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

3

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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 mm²), volume 8h after initiation of imbibition (Imb_Vol; in mm³), 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[®] 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). 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 α -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 β -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.

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

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

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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 (%)
Bacteria				
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
Fungi				
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 (log₁₀) 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 (log₁₀) 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.

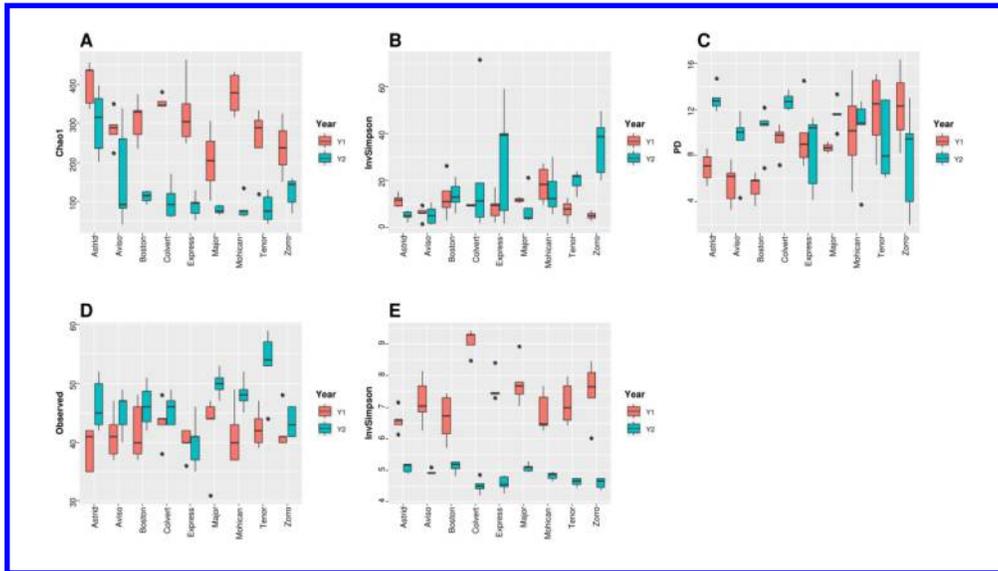


Fig. 1 Diversity of seed-associated microbial assemblages of *B. napus*. Seed samples of nine genotypes were collected during two consecutive years (Y1 and Y2). Community profiling of the seed microbiota was performed on five seed subsamples per genotype. (A) Estimated richness (Chao1 index), (B) diversity (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)

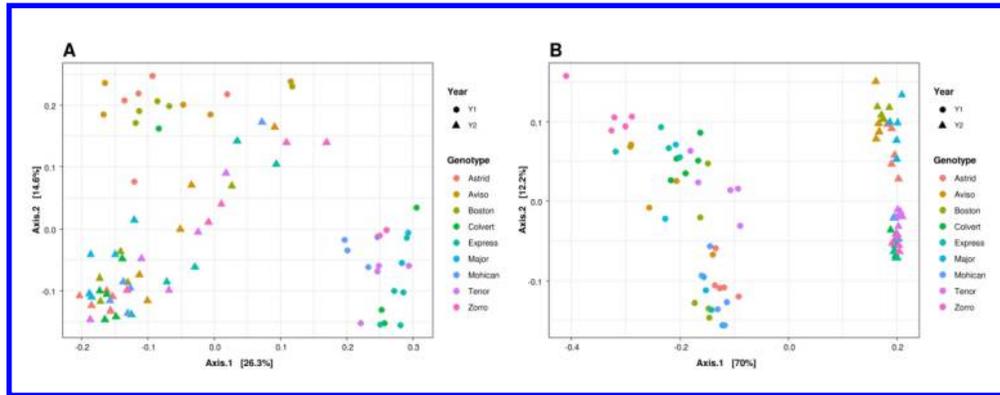


Fig. 2 Similarities in microbial composition between *B. napus* genotypes seed-associated microbial assemblages. Similarities in seed microbial composition were estimated with weighted UniFrac distance (A) and Bray-Curtis index for bacterial and fungal assemblages (B), respectively. Principal coordinate 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)

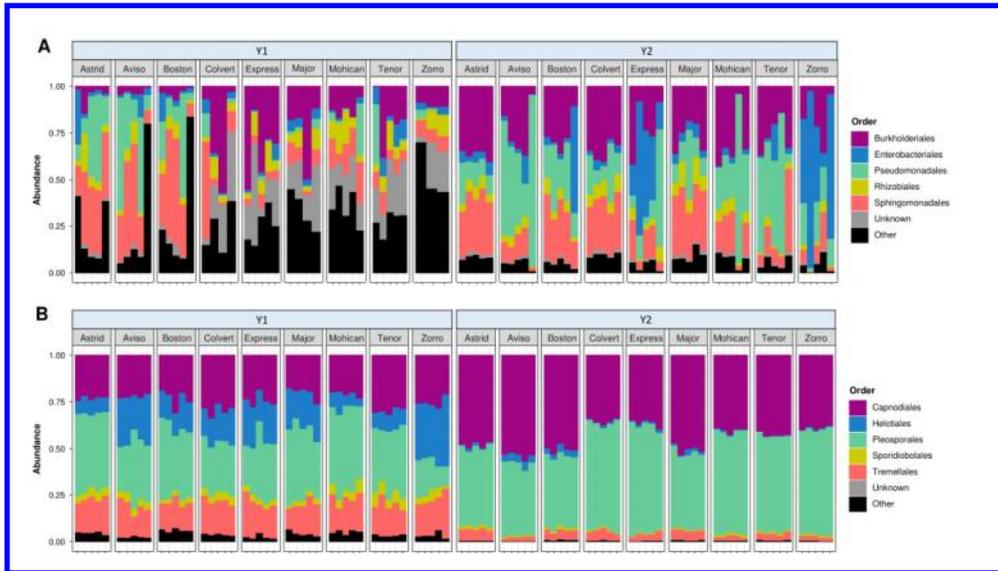


Fig. 3 Taxonomic composition of *B. napus* seed microbiota. Relative abundance of the most abundant bacterial (A) and fungal (B) order within *B. napus* seed samples collected from nine genotypes across two successive generations. Taxonomic affiliation of bacterial and fungal ASVs was performed with 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)

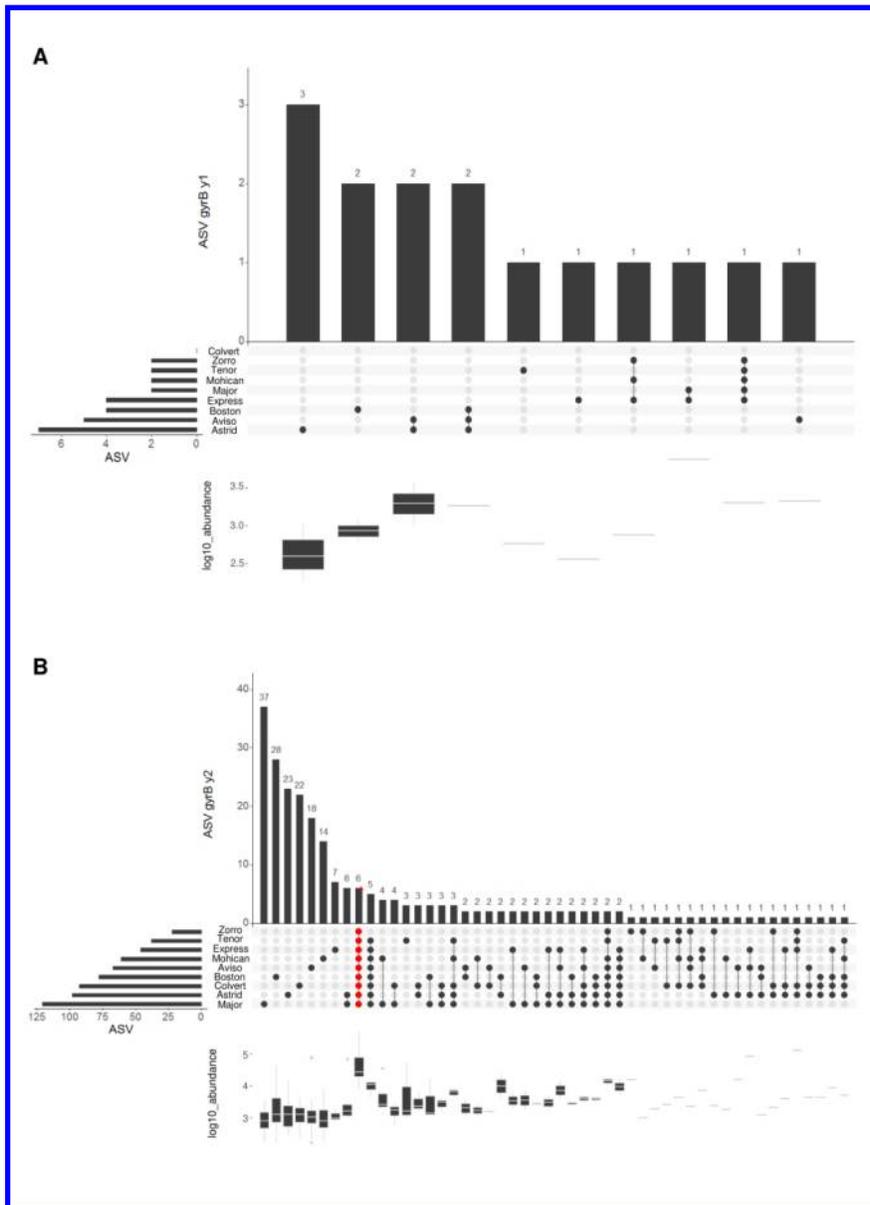


Fig. 4 Generalist and specific bacterial ASVs. Prevalence of bacterial ASVs across plant genotypes were investigated with UpSetR (Lex et al., 2014). Upper histograms represented the number of ASVs associated to one specific intersection in Y1 (A) or Y2 (B). Plant genotype associated with each particular intersection is 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 (log10) of bacterial ASVs associated with each intersection.

680x940mm (72 x 72 DPI)

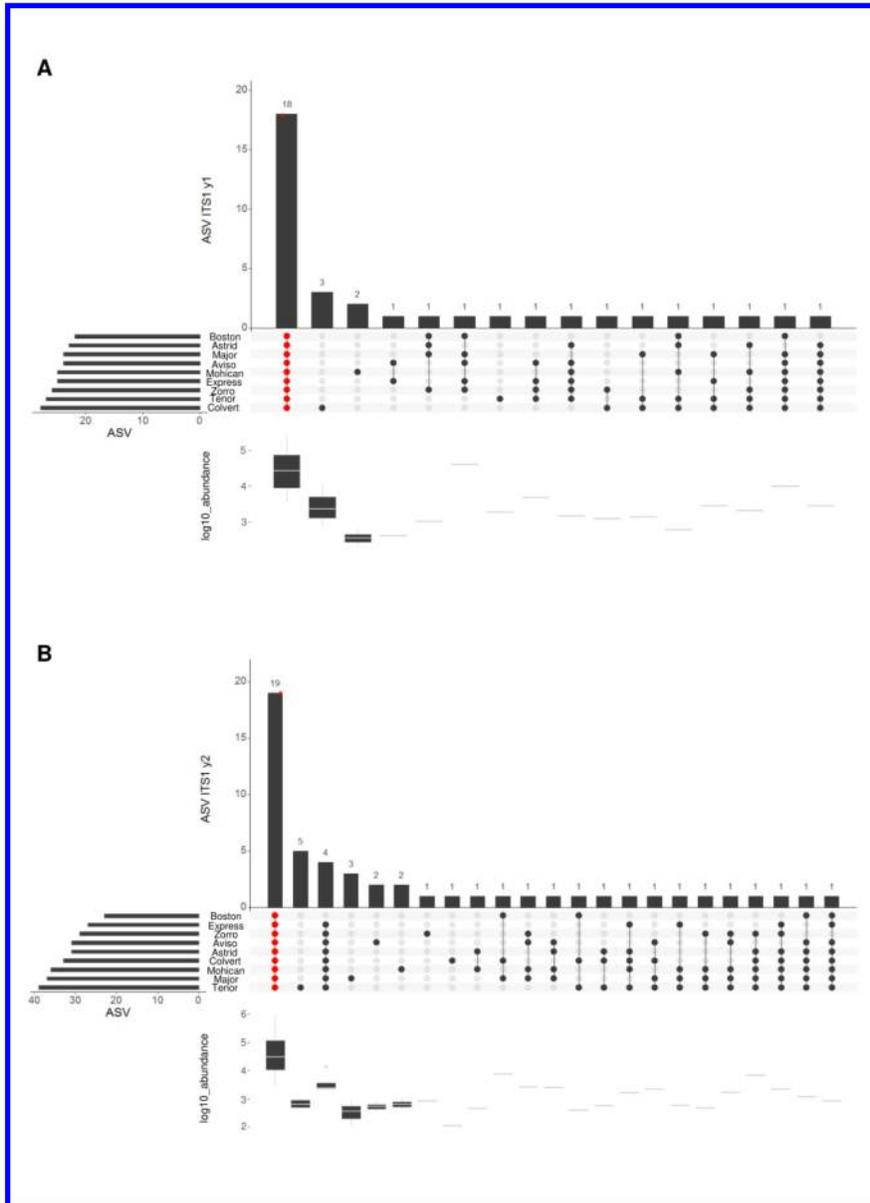


Fig. 5 Generalist and specific fungal ASVs. Prevalence of fungal ASVs across plant genotypes were investigated with UpSetR (Lex et al., 2014). Upper histograms represent the number of ASVs associated with one specific intersection in Y1 (A) or Y2 (B). Plant genotype(s) associated with each particular 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 (log₁₀) of fungal ASVs associated with each intersection.

680x940mm (72 x 72 DPI)

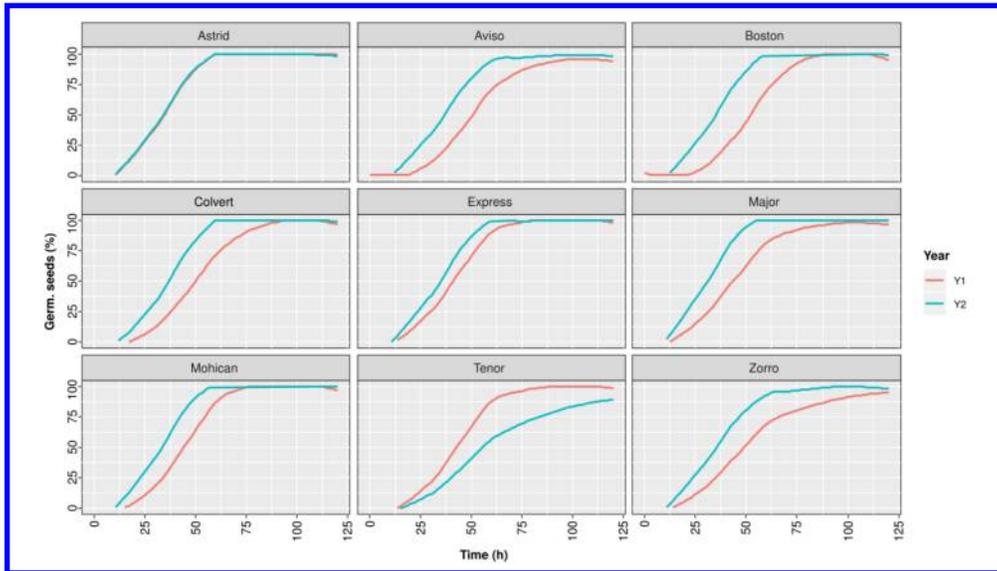


Fig. 6 Dynamics of seed germination of each genotype and year. Germination was monitored in vitro with an automated phenotyping platform (Phenotic; Ducournau et al., 2004, 2005; Wagner et al., 2012). Four subsamples of 25 seeds by genotype and year (Y1 and Y2) were incubated at 20°C on germination paper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

940x529mm (72 x 72 DPI)

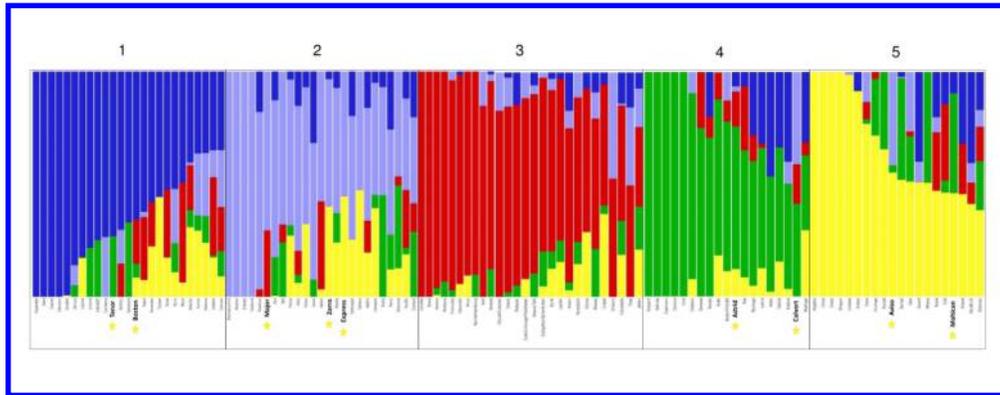


Fig. S1 Distribution of the 116 genotypes representing the diversity of WOSR in Western Europe, among the 5 WOSR genetic groups. Cultivars were genotyped using 628 SNP (Clarke et al., 2016). The structure of the collection was studied using ADMIXTURE (Alexander et al., 2009). Each genetic group is represented 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)

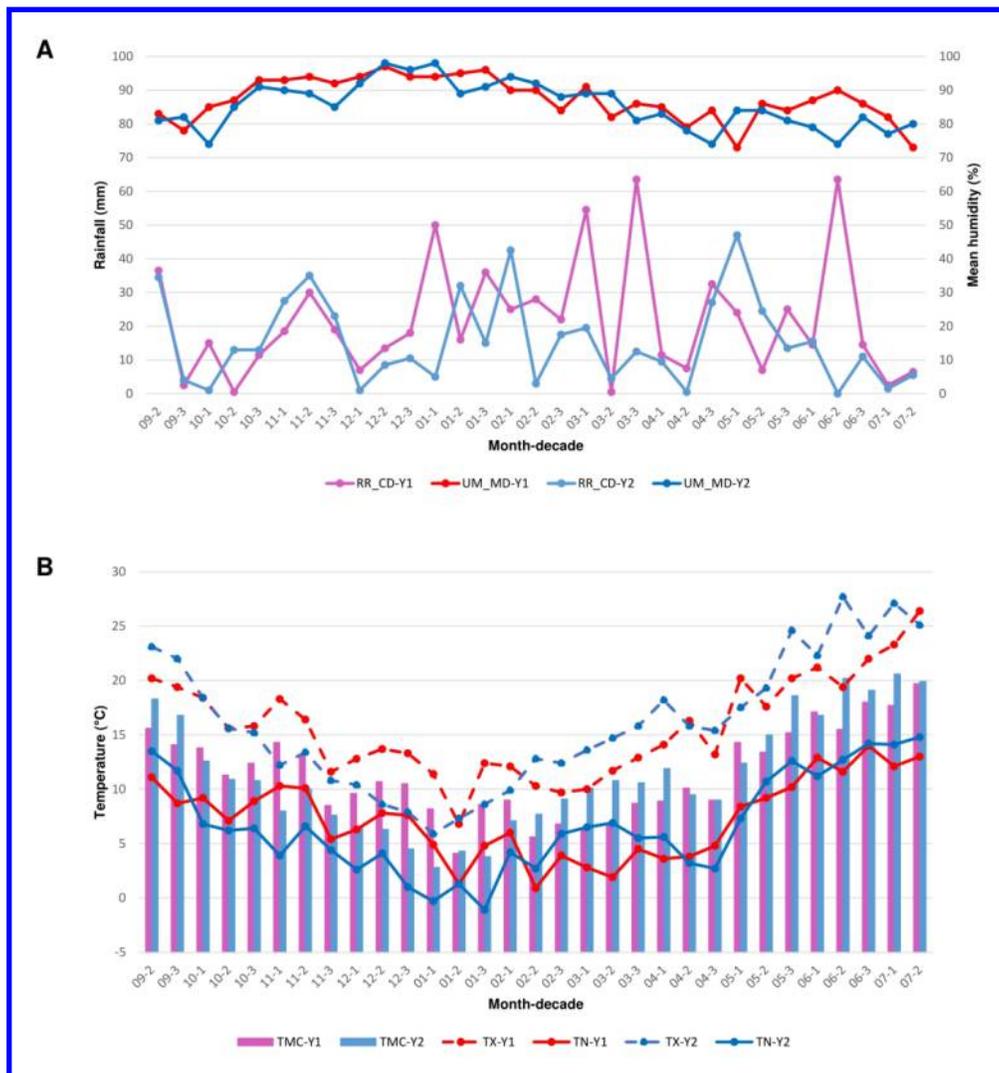


Fig. S2 Climatic data for the cropping periods Y1 and Y2. (A) Rainfall level in mm (RR-DC) and mean humidity in % (UM-MD). (B) Mean calculated temperature (TMC), minimal (TN) and maximal (TX) temperatures in °C. All data are reported for 10 days (decade) during the years 2015-2016 (Y1) and 2016-2017 (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)

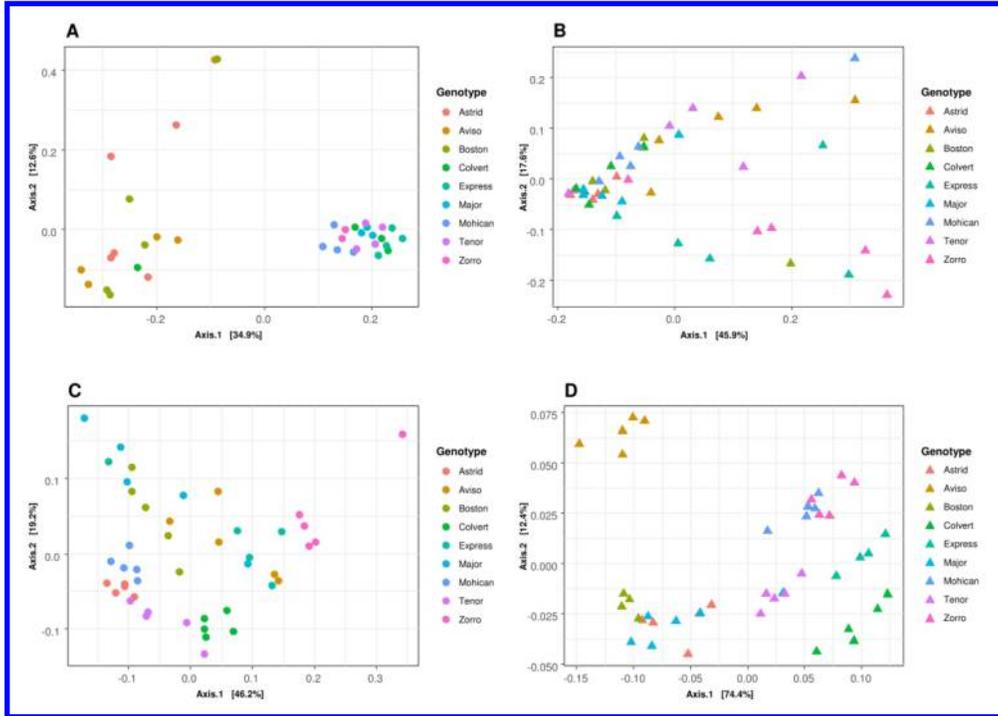


Fig. S3 Similarities in microbial composition between *B. napus* genotypes seed-associated microbial assemblages, per year. Similarities in seed microbial composition were estimated with weighted UniFrac distance (A, B) and Bray-Curtis index (C, D) for bacterial and fungal assemblages, respectively. 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)

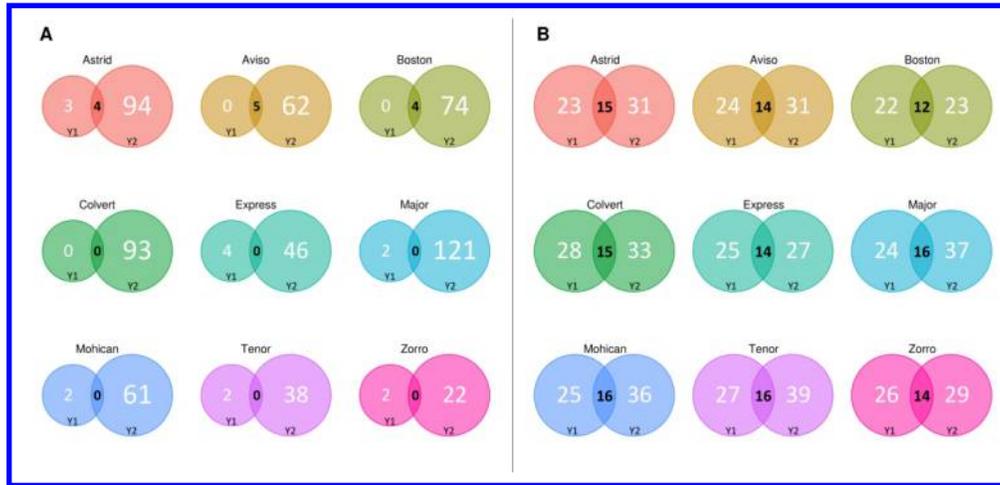


Fig. S4 Number of bacterial (A) and fungal (B) ASVs shared between years or specific of one year, for each genotype.

940x445mm (72 x 72 DPI)

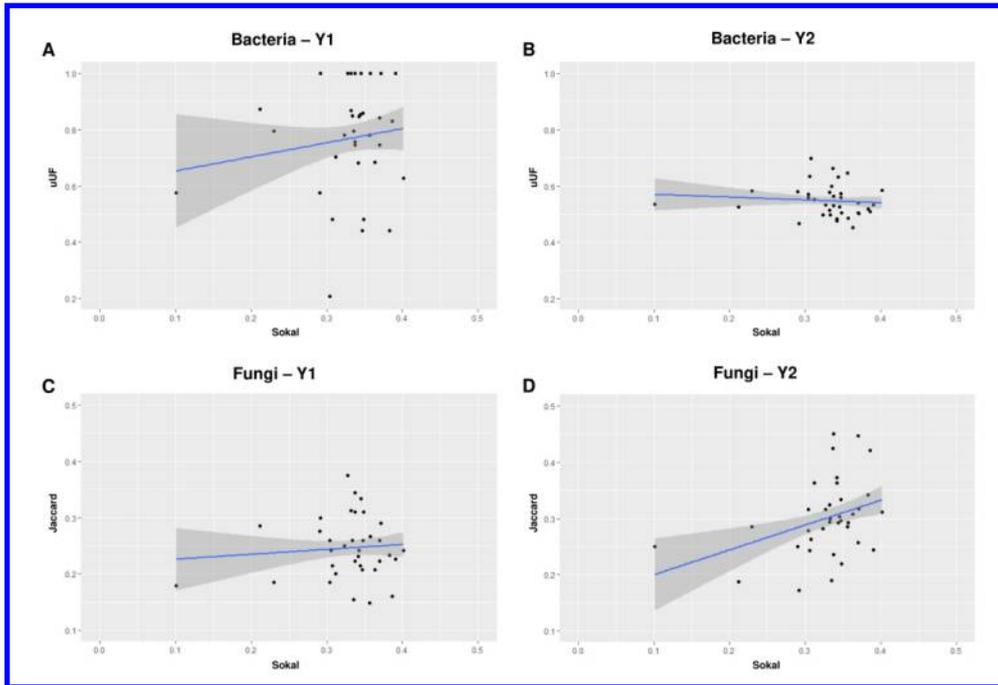


Fig. S5 Relationships between *B. napus* genetic distances and similarities of microbial assemblage compositions. The relationship between plant genotype and microbial assemblage composition was estimated by fitting a linear regression model between Sokal-Michener genetic distance and unweighted UniFrac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

861x583mm (72 x 72 DPI)

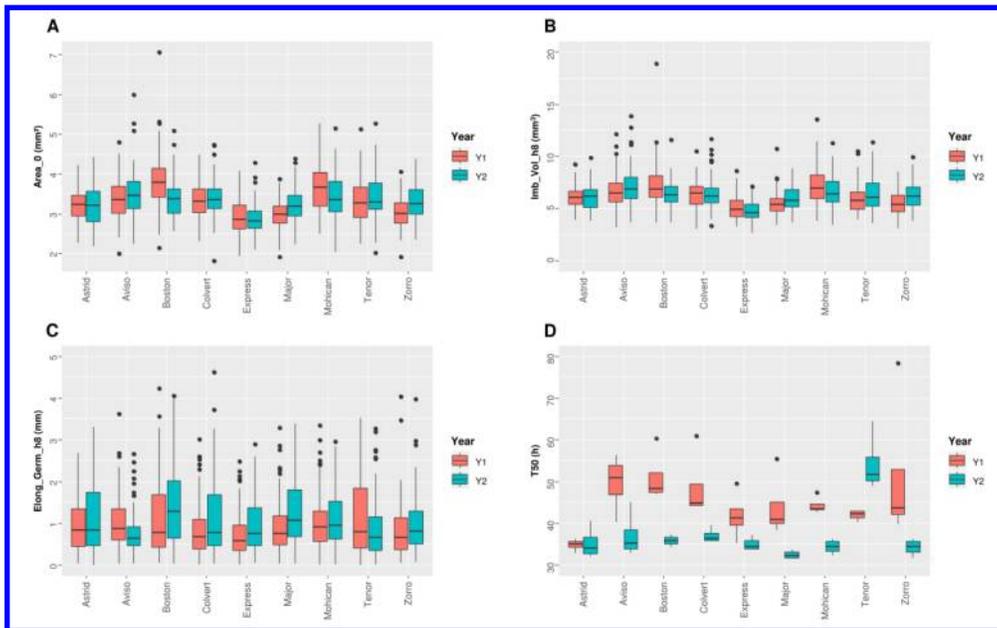


Fig. S6 Different phenotyping parameters calculated for seeds of each year and genotype. (A) Seed surface at initial time; (B) Seed imbibition 8h after initiation of imbibition; (C) Radicle elongation 8h after initiation of germination; (D) Time at which a half of the seeds have germinated. Significant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

Name	Quality	Year of creation	Breeder (country)	WOSR genetic group
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 ¹	Y1	Y2
Y0	0		
Y1	170	0	
Y2	966	860	0

Cropping history					
Years	Year code	WOSR Genotypes	Sowing date	Date of self-pollination bag attach ²	Harvest date
2015-2016	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
2016-2017	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

¹Self-pollination with cages of 1.80 m height, 0.65 m diameter, covered with a polyester veil, Lenglard, France²30x70 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 ₁₀)	ASV	Taxonomy	Ab (log ₁₀)
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