand M,  
565 Laurent E, Hunault G, Simoneau P, *et al.* 2016. Differences in stability of seed-associated  
566 microbial assemblages in response to invasion by phytopathogenic microorganisms.  
567 *PeerJ* 4: e1923.
- 568 Rezki S, Champion C, Simoneau P, Jacques M-A, Shade A, Barret M. 2018. Assembly of seed-  
569 associated microbial communities within and across successive plant generations. *Plant*  
570 *and Soil* 422: 67–79.
- 571 Rodrigues Pereira AS, Houwen PJW, Deurenberg-Vos HWJ, Pey EBF. 1972. Cytokinins and the  
572 bacterial symbiosis of *Ardisia* species. *Zeitschrift für Pflanzenphysiologie* 68: 170–177.
- 573 Rodríguez CE, Mitter B, Barret M, Sessitsch A, Compant S. 2018. Commentary: seed bacterial  
574 inhabitants and their routes of colonization. *Plant and Soil* 422: 129–134.
- 575 Rybakova D, Mancinelli R, Wikström M, Birch-Jensen A-S, Postma J, Ehlers R-U, Goertz S,  
576 Berg G. 2017. The structure of the *Brassica napus* seed microbiome is cultivar-  
577 dependent and affects the interactions of symbionts and pathogens. *Microbiome* 5.
- 578 Sasse J, Martinoia E, Northen T. 2018. Feed Your Friends: Do Plant Exudates Shape the Root  
579 Microbiome? *Trends in Plant Science* 23: 25–41.
- 580 Schlaeppi K, Dombrowski N, Oter RG, Ver Loren van Themaat E, Schulze-Lefert P. 2014.  
581 Quantitative divergence of the bacterial root microbiota in *Arabidopsis thaliana*  
582 relatives. *Proceedings of the National Academy of Sciences* 111: 585–592.

- 583 Sturrock CJ, Woodhall J, Brown M, Walker C, Mooney SJ, Ray RV. 2015. Effects of damping-  
584 off caused by *Rhizoctonia solani* anastomosis group 2-1 on roots of wheat and oil seed  
585 rape quantified using X-ray Computed Tomography and real-time PCR. *Frontiers in Plant*  
586 *Science* 6.
- 587 Vannette RL, Fukami T. 2017. Dispersal enhances beta diversity in nectar microbes. *Ecology*  
588 *Letters* 20: 901–910.
- 589 Wagner M-H, Ducournau S, Luciani A, Léchappé J. 2012. From knowledge-based research  
590 towards accurate and rapid testing of seed quality in winter rape. *Seed Science Research*  
591 22: S80–S85.
- 592 Wallace JG, Kremling KA, Kovar LL, Buckler ES. 2018. Quantitative Genetics of the Maize Leaf  
593 Microbiome. *Phytobiomes Journal* 2: 208–224.
- 594 Walters WA, Jin Z, Youngblut N, Wallace JG, Sutter J, Zhang W, González-Peña A, Peiffer J,  
595 Koren O, Shi Q, *et al.* 2018. Large-scale replicated field study of maize rhizosphere  
596 identifies heritable microbes. *Proceedings of the National Academy of Sciences* 115:  
597 7368–7373.
- 598 Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve Bayesian Classifier for Rapid  
599 Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl. Environ.*  
600 *Microbiol.* 73: 5261–5267.
- 601 Zhao Q, Xiong W, Xing Y, Sun Y, Lin X, Dong Y. 2018. Long-Term Coffee Monoculture Alters  
602 Soil Chemical Properties and Microbial Communities. *Scientific Reports* 8: 6116.



603 **Table 1** Percentage of explained variance for beta-diversity estimators. Percentage of  
 604 explained variance estimated with PERMANOVA analysis ( $P < 0.01$ ) for  $\beta$ -diversity of  
 605 bacterial (*gyrB*) and fungal (ITS1) assemblages.

	<b>Year</b>	<b>Genotype</b>	<b>Year:Genotype</b>	<b>Genotype (Y1)</b>	<b>Genotype (Y2)</b>
<b><i>gyrB</i></b>	16.4	19.3	16.6	44	40.8
<b>ITS1</b>	65.5	11.5	13.3	66.2	90.7

606

607

608

609

610

611

612

613

614

615

616

617

618

619

621

622 **Table 2** Core members of *Brassica napus* seed microbiota. Prevalence (expressed as a  
 623 percentage) of each amplicon sequence variants (ASV) in *B. napus* seed samples collected in  
 624 year 1 (Y1), year 2 (Y2) and in seed samples (Others) of *Brassica oleracea*, *Brassica rapa* and  
 625 *Raphanus sativus* reported by Barret *et al.*, 2015; Rezki *et al.*, 2016; Rezki *et al.*, 2018.

ASV	Taxonomy	Y1 (%)	Y2 (%)	Others (%)
<b>Bacteria</b>				
ASV00001	<i>Pantoea agglomerans</i>	0.0	100.0	90.6
ASV00003	<i>Ralstonia pickettii</i>	0.0	100.0	0.6
ASV00028	<i>Pseudomonas</i>	20.0	100.0	34.9
ASV00038	<i>Sphingomonas</i>	0.0	100.0	10.6
ASV00046	<i>Frigoribacterium</i>	10.0	100.0	11.1
ASV00124	<i>Burkholderiales</i>	0.0	100.0	0.0
<b>Fungi</b>				
ASV0001	<i>Alternaria infectoria</i>	100.0	100.0	72.6
ASV0002	<i>Cladosporium</i>	100.0	100.0	97.6
ASV0003	<i>Cladosporium delicatulum</i>	100.0	100.0	100.0
ASV0004	<i>Botrytis fabae</i>	100.0	100.0	47.9
ASV0005	<i>Sporidiobolales sp.</i>	100.0	100.0	60.1
ASV0006	<i>Alternaria infectoria</i>	100.0	100.0	66.2
ASV0007	<i>Alternaria brassicae</i>	100.0	90.0	95.4
ASV0008	<i>Cryptococcus victoriae</i>	100.0	0.0	34.8
ASV0009	<i>Cryptococcus victoriae</i>	100.0	0.0	37.5
ASV0010	<i>Cryptococcus oeirensis</i>	100.0	0.0	37.8
ASV0011	<i>Filobasidium stepposum</i>	100.0	100.0	95.4
ASV0012	<i>Alternaria infectoria</i>	100.0	100.0	62.8
ASV0013	<i>Alternaria infectoria</i>	0.0	100.0	47.0
ASV0014	<i>Bulleromyces sp.</i>	100.0	0.0	0.3
ASV0015	<i>Bensingtonia sp.</i>	100.0	90.0	58.5
ASV0017	<i>Erysiphe cruciferarum</i>	100.0	20.0	12.8
ASV0018	<i>Cryptococcus oeirensis</i>	0.0	100.0	0.0
ASV0019	<i>Udeniomyces pannonicus</i>	60.0	100.0	14.0
ASV0021	<i>Cladosporium aggregatocaticratum</i>	100.0	100.0	47.3
ASV0022	<i>Alternaria</i>	90.0	100.0	0.3
ASV0025	<i>Cryptococcus victoriae</i>	0.0	100.0	0.0
ASV0028	<i>Cryptococcus tephrensensis</i>	100.0	90.0	32.9
ASV0029	<i>Cryptococcus carnescens</i>	90.0	100.0	32.9
ASV0030	<i>Cladosporium perangustum</i>	0.0	100.0	4.6
ASV0038	<i>Phaeosphaeria caricicola</i>	100.0	0.0	11.9
ASV0041	<i>Cladosporium ramotenellum</i>	0.0	100.0	5.2
ASV0044	<i>Cryptococcus victoriae</i>	0.0	100.0	0.0
ASV0054	<i>Ramularia vizellae</i>	0.0	100.0	0.9

626

627 **Figures, Tables and Supporting information captions.**

628 **Fig. 1 Diversity of seed-associated microbial assemblages of *B. napus*.** Seed samples of nine  
629 genotypes were collected during two consecutive years (Y1 and Y2). Community profiling of  
630 the seed microbiota was performed on five seed subsamples per genotype. (A) Estimated  
631 richness (Chao1 index), (B) diversity (Inverse Simpson's index) and (C) phylogenetic diversity  
632 (Faith's phylogenetic diversity index) were monitored with *gyrB* ASVs for bacteria. (D)  
633 Observed richness (number of detected ASVs) and (E) diversity (Inverse Simpson's index)  
634 were assessed with ITS1 ASVs for fungi.

635

636 **Fig. 2 Similarities in microbial composition between *B. napus* genotypes seed-associated**  
637 **microbial assemblages.** Similarities in seed microbial composition were estimated with  
638 weighted UniFrac distance (A) and Bray-Curtis index for bacterial and fungal assemblages (B),  
639 respectively. Principal coordinate analysis (PCoA) was used for ordination of weighted  
640 UniFrac distance and Bray-Curtis index. Samples are coloured according to their genotypes,  
641 while shape corresponded to the two years of production.

642

643 **Fig. 3 Taxonomic composition of *B. napus* seed microbiota.** Relative abundance of the most  
644 abundant bacterial (A) and fungal (B) order within *B. napus* seed samples collected from nine  
645 genotypes across two successive generations. Taxonomic affiliation of bacterial and fungal  
646 ASVs was performed with an in-house *gyrB* database (Bartoli *et al.*, 2018) and the UNITE v7.1  
647 database (Abarenkov *et al.*, 2010), respectively. Unknown taxa represented ASVs that are  
648 not affiliated at the order level.

649

650 **Fig. 4 Generalist and specific bacterial ASVs.** Prevalence of bacterial ASVs across plant  
651 genotypes were investigated with UpSetR (Lex *et al.*, 2014). Upper histograms represented  
652 the number of ASVs associated to one specific intersection in Y1 (A) or Y2 (B). Plant genotype  
653 associated with each particular intersection is highlighted with a dot. Intersection associated  
654 with all genotypes is displayed in red. The histogram located on the left of each UpSetR  
655 representation indicated the number of ASVs per genotype. Finally, boxplot represented the  
656 median relative abundance (log<sub>10</sub>) of bacterial ASVs associated with each intersection.

657

658 **Fig. 5 Generalist and specific fungal ASVs.** Prevalence of fungal ASVs across plant genotypes  
659 were investigated with UpSetR (Lex *et al.*, 2014). Upper histograms represent the number of  
660 ASVs associated with one specific intersection in Y1 (A) or Y2 (B). Plant genotype(s)  
661 associated with each particular intersection is (are) highlighted with a dot. Intersection  
662 associated with all genotypes is displayed in red. The histogram located on the left of each  
663 UpSetR representation indicated the number of ASVs per genotype. Finally, boxplot  
664 represented the median relative abundance (log<sub>10</sub>) of fungal ASVs associated with each  
665 intersection.

666

667 **Fig. 6 Dynamics of seed germination of each genotype and year.** Germination was  
668 monitored *in vitro* with an automated phenotyping platform (Phenotic; Ducournau *et al.*,  
669 2004, 2005; Wagner *et al.*, 2012). Four subsamples of 25 seeds by genotype and year (Y1 and  
670 Y2) were incubated at 20°C on germination paper (GE Healthcare, type 3644) continuously  
671 moistened for 5 days and image acquisition was performed every two hours.

672

673 **Fig. S1** Distribution of the 116 genotypes representing the diversity of WOSR in Western  
674 Europe, among the 5 WOSR genetic groups. Cultivars were genotyped using 628 SNP (Clarke  
675 *et al.*, 2016). The structure of the collection was studied using ADMIXTURE (Alexander *et al.*,  
676 2009). Each genetic group is represented with a colour. A cultivar belongs to the group of  
677 which the colour is majority. The 9 selected genotypes of the study are marked with a yellow  
678 star.

679

680 **Fig. S2** Climatic data for the cropping periods Y1 and Y2. (A) Rainfall level in mm (RR-DC) and  
681 mean humidity in % (UM-MD). (B) Mean calculated temperature (TMC), minimal (TN) and  
682 maximal (TX) temperatures in °C. All data are reported for 10 days (decade) during the years  
683 2015-2016 (Y1) and 2016-2017 (Y2) from records at Le Rheu station 35240002 – La Grande-  
684 Verrière – (48,113°N, -1,781°E) (INRA Agroclim network)

685

686 **Fig. S3 Similarities in microbial composition between *B. napus* genotypes seed-associated**  
687 **microbial assemblages, per year.** Similarities in seed microbial composition were estimated  
688 with weighted UniFrac distance (A, B) and Bray-Curtis index (C, D) for bacterial and fungal  
689 assemblages, respectively. Principal coordinate analysis (PCoA) was used for ordination of  
690 weighted UniFrac distance and Bray-Curtis index. Samples are colored according to their  
691 genotypes, while shape corresponded to the year (●=Y1; ▲=Y2).

692

693 **Fig. S4** Number of bacterial (A) and fungal (B) ASVs shared between years or specific of one  
694 year, for each genotype.

695

696 **Fig. S5** Relationships between *B. napus* genetic distances and similarities of microbial  
697 assemblage compositions. The relationship between plant genotype and microbial  
698 assemblage composition was estimated by fitting a linear regression model between Sokal-  
699 Michener genetic distance and unweighted UniFrac distance (bacteria; A, B) or Jaccard index  
700 (fungi; C, D) for Y1 (A, C) and Y2 (B, D).

701

702 **Fig. S6** Different phenotyping parameters calculated for seeds of each year and genotype.  
703 (A) Seed surface at initial time; (B) Seed imbibition 8h after initiation of imbibition; (C)  
704 Radicle elongation 8h after initiation of germination; (D) Time at which a half of the seeds  
705 have germinated. Significant results are marked with a red star.

706

707 **Table S1** Characteristics of the nine *B. napus* genotypes. Quality: 00 or ++ is for absence or  
708 presence of Glucosinolate-Erucic acid. WOSR groups are defined from the genetic diversity  
709 study of 116 WOSR accessions with 628 SNPs (Fig. S1).

710

711 **Table S2** Cropping conditions for the *B. napus* seed production.

712

713 **Table S3** Genotype-specific bacterial and fungal ASVs for Y1.

714

715 **Table S4** Genotype-specific bacterial and fungal ASVs for Y2.

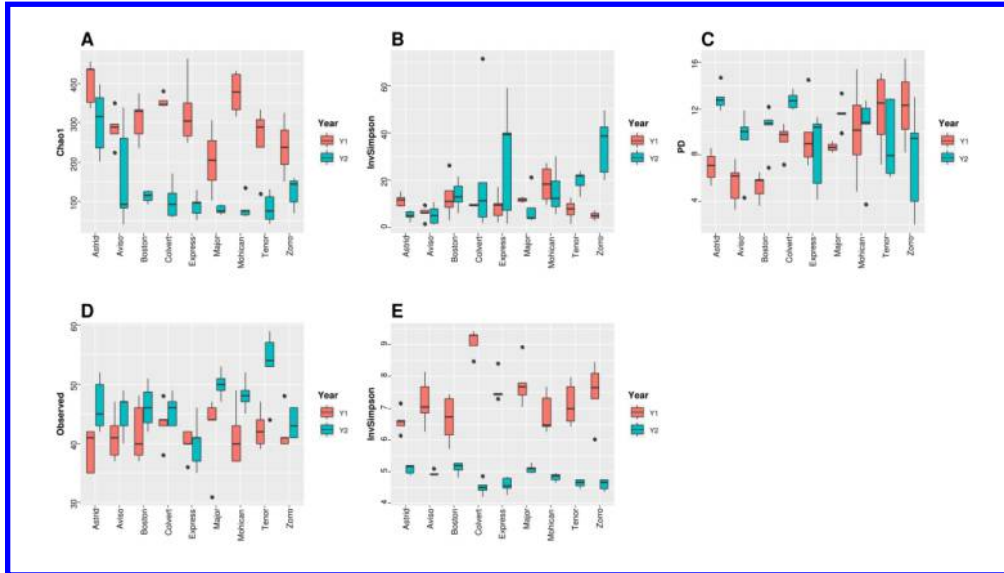


Fig. 1 Diversity of seed-associated microbial assemblages of *B. napus*. Seed samples of nine genotypes were collected during two consecutive years (Y1 and Y2). Community profiling of the seed microbiota was performed on five seed subsamples per genotype. (A) Estimated richness (Chao1 index), (B) diversity (Inverse Simpson's index) and (C) phylogenetic diversity (Faith's phylogenetic diversity index) were monitored with *gyrB* ASVs for bacteria. (D) Observed richness (number of detected ASVs) and (E) diversity (Inverse Simpson's index) were assessed with ITS1 ASVs for fungi.

940x529mm (72 x 72 DPI)

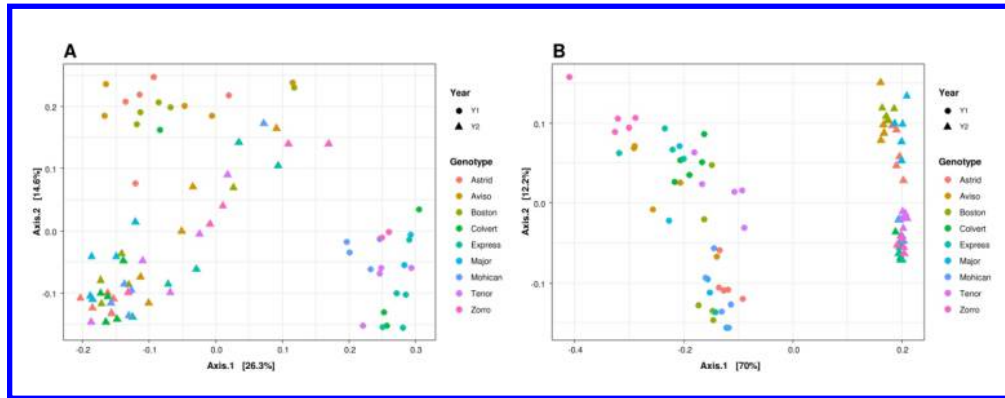


Fig. 2 Similarities in microbial composition between *B. napus* genotypes seed-associated microbial assemblages. Similarities in seed microbial composition were estimated with weighted UniFrac distance (A) and Bray-Curtis index for bacterial and fungal assemblages (B), respectively. Principal coordinate analysis (PCoA) was used for ordination of weighted UniFrac distance and Bray-Curtis index. Samples are coloured according to their genotypes, while shape corresponded to the two years of production.

940x361mm (72 x 72 DPI)



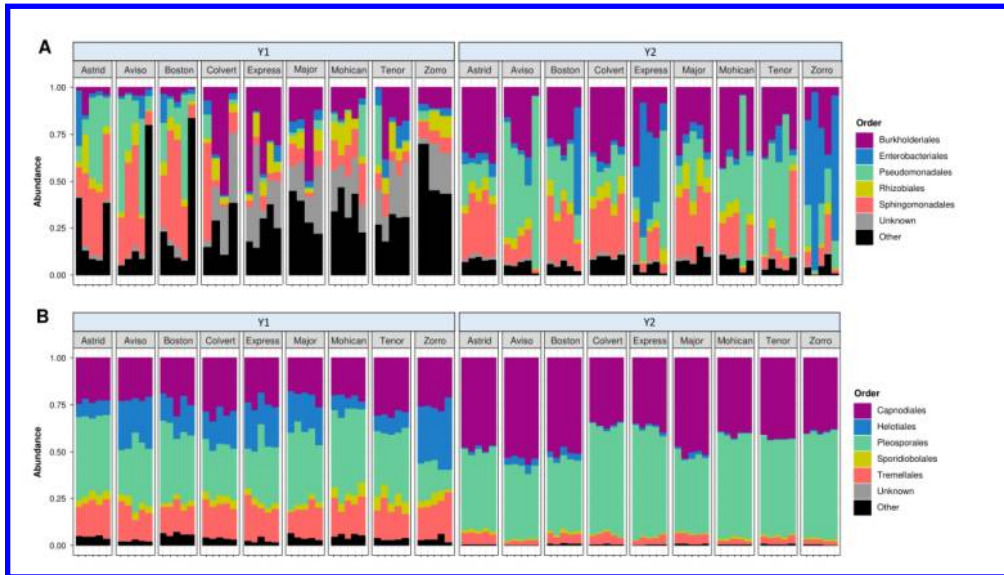


Fig. 3 Taxonomic composition of *B. napus* seed microbiota. Relative abundance of the most abundant bacterial (A) and fungal (B) order within *B. napus* seed samples collected from nine genotypes across two successive generations. Taxonomic affiliation of bacterial and fungal ASVs was performed with an in-house gyrB database (Bartoli et al., 2018) and the UNITE v7.1 database (Abarenkov et al., 2010), respectively. Unknown taxa represented ASVs that are not affiliated at the order level.

940x529mm (72 x 72 DPI)



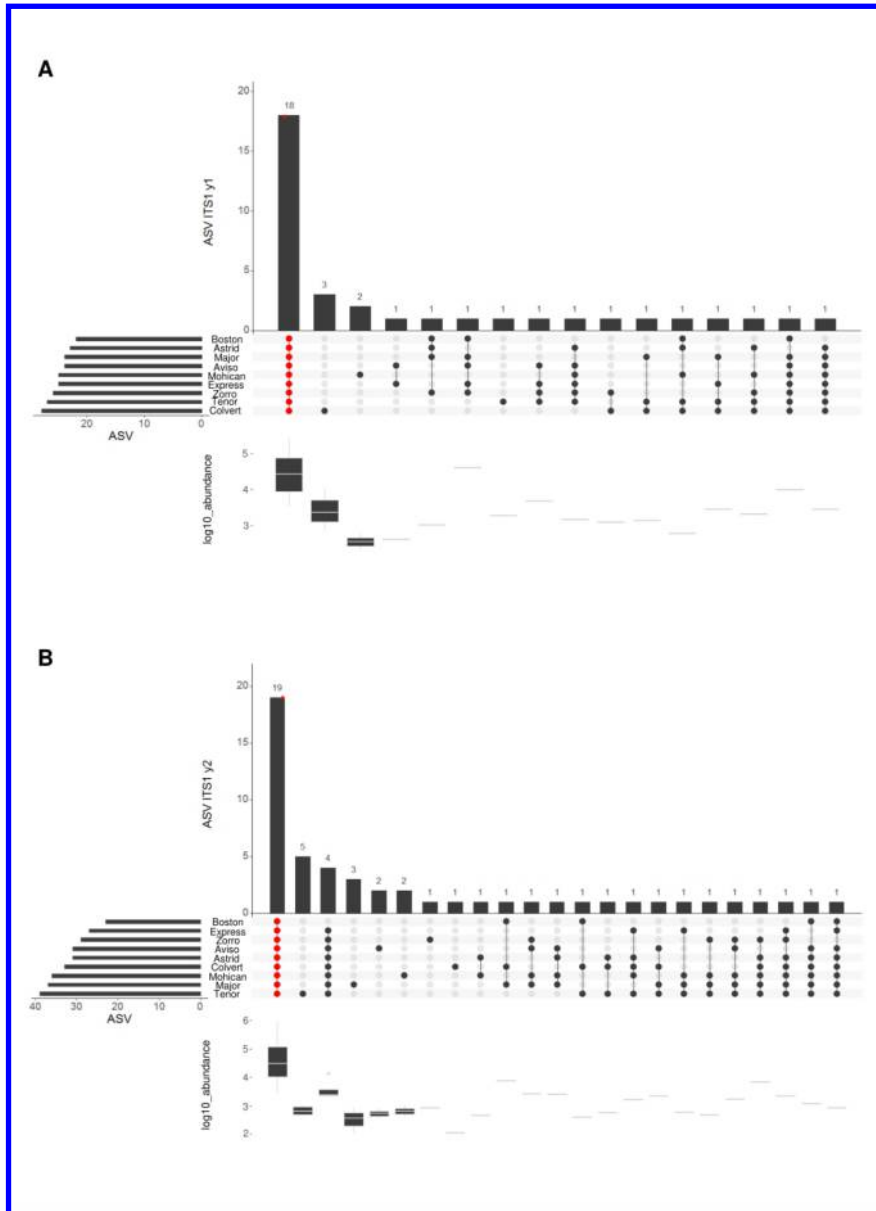


Fig. 5 Generalist and specific fungal ASVs. Prevalence of fungal ASVs across plant genotypes were investigated with UpSetR (Lex et al., 2014). Upper histograms represent the number of ASVs associated with one specific intersection in Y1 (A) or Y2 (B). Plant genotype(s) associated with each particular intersection is (are) highlighted with a dot. Intersection associated with all genotypes is displayed in red. The histogram located on the left of each UpSetR representation indicated the number of ASVs per genotype. Finally, boxplot represented the median relative abundance ( $\log_{10}$ ) of fungal ASVs associated with each intersection.

680x940mm (72 x 72 DPI)

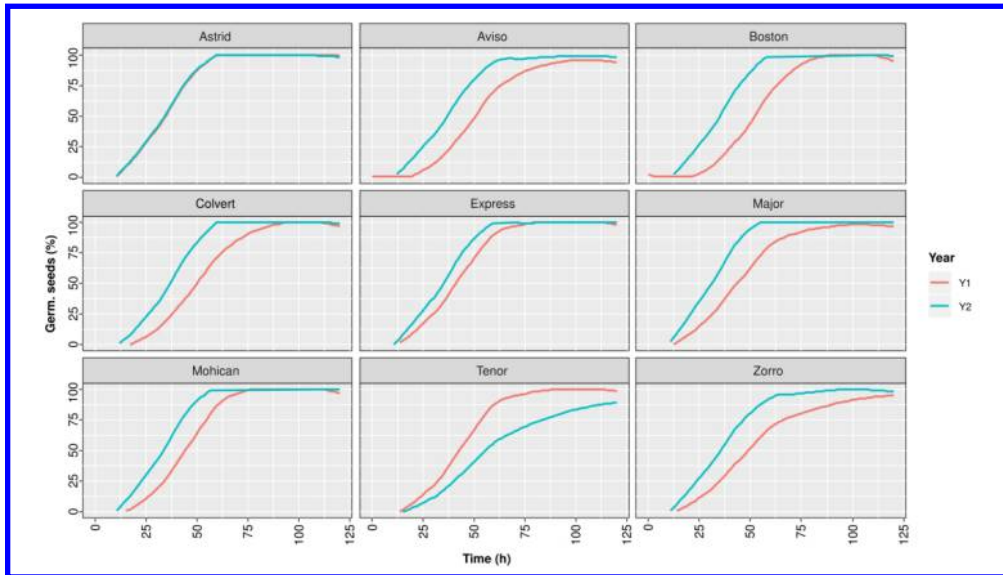


Fig. 6 Dynamics of seed germination of each genotype and year. Germination was monitored in vitro with an automated phenotyping platform (Phenotic; Ducournau et al., 2004, 2005; Wagner et al., 2012). Four subsamples of 25 seeds by genotype and year (Y1 and Y2) were incubated at 20°C on germination paper (GE Healthcare, type 3644) continuously moistened for 5 days and image acquisition was performed every two hours.

940x529mm (72 x 72 DPI)

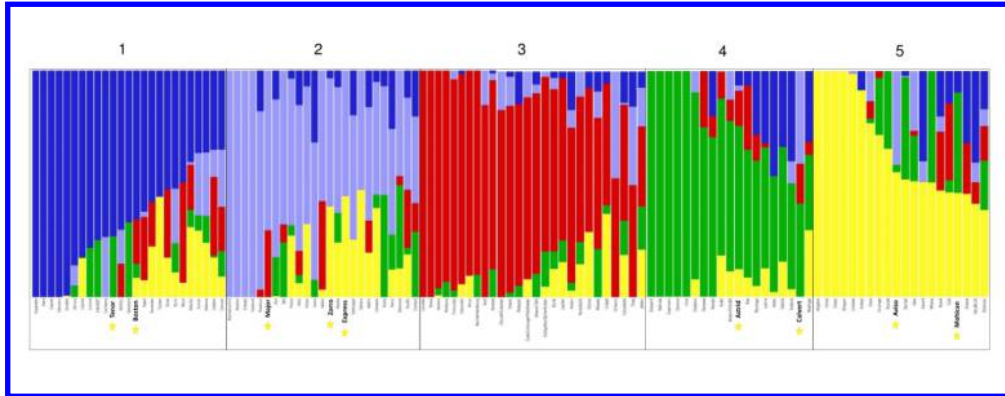


Fig. S1 Distribution of the 116 genotypes representing the diversity of WOSR in Western Europe, among the 5 WOSR genetic groups. Cultivars were genotyped using 628 SNP (Clarke et al., 2016). The structure of the collection was studied using ADMIXTURE (Alexander et al., 2009). Each genetic group is represented with a colour. A cultivar belongs to the group of which the colour is majority. The 9 selected genotypes of the study are marked with a yellow star.

3305x1275mm (72 x 72 DPI)

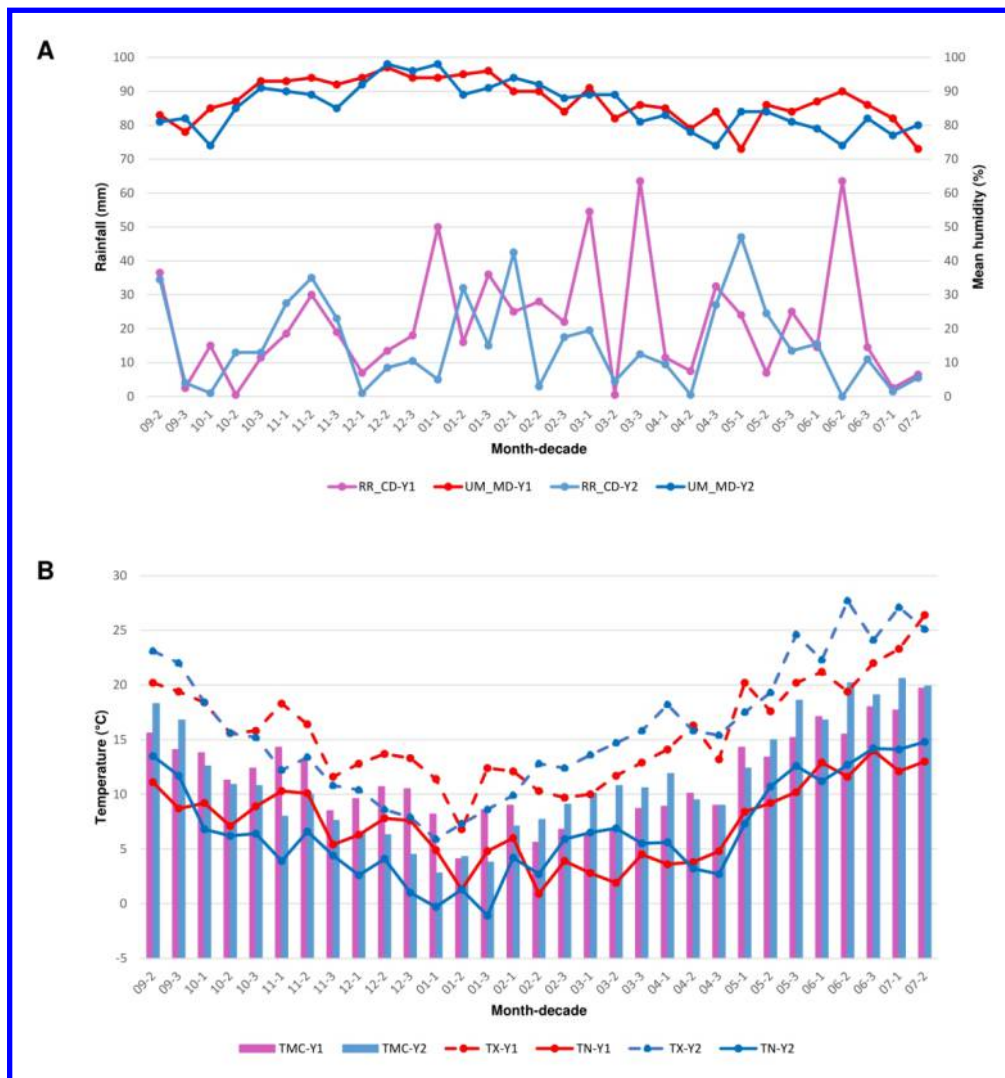


Fig. S2 Climatic data for the cropping periods Y1 and Y2. (A) Rainfall level in mm (RR-DC) and mean humidity in % (UM-MD). (B) Mean calculated temperature (TMC), minimal (TN) and maximal (TX) temperatures in °C. All data are reported for 10 days (decade) during the years 2015-2016 (Y1) and 2016-2017 (Y2) from records at Le Rheu station 35240002 – La Grande-Verrière – (48,113°N, -1,781°E) (INRA Agroclim network)

694x740mm (72 x 72 DPI)

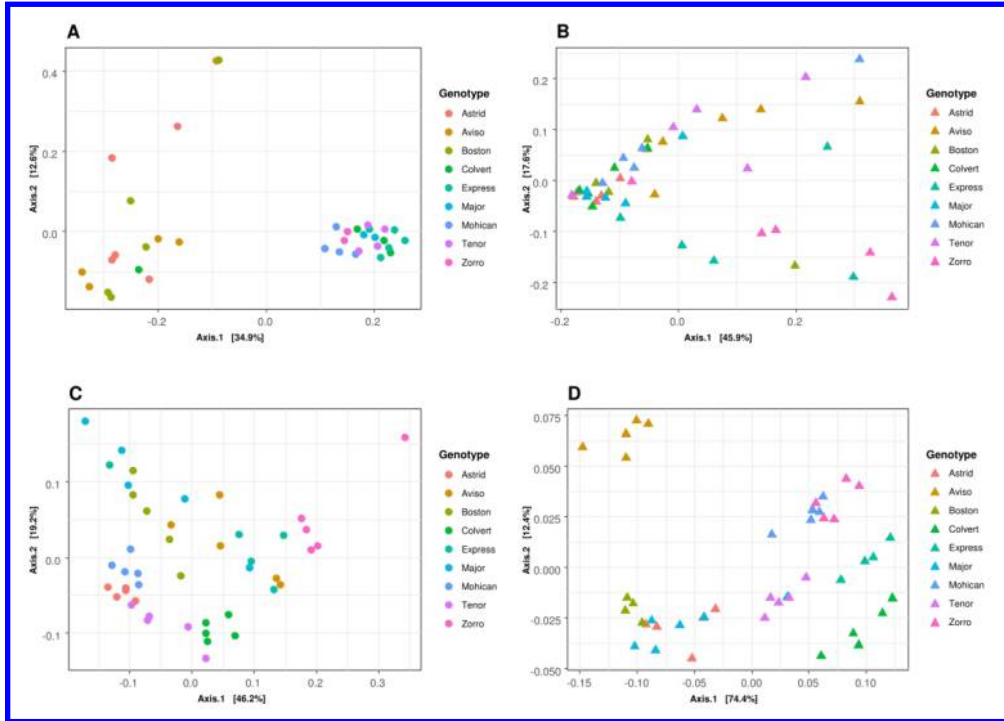


Fig. S3 Similarities in microbial composition between *B. napus* genotypes seed-associated microbial assemblages, per year. Similarities in seed microbial composition were estimated with weighted UniFrac distance (A, B) and Bray-Curtis index (C, D) for bacterial and fungal assemblages, respectively. Principal coordinate analysis (PCoA) was used for ordination of weighted UniFrac distance and Bray-Curtis index. Samples are colored according to their genotypes, while shape corresponded to the year ( $\square$ =Y1;  $\triangle$ =Y2).

940x666mm (72 x 72 DPI)

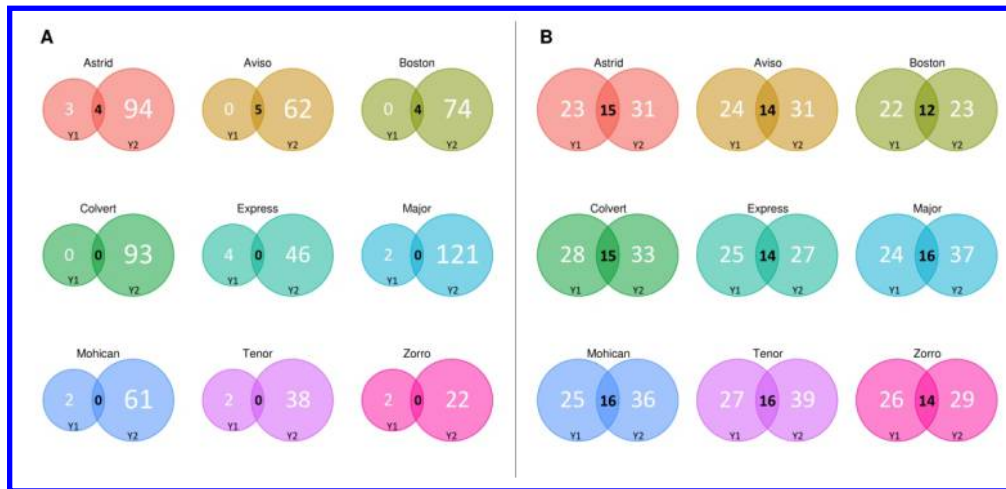


Fig. S4 Number of bacterial (A) and fungal (B) ASVs shared between years or specific of one year, for each genotype.

940x445mm (72 x 72 DPI)



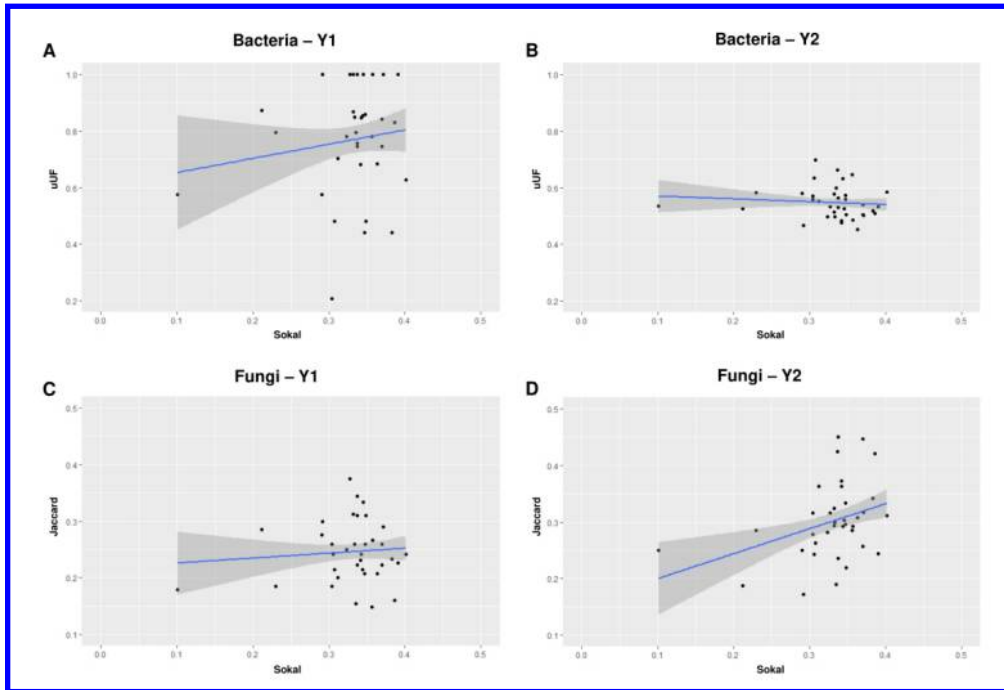


Fig. S5 Relationships between *B. napus* genetic distances and similarities of microbial assemblage compositions. The relationship between plant genotype and microbial assemblage composition was estimated by fitting a linear regression model between Sokal-Michener genetic distance and unweighted UniFrac distance (bacteria; A, B) or Jaccard index (fungi; C, D) for Y1 (A, C) and Y2 (B, D).

861x583mm (72 x 72 DPI)

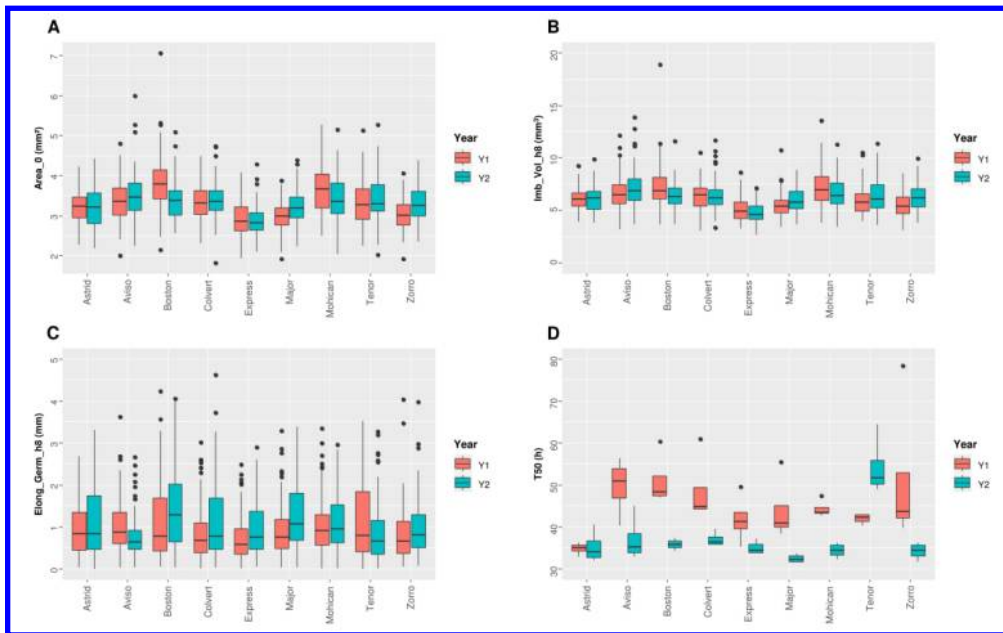


Fig. S6 Different phenotyping parameters calculated for seeds of each year and genotype. (A) Seed surface at initial time; (B) Seed imbibition 8h after initiation of imbibition; (C) Radicle elongation 8h after initiation of germination; (D) Time at which a half of the seeds have germinated. Significant results are marked with a red star.

987x611mm (72 x 72 DPI)

**Table S1** Characteristics of the nine *B. napus* genotypes. Quality: 00 or ++ is for absence or presence of Glucosinolate-Erucic acid. WOSR groups are defined from the genetic diversity study of 116 WOSR accessions with 628 SNPs (Fig. S1).

<b>Name</b>	<b>Quality</b>	<b>Year of creation</b>	<b>Breeder (country)</b>	<b>WOSR genetic group</b>
Astrid	00	2003	Euralis (UK )	4
Aviso	00	2000	Danisco (France)	5
Boston	00	2000	Limagrain (UK)	1
Colvert	00	1997	Limagrain (UK)	4
Express	00	1993	NPZ (Germany)	2
Major	++	1977	INRA (France)	2
Mohican	00	1995	Euralis (France)	5
Tenor	00	1999	Momont/Limagrain (France)	1
Zorro	00	1994	NPZ (Germany)	2

**Table S2** Cropping conditions for the *B. napus* seed production.

Location of INRA fields in Le Rheu, France			
Years	Year code	GPS coordinates	
2012-2013	Y0	48°06'36.29"N	1°46'22.04"O
2015-2016	Y1	48°06'40.86"N	1°46'26.08"O
2016-2017	Y2	48°06'37.21"N	1°47'07.98"O

Distance between fields in meters			
	Y0 <sup>1</sup>	Y1	Y2
<b>Y0</b>	0		
<b>Y1</b>	170	0	
<b>Y2</b>	966	860	0

Cropping history					
Years	Year code	WOSR Genotypes	Sowing date	Date of self-pollination bag attach <sup>2</sup>	Harvest date
<b>2015-2016</b>	Y1				
		Astrid	11/09/2015	04/04/2016	15/07/2016
		Aviso	11/09/2015	10/04/2016	15/07/2016
		Boston	11/09/2015	10/04/2016	15/07/2016
		Colvert	11/09/2015	07/04/2016	15/07/2016
		Express	11/09/2015	07/04/2016	15/07/2016
		Major	11/09/2015	10/04/2016	15/07/2016
		Mohican	11/09/2015	08/04/2016	15/07/2016
		Tenor	11/09/2015	10/04/2016	15/07/2016
		Zorro	11/09/2015	10/04/2016	15/07/2016
<b>2016-2017</b>	Y2				
		Astrid	22/09/2016	04/04/2017	04/07/2017
		Aviso	22/09/2016	07/04/2017	04/07/2017
		Boston	22/09/2016	07/04/2017	04/07/2017
		Colvert	22/09/2016	05/04/2017	04/07/2017
		Express	22/09/2016	05/04/2017	04/07/2017
		Major	22/09/2016	07/04/2017	04/07/2017
		Mohican	22/09/2016	05/04/2017	04/07/2017
		Tenor	22/09/2016	06/04/2017	04/07/2017
		Zorro	22/09/2016	06/04/2017	04/07/2017

<sup>1</sup>Self-pollination with cages of 1.80 m height, 0.65 m diameter, covered with a polyester veil, Lenglard, France<sup>2</sup>30x70 mm bags ref SM570Y, Sealed air Cryovac, France

**Table S3** Genotype-specific bacterial and fungal ASVs for Y1.

Genotype	Bacterial ASVs			Fungal ASVs		
	ASV	Taxonomy	Ab (log10)	ASV	Taxonomy	Ab (log10)
Astrid	ASV00217	<i>Sphingomonas</i>	2.6	-	-	-
	ASV00220	<i>Sphingomonas</i>	3	-	-	-
	ASV00704	<i>Sphingomonas</i>	2.3	-	-	-
Aviso	ASV00047	<i>Sphingomonas</i>	3.3	-	-	-
Boston	ASV00046	<i>Frigoribacterium</i>	3.1	-	-	-
	ASV00178	<i>Sphingomonas</i>	2.8	-	-	-
Colvert	-	-	-	ASV0037	<i>Sclerotinia</i>	4
	-	-	-	ASV0047	<i>Cryptococcus</i>	3.4
	-	-	-	ASV0043	<i>Cystofilobasidium macerans</i>	2.9
Express	ASV00993	<i>Comamonadaceae</i>	2.6	-	-	-
Major	-	-	-	-	-	-
Mohican	-	-	-	ASV0023	<i>Holtermanniales</i>	2.8
	-	-	-	ASV0076	<i>Entylomatales</i>	2.3
Tenor	ASV00020	<i>Propionibacterium acnes</i>	2.7	ASV0062	<i>Cryptococcus victoriae</i>	3.3
Zorro	-	-	-	-	-	-

**Table S4** Genotype-specific bacterial and fungal ASVs for Y2.

Genotype	Bacterial ASVs			Fungal ASVs		
	ASV	Taxonomy	Ab (log <sub>10</sub> )	ASV	Taxonomy	Ab (log <sub>10</sub> )
Astrid	ASV00004	<i>Pantoea agglomerans</i>	4.2	-	-	-
	ASV00014	<i>Pseudomonas viridiflava</i>	3.7	-	-	-
	ASV00100	<i>Pseudomonas fluorescens</i>	4.0	-	-	-
	ASV00216	<i>Oxalobacteraceae</i>	3.4	-	-	-
	ASV00243	<i>Microbacteriaceae</i>	3.3	-	-	-
	ASV00268	<i>Pseudomonas</i>	3.4	-	-	-
	ASV00300	<i>Sphingomonas</i>	3.4	-	-	-
	ASV00309	<i>Oxalobacteraceae</i>	3.4	-	-	-
	ASV00330	<i>Sphingomonas</i>	3.2	-	-	-
	ASV00339	<i>Sphingomonas</i>	3.3	-	-	-
	ASV00446	<i>Sphingomonas</i>	3.1	-	-	-
	ASV00457	<i>Sphingomonas</i>	3.1	-	-	-
	ASV00482	<i>Oxalobacteraceae</i>	3.1	-	-	-
	ASV00510	<i>Frigoribacterium</i>	3.0	-	-	-
	ASV00637	<i>Sphingomonas</i>	2.9	-	-	-
	ASV00709	<i>Oxalobacteraceae</i>	2.8	-	-	-
	ASV00724	<i>Oxalobacteraceae</i>	2.8	-	-	-
	ASV00782	<i>Sphingomonas</i>	2.6	-	-	-
	ASV00804	<i>Sanguibacter keddieii</i>	2.7	-	-	-
	ASV00933	<i>Oxalobacteraceae</i>	2.5	-	-	-
ASV00944	<i>Actinobacteria</i>	2.6	-	-	-	
ASV00995	<i>Sphingomonas</i>	2.5	-	-	-	
ASV01202	<i>Sphingomonas</i>	2.5	-	-	-	
Aviso	ASV00016	<i>Pseudomonas</i>	4.9	ASV0089	<i>Alternaria infectoria</i>	2.9
	ASV00018	<i>Pseudomonas tolaasii</i>	4.9	ASV0114	<i>Alternaria infectoria</i>	2.6
	ASV00068	<i>Pantoea agglomerans</i>	3.1	-	-	-
	ASV00110	<i>Pseudomonas proteolytica</i>	3.1	-	-	-
	ASV00164	<i>Pseudomonas</i>	3.6	-	-	-
	ASV00285	<i>Rhizobium</i>	3.4	-	-	-
	ASV00297	<i>Sphingomonas</i>	3.3	-	-	-
	ASV00410	<i>Pseudomonas proteolytica</i>	3.2	-	-	-
	ASV00424	<i>Sphingomonas</i>	3.0	-	-	-
	ASV00449	<i>Pseudomonas</i>	2.9	-	-	-
	ASV00493	<i>Sphingomonas</i>	3.1	-	-	-
	ASV00540	<i>Curtobacterium</i>	3.0	-	-	-
	ASV00653	<i>Oxalobacteraceae</i>	2.9	-	-	-
	ASV00673	<i>Frigoribacterium</i>	2.8	-	-	-
	ASV00697	<i>Actinobacteria</i>	2.8	-	-	-
	ASV00737	<i>Pseudomonas fluorescens</i>	2.8	-	-	-
	ASV01479	<i>Frigoribacterium</i>	2.3	-	-	-
ASV01639	<i>Sphingomonas</i>	2.2	-	-	-	
Boston	ASV00030	<i>Erwinia billingiae</i>	4.6	-	-	-
	ASV00062	<i>Pseudomonas</i>	3.7	-	-	-
	ASV00064	<i>Pseudomonas</i>	3.1	-	-	-
	ASV00085	<i>Pseudomonas viridiflava</i>	3.6	-	-	-
	ASV00108	<i>Pseudomonas</i>	4.0	-	-	-
	ASV00137	<i>Pseudomonas</i>	3.8	-	-	-
	ASV00193	<i>Rhizobium</i>	3.4	-	-	-
	ASV00197	<i>Pseudomonas fluorescens</i>	3.7	-	-	-
	ASV00202	<i>Oxalobacteraceae</i>	3.7	-	-	-

	ASV00214	<i>Massilia</i>	3.6	-	-	-
	ASV00215	<i>Rhizobium</i>	3.4	-	-	-
	ASV00244	<i>Rahnella aquatilis</i>	2.8	-	-	-
	ASV00283	<i>Rhizobium</i>	3.4	-	-	-
	ASV00302	<i>Rhizobium</i>	3.2	-	-	-
	ASV00358	<i>Rhizobium</i>	3.3	-	-	-
	ASV00438	<i>Pseudomonas</i>	3.1	-	-	-
	ASV00473	<i>Sphingomonas</i>	3.0	-	-	-
	ASV00491	<i>Pseudomonas</i>	2.6	-	-	-
	ASV00523	<i>Sphingomonas</i>	2.9	-	-	-
	ASV00524	<i>Oxalobacteraceae</i>	3.0	-	-	-
	ASV00531	<i>Comamonadaceae</i>	3.0	-	-	-
	ASV00573	<i>Sphingomonas</i>	2.9	-	-	-
	ASV00610	<i>Rhizobium</i>	2.9	-	-	-
	ASV00903	<i>Oxalobacteraceae</i>	2.6	-	-	-
	ASV01010	<i>Oxalobacteraceae</i>	2.5	-	-	-
	ASV01142	<i>Oxalobacteraceae</i>	2.5	-	-	-
	ASV01318	<i>Morganella morganii</i>	2.4	-	-	-
	ASV01439	<i>Microbacterium</i>	2.2	-	-	-
Colvert	ASV00034	<i>Pseudomonas</i>	3.3	ASV0076	<i>Entylomatales</i>	2.0
	ASV00200	<i>Pseudomonas</i>	3.6	-	-	-
	ASV00207	<i>Sphingomonas</i>	3.3	-	-	-
	ASV00227	<i>Sphingomonas</i>	3.6	-	-	-
	ASV00270	<i>Rhizobium</i>	3.5	-	-	-
	ASV00296	<i>Sphingomonas</i>	3.4	-	-	-
	ASV00321	<i>Oxalobacteraceae</i>	3.0	-	-	-
	ASV00347	<i>Rhodobacteraceae</i>	3.3	-	-	-
	ASV00364	<i>Sphingomonas</i>	3.3	-	-	-
	ASV00377	<i>Sphingomonas</i>	3.1	-	-	-
	ASV00392	<i>Frigoribacterium</i>	3.2	-	-	-
	ASV00432	<i>Oxalobacteraceae</i>	3.2	-	-	-
	ASV00483	<i>Sphingomonas</i>	3.1	-	-	-
	ASV00549	<i>Oxalobacteraceae</i>	3.0	-	-	-
	ASV00557	<i>Sphingomonas</i>	3.0	-	-	-
	ASV00612	<i>Sphingomonas</i>	2.9	-	-	-
	ASV00615	<i>Rhizobium</i>	2.6	-	-	-
	ASV00664	<i>Methylobacterium</i>	2.8	-	-	-
	ASV00736	<i>Sphingomonadaceae</i>	2.7	-	-	-
	ASV00829	<i>Frigoribacterium</i>	2.7	-	-	-
	ASV01320	<i>Frigoribacterium</i>	2.4	-	-	-
	ASV01377	<i>Rhizobiales</i>	2.3	-	-	-
Express	ASV00037	<i>Pseudomonas</i>	3.0	-	-	-
	ASV00326	<i>Sphingomonas</i>	3.3	-	-	-
	ASV00371	<i>Sphingomonas</i>	3.3	-	-	-
	ASV00403	<i>Sphingomonas</i>	3.0	-	-	-
	ASV00525	<i>Sphingomonas</i>	2.9	-	-	-
	ASV00535	<i>Sphingomonas</i>	3.0	-	-	-
	ASV00608	<i>Oxalobacteraceae</i>	2.9	-	-	-
Major	ASV00017	<i>Pseudomonas fluorescens</i>	3.4	ASV0037	<i>Sclerotinia</i>	2.6
	ASV00128	<i>Sphingomonas</i>	3.0	ASV0047	<i>Cryptococcus</i>	2.9
	ASV00252	<i>Rhodococcus</i>	3.4	ASV0135	<i>Ulocladium chartarum</i>	2.0
	ASV00253	<i>Sphingomonas</i>	3.5	-	-	-
	ASV00261	<i>Sanguibacter keddieii</i>	2.5	-	-	-
	ASV00273	<i>Variovorax</i>	3.1	-	-	-
	ASV00275	<i>Rhizobium</i>	3.1	-	-	-

	ASV00284	<i>Sphingomonas</i>	3.4	-	-	-
	ASV00303	<i>Sphingomonas</i>	3.2	-	-	-
	ASV00322	<i>Sphingomonas</i>	3.3	-	-	-
	ASV00332	<i>Sphingomonas</i>	3.3	-	-	-
	ASV00363	<i>Methylobacterium</i>	3.3	-	-	-
	ASV00379	<i>Agrobacterium tumefaciens</i>	3.0	-	-	-
	ASV00389	<i>Sphingomonas</i>	3.2	-	-	-
	ASV00397	<i>Pseudomonas fluorescens</i>	3.2	-	-	-
	ASV00404	<i>Sphingomonas</i>	3.1	-	-	-
	ASV00490	<i>Microbacteriaceae</i>	3.0	-	-	-
	ASV00506	<i>Pseudomonas</i>	2.6	-	-	-
	ASV00515	<i>Oxalobacteraceae</i>	3.0	-	-	-
	ASV00572	<i>Sphingomonas</i>	2.9	-	-	-
	ASV00579	<i>Microbacteriaceae</i>	2.9	-	-	-
	ASV00588	<i>Rhizobium</i>	2.8	-	-	-
	ASV00603	<i>Sphingomonas</i>	2.9	-	-	-
	ASV00621	<i>Oxalobacteraceae</i>	2.9	-	-	-
	ASV00685	<i>Pseudomonas fluorescens</i>	2.8	-	-	-
	ASV00727	<i>Oxalobacteraceae</i>	2.8	-	-	-
	ASV00730	<i>Methylibium</i>	2.8	-	-	-
	ASV00738	<i>Sphingomonas</i>	2.8	-	-	-
	ASV00745	<i>Sphingomonas</i>	2.8	-	-	-
	ASV00773	<i>Sphingomonas</i>	2.7	-	-	-
	ASV00863	<i>Actinobacteria</i>	2.7	-	-	-
	ASV00875	<i>Frigoribacterium</i>	2.7	-	-	-
	ASV00916	<i>Clavibacter michoganensis</i>	2.6	-	-	-
	ASV00947	<i>Sphingomonas</i>	2.6	-	-	-
	ASV00966	<i>Alphaproteobacteria</i>	2.6	-	-	-
	ASV01070	<i>Oxalobacteraceae</i>	2.5	-	-	-
	ASV01327	<i>Curtobacterium</i>	2.4	-	-	-
	ASV01608	<i>Pseudomonas fluorescens</i>	2.2	-	-	-
Mohican	ASV00044	<i>Pseudomonas lurida</i>	3.3	ASV0081	<i>Alternaria infectoria</i>	3.0
	ASV00054	<i>Stenotrophomonas maltophilia</i>	3.0	ASV0110	<i>Gibellulopsis nigrescens</i>	2.6
	ASV00122	<i>Pseudomonas</i>	3.9	-	-	-
	ASV00239	<i>Agrobacterium</i>	3.2	-	-	-
	ASV00368	<i>Oxalobacteraceae</i>	3.3	-	-	-
	ASV00375	<i>Actinobacteria</i>	3.3	-	-	-
	ASV00414	<i>Sphingomonas</i>	3.2	-	-	-
	ASV00562	<i>Oxalobacteraceae</i>	3.0	-	-	-
	ASV00633	<i>Actinomycetales</i>	2.7	-	-	-
	ASV00639	<i>Actinomycetales</i>	2.8	-	-	-
	ASV00668	<i>Actinomycetales</i>	2.8	-	-	-
	ASV00743	<i>Rhizobiales</i>	2.7	-	-	-
	ASV00865	<i>Sphingomonas</i>	2.6	-	-	-
	ASV01174	<i>Hymenobacter</i>	2.4	-	-	-
	ASV01574	<i>Sphingomonas</i>	2.3	-	-	-
Tenor	ASV00022	<i>Pseudomonas fluorescens</i>	4.7	ASV0080	<i>Alternaria infectoria</i>	3.0
	ASV00289	<i>Pseudomonas rhizosphaerae</i>	3.2	ASV0083	<i>Pleosporaceae</i>	3.0
	ASV00562	<i>Oxalobacteraceae</i>	3.0	ASV0093	<i>Alternaria infectoria</i>	2.8
	-	-	-	ASV0100	<i>Alternaria triticina</i>	2.7
	-	-	-	ASV0108	<i>Alternaria</i>	2.6
Zorro	ASV00070	<i>Pseudomonas fluorescens</i>	4.2	ASV0086	<i>Pleosporaceae</i>	2.9