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

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ABSTRACT

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

Selection of winter oilseed rape (WOSR) genotypes.

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

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

Seed production.

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

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

Seed phenotyping.

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

Microbial DNA sample preparation.

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

Libraries construction and sequencing.

An initial PCR amplification was performed with the primer sets gyrB_aF64/gyrB_aR553 (Barret *et al.*, 2015) and ITS1F/ITS2 (Buée *et al.*, 2009), which target a portion of *gyrB* and the fungal ITS1 region, respectively. The cycling conditions for ITS1F/ITS2, were an initial denaturation at 94°C for 3 min, followed by 35 cycles of amplification at 94°C (30 s), 50°C (45 s) and 68°C (90 s), and a final elongation step at 68°C for 10 min. The cycling conditions for *gyrB* were: initial denaturation at 94°C for 3 min, 35 cycles of amplification at 94°C (30 s), 55°C (45 s) and 68°C (90 sec), and final elongation step at 68°C for 10 min. Amplicons were purified with magnetic beads (Sera-Mag^{TM}, Merck). A second PCR amplification was performed to incorporate Illumina adapters and barcodes. PCR cycling conditions were identical for the two molecular markers: a first denaturation step at 94°C (1 min), followed

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 10 min. Amplicons were purified as previously described and quantified with the QuantIT PicoGreen ds DNA Assay Kit (ThermoFisher Scientific). All the amplicons were pooled in equimolar concentrations and the concentration of the equimolar pool was monitored with quantitative PCR (KAPA SYBR® FAST, Merck). Amplicon libraries were mixed with 10% PhiX and sequenced with MiSeq reagent kit v2 500 cycles (one sequencing cartridge per year).

Sequence processing.

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

Microbial community analyses.

Analyses of diversity were conducted with the R package Phyloseq version 1.22.3 (McMurdie & Holmes, 2013). Data were normalized based on sequencing depth, using a rarefaction of 1,100 and 15,000 sequences per sample for *gyrB* and ITS1, respectively. Observed richness (number of ASV per sample), estimated richness (Chao1 index), and inverse Simpson's index were calculated with Phyloseq. Faith's phylogenetic diversity was calculated on *gyrB* dataset 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).

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

RESULTS

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

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

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

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

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

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

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

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

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

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

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

To assess if the composition of the seed microbiota could be related to genetic relatedness of *B. napus*, we compared Sokal-Michener genetic distance to Jaccard and uUF distances calculated with ITS1 and *gyrB* sequences, respectively (Supplementary Fig. S5). Coefficient of determination between Jaccard/uUF distances and Sokal-Michener distance were low for 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) assemblages. While a significant (P = 0.002) positive correlation between genetic distance and fungal assemblage composition was detected in Y2, the overall genetic similarities between the *B. napus* genotypes employed in this work is not a robust predictor of seed microbiota composition**.**

Impact of production year and host genotype on seed phenotypes.

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

DISCUSSION

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

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

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

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

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

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

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

In conclusion, the microbial assemblages of *B. napus* seeds are mainly influenced by the environment but to a lesser extent by the plant genotype. The inheritance of some abundant bacterial and fungal ASVs also differs between genotypes. Further studies will explore more extensively the host genetic determinants driving the microbial assemblages and the impact 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)
gyrB	16.4	19.3	16.6	44	40.8
ITS1	65.5	11.5	13.3	66.2	90.7

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

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

Figures, Tables and Supporting information captions.

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.

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.

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.

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.

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.

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.

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.

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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

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).

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.

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).

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

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

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

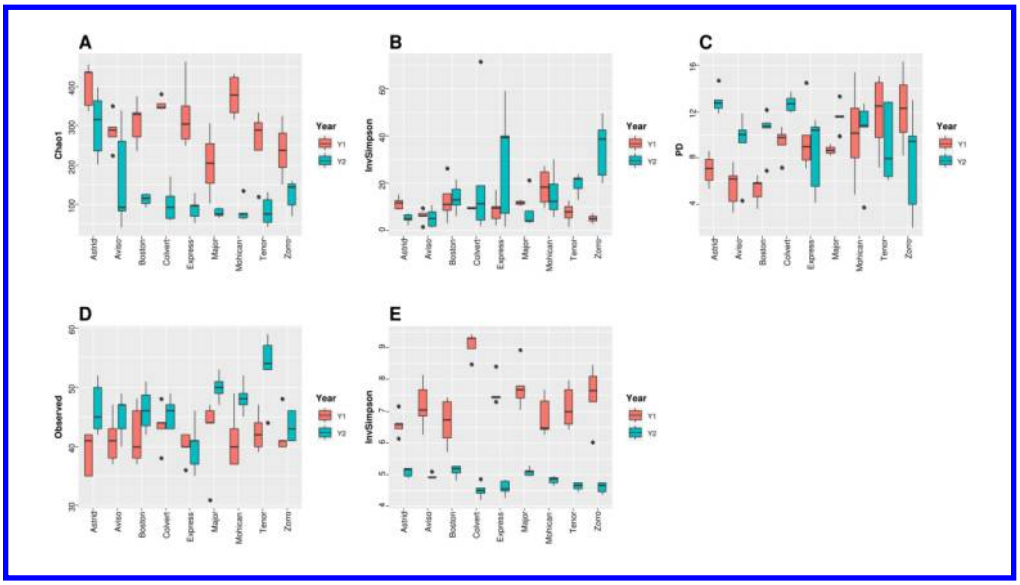


Fig. 1 Diversity of seed-associated microbial assemblages of B. napus. Seed samples of nine genotypes were collected during two consecutive years (Y1 and Y2). Community profiling of the seed microbiota was performed on five seed subsamples per genotype. (A) Estimated richness (Chao1 index), (B) diversity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

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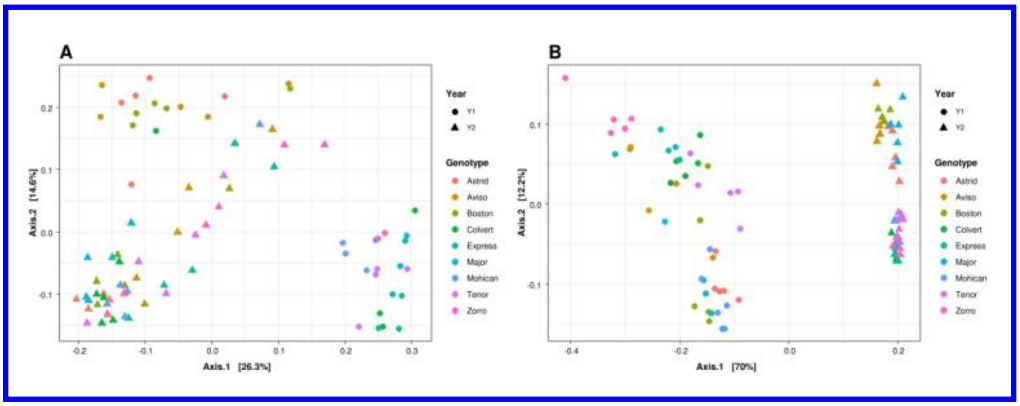


Fig. 2 Similarities in microbial composition between B. napus genotypes seed-associated microbial assemblages. Similarities in seed microbial composition were estimated with weighted UniFrac distance (A) and Bray-Curtis index for bacterial and fungal assemblages (B), respectively. Principal coordinate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

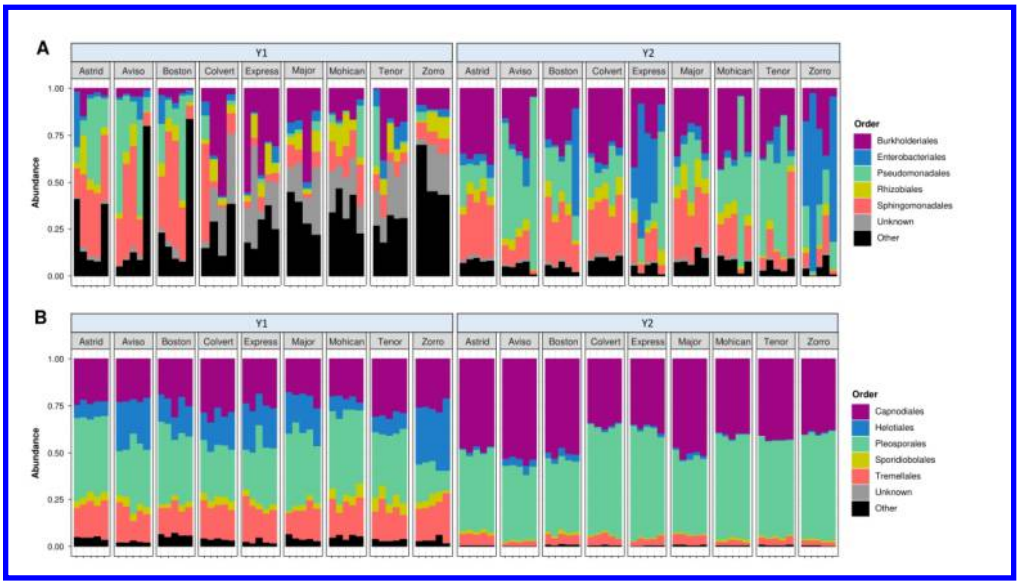


Fig. 3 Taxonomic composition of B. napus seed microbiota. Relative abundance of the most abundant bacterial (A) and fungal (B) order within B. napus seed samples collected from nine genotypes across two successive generations. Taxonomic affiliation of bacterial and fungal ASVs was performed with an in-house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

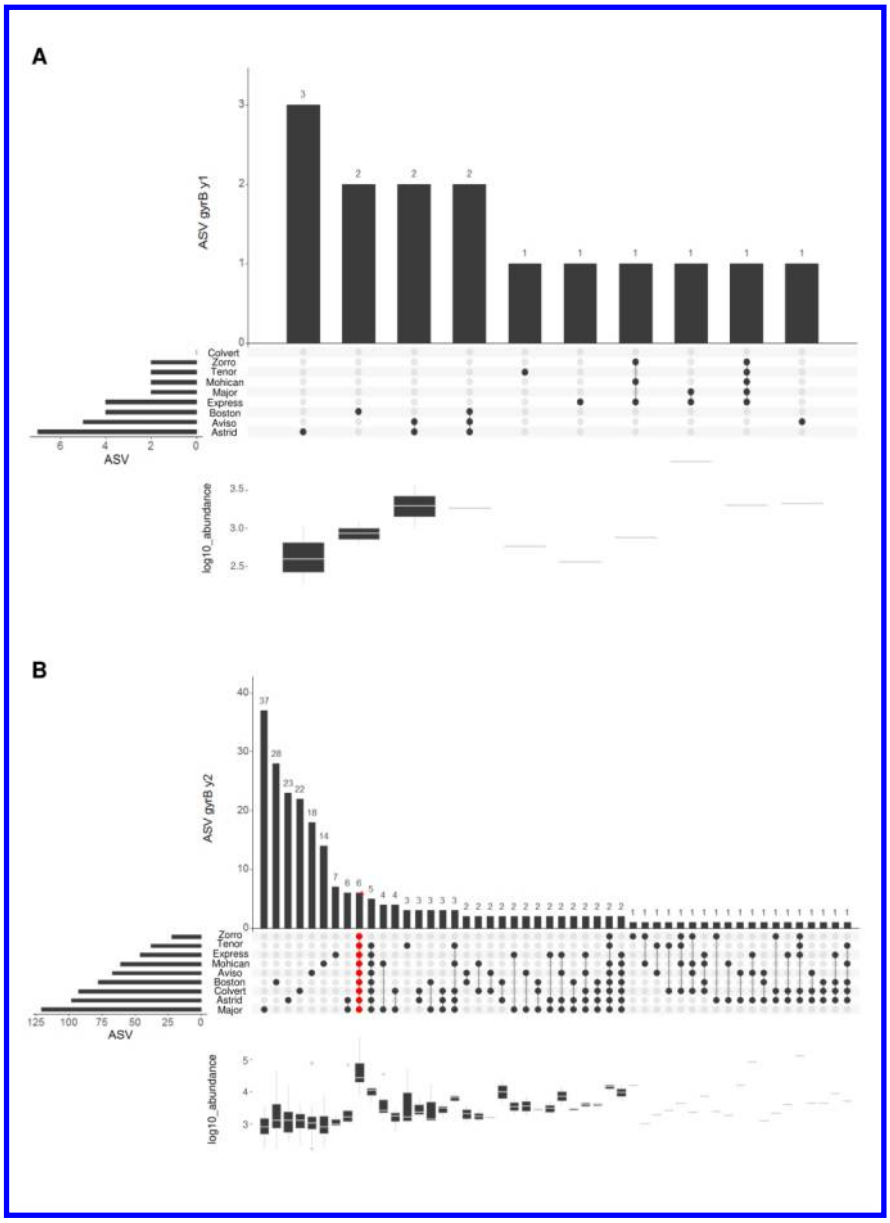


Fig. 4 Generalist and specific bacterial ASVs. Prevalence of bacterial ASVs across plant genotypes were investigated with UpSetR (Lex et al., 2014). Upper histograms represented the number of ASVs associated to one specific intersection in Y1 (A) or Y2 (B). Plant genotype associated with each particular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

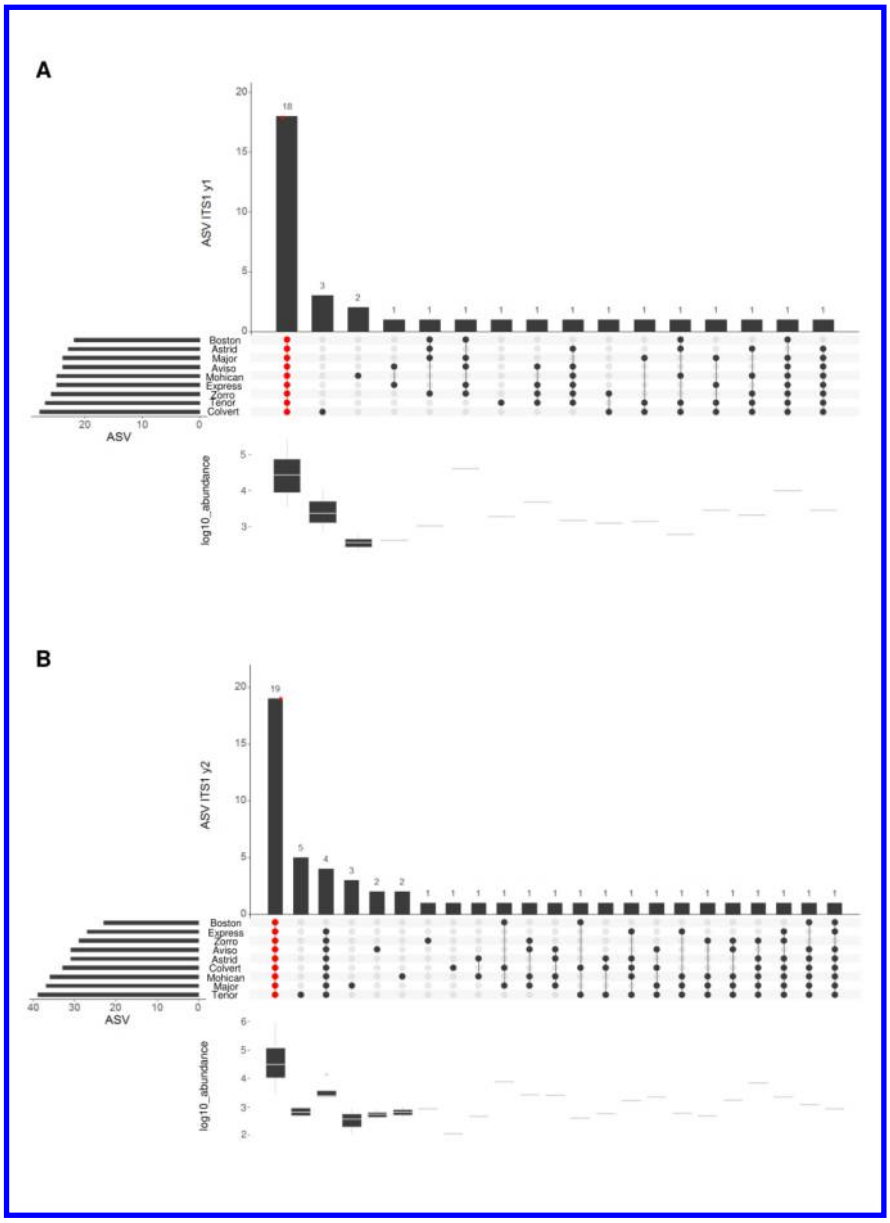


Fig. 5 Generalist and specific fungal ASVs. Prevalence of fungal ASVs across plant genotypes were investigated with UpSetR (Lex et al., 2014). Upper histograms represent the number of ASVs associated with one specific intersection in Y1 (A) or Y2 (B). Plant genotype(s) associated with each particular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

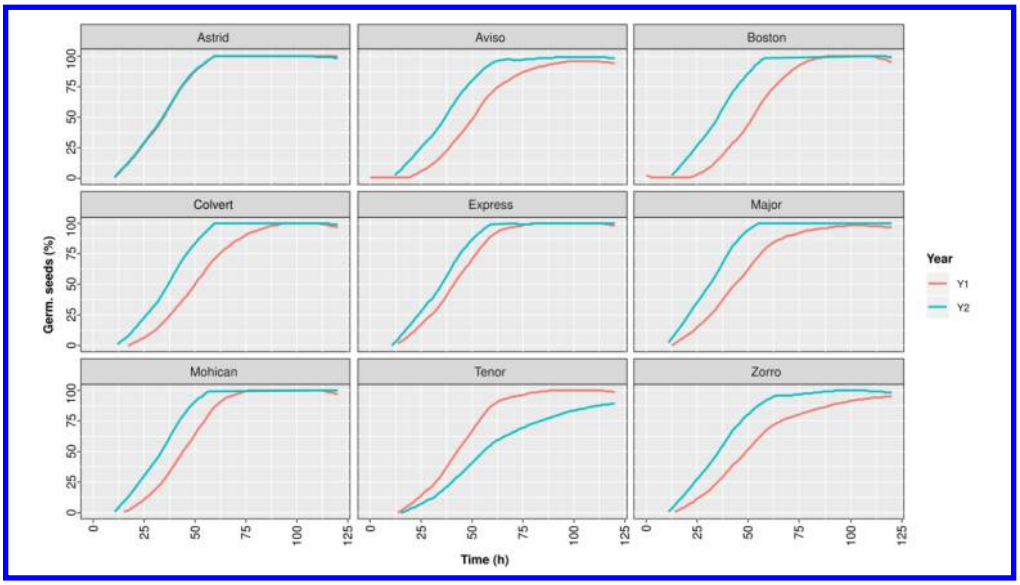


Fig. 6 Dynamics of seed germination of each genotype and year. Germination was monitored in vitro with an automated phenotyping platform (Phenotic; Ducournau et al., 2004, 2005; Wagner et al., 2012). Four subsamples of 25 seeds by genotype and year (Y1 and Y2) were incubated at 20°C on germination paper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

940x529mm (72 x 72 DPI)

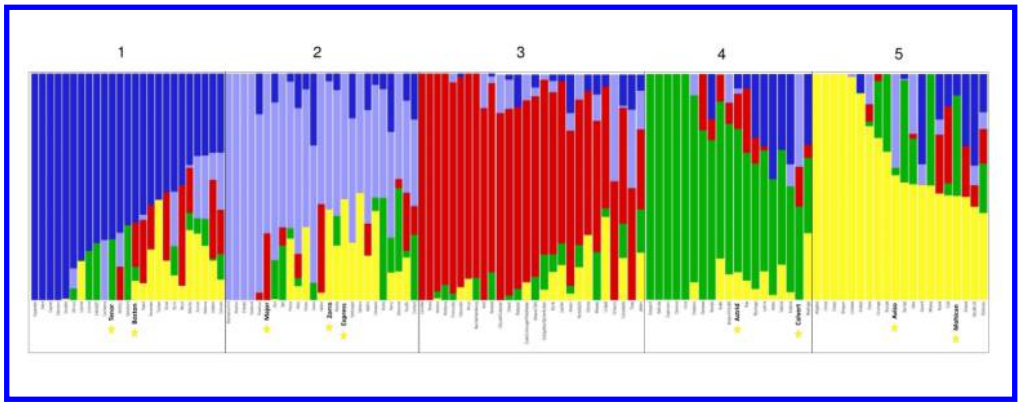


Fig. S1 Distribution of the 116 genotypes representing the diversity of WOSR in Western Europe, among the 5 WOSR genetic groups. Cultivars were genotyped using 628 SNP (Clarke et al., 2016). The structure of the collection was studied using ADMIXTURE (Alexander et al., 2009). Each genetic group is represented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

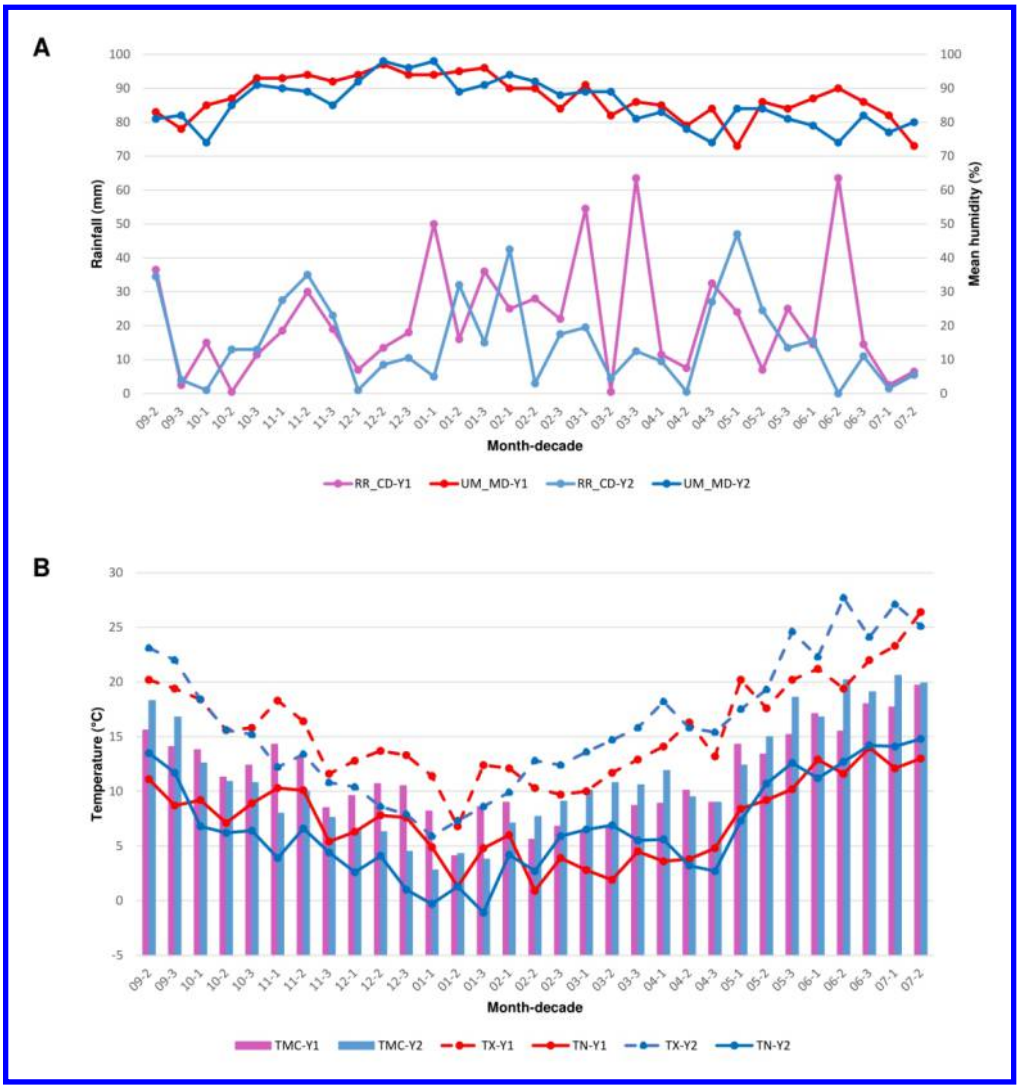


Fig. S2 Climatic data for the cropping periods Y1 and Y2. (A) Rainfall level in mm (RR-DC) and mean humidity in % (UM-MD). (B) Mean calculated temperature (TMC), minimal (TN) and maximal (TX) temperatures in °C. All data are reported for 10 days (decade) during the years 2015-2016 (Y1) and 2016-2017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

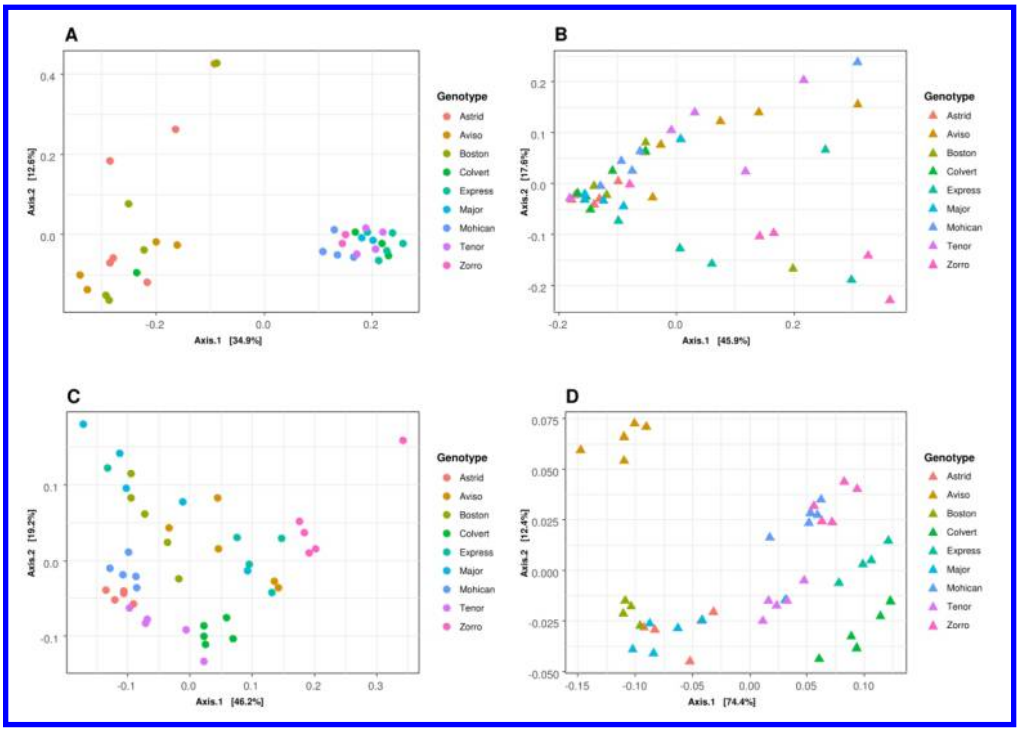


Fig. S3 Similarities in microbial composition between B. napus genotypes seed-associated microbial assemblages, per year. Similarities in seed microbial composition were estimated with weighted UniFrac distance (A, B) and Bray-Curtis index (C, D) for bacterial and fungal assemblages, respectively. Principal 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 (☐=Y1; ☐=Y2).

940x666mm (72 x 72 DPI)

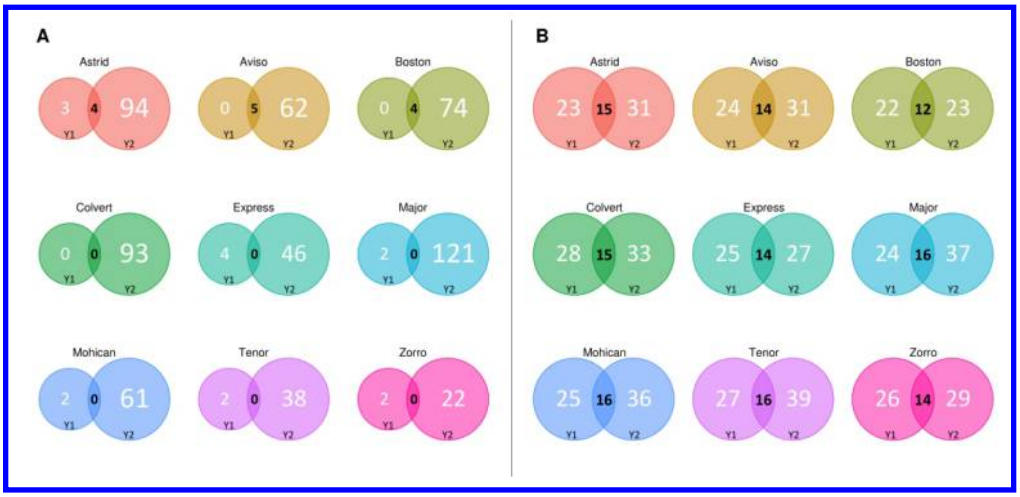


Fig. S4 Number of bacterial (A) and fungal (B) ASVs shared between years or specific of one year, for each genotype.

940x445mm (72 x 72 DPI)

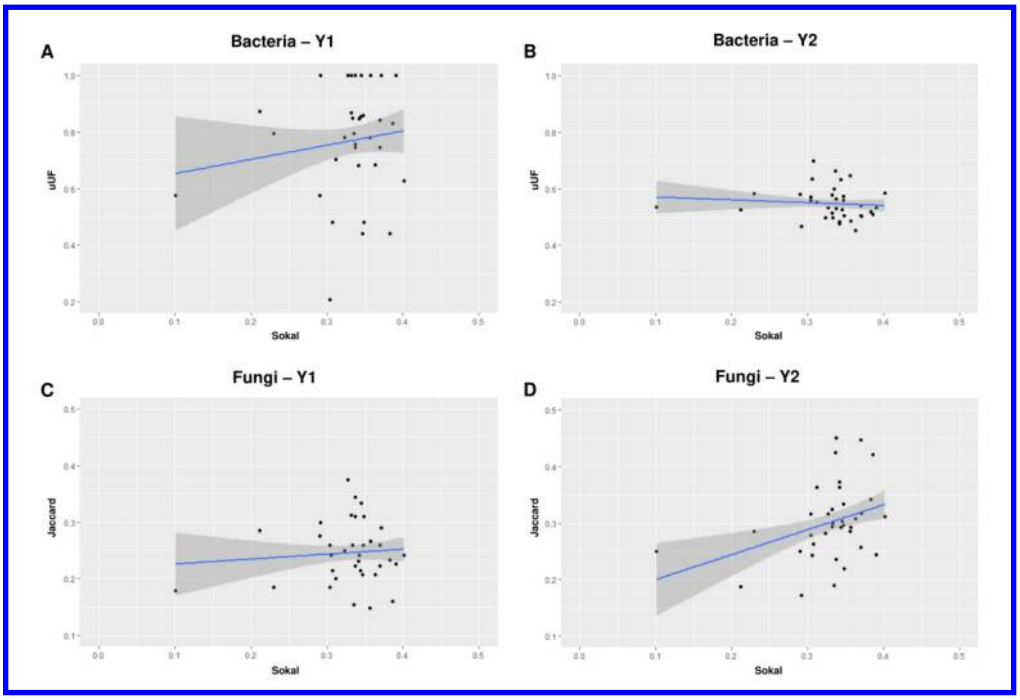


Fig. S5 Relationships between B. napus genetic distances and similarities of microbial assemblage compositions. The relationship between plant genotype and microbial assemblage composition was estimated by fitting a linear regression model between Sokal-Michener genetic distance and unweighted UniFrac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

861x583mm (72 x 72 DPI)

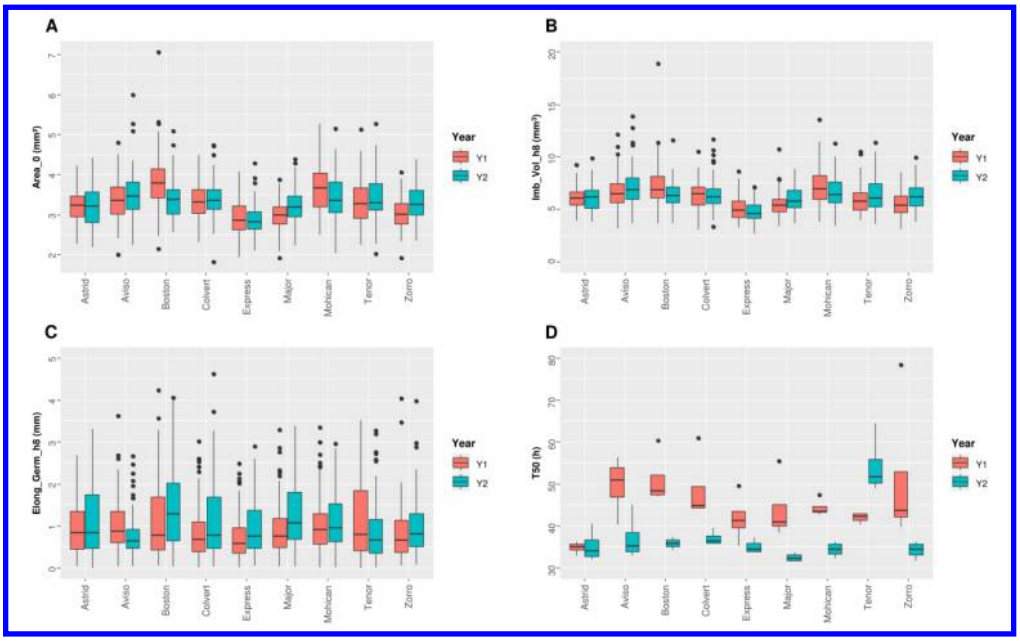


Fig. S6 Different phenotyping parameters calculated for seeds of each year and genotype. (A) Seed surface at initial time; (B) Seed imbibition 8h after initiation of imbibition; (C) Radicle elongation 8h after initiation of germination; (D) Time at which a half of the seeds have germinated. Significant 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''0
2015-2016	Y1	48°06'40.86''N	1°46'26.08''0
2016-2017	Y2	48°06'37.21''N	1°47'07.98''0

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

[1]Self-pollination with cages of 1.80 m height, 0.65 m diameter, covered with a polyester veil, Lenglart, France
[2]30x70 mm bags ref SM570Y, Sealed air Cryovac, France

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

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

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

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

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

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