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REGULAR ARTICLE

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

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

Methods Structure of seed microbial communities were estimated on individual plants through amplification and sequencing of genes that are markers of taxonomic diversity for bacteria (*gyrB*) and fungi (ITS1). The relative contribution of dispersal and ecological drift in inter-individual fluctuations were estimated with a neutral community model.

Results Seed microbial communities of radish display a low heritability across plant generations. Fluctuations in microbial community profiles were related to changes in community membership and composition across plant generations, but also to variation between individual plants. Ecological drift was an important driver of the structure of seed bacterial communities, while dispersal was involved in the assembly of the fungal fraction of the seed microbiota.

Conclusions These results provide a first glimpse of the governing processes driving the assembly of the seed microbiota.

Keywords Seed-associated microbial community · Heritability · Community assembly · Dispersal · Ecological drift

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Introduction

Plants have co-evolved with complex microbial communities which are known collectively as the plant microbiota. The plant microbiota can influence multiple plant traits such as biomass accumulation (Sugiyama et al. 2013), metabolite production (Badri et al. 2013),

drought tolerance (Lau and Lennon 2012; Rolli et al. 2015), flowering time (Wagner et al. 2014; Panke-Buisse et al. 2015; Dombrowski et al. 2017) and disease resistance (Busby et al. 2016; Ritpitakphong et al. 2016; Mendes et al. 2011; Santhanam et al. 2015).

Because the microbiota can benefit plant fitness, deciphering the processes that drive microbiota assembly over space and time is of great interest (Paredes and Lebeis 2016). Community assembly processes could be divided into four main forces: dispersal, diversification, ecological drift and selection (Nemergut et al. 2013). Recently, many research groups have investigated the importance of selection in determining community assembly in the rhizosphere and the phyllosphere (Bulgarelli et al. 2013; Müller et al., 2016). For instance, it has been shown that selection by the environment (e.g. soil physiochemical properties) and to a lesser extend selection by the host (e.g. plant genotype) influence the variation of bacterial root microbiota profiles (Bulgarelli et al. 2012; Lundberg et al. 2012; Peiffer et al. 2013; Edwards et al. 2015; Dombrowski et al. 2017). Selection by the environment and the host are also important drivers of leaf-associated microbiota composition (Bodenhausen et al. 2014; Horton et al. 2014; Wagner et al. 2016). In comparison to selection, the relative importance of dispersal, diversification and ecological drift in driving assembly of plant-associated microbiota has not been well investigated. To our best knowledge, only one study has investigated the relative importance of selection, dispersal and ecological drift in the assembly of the leaf microbiota (Maignien et al. 2014). This study found that community membership of the phyllosphere microbiota is shaped by selection, while community composition is driven by dispersal (Maignien et al. 2014).

In comparison to the phyllosphere and the rhizosphere, our knowledge of microbial communities associated to the other plant habitats such as the anthosphere, carposphere and seed habitat is quite limited. However, seed-associated microbial communities are ecologically interesting because they both represent an endpoint and a starting point for community assembly of the plant microbiota (Shade et al. 2017). Moreover seed-associated micro-organisms contribute to seed preservation (Chee-Sanford et al. 2006) and the release of seeds from dormancy (Goggin et al. 2015); they also have been associated with decreased germination rates (Nelson 2004; Munkvold 2009) and enhancement of ear emergence (Mitter et al. 2017). Recent diversity surveys of the seed microbiota revealed a high level of variation in microbial assemblages composition (Lopez-Velasco et al. 2013; Links et al. 2014; Barret et al. 2015; Klaedtke et al. 2016; Rezki et al. 2016). Based on these previous studies, it seems that selection by the host plant is not a major driver of seed community assembly (Barret et al. 2015; Klaedtke et al. 2016). In contrast, selection by the environment has been shown to shape the structure of seed-associated fungal microbiota but not seed-associated bacterial microbiota (Klaedtke et al. 2016). Hence variation in microbiota composition observed across seed samples could be due to other ecological forces such as dispersal, diversification or ecological drift.

Although the contributions of seed-associated microorganisms to the composition of the plant microbiota is mostly unknown, there are observations of seed-associated microorganisms that are conserved across multiple plant generations (Johnston-Monje and Raizada 2011; Hardoim et al. 2015). These observations suggest that some members of the plant microbiota are vertically transmitted. The initial objective of the present work was to assess the heritability of the radish seed microbiota during multiple plant generations. The structure and diversity of seed-associated microbial communities were analysed on radish seed samples collected on individual plants during three successive generations that were grown on the same experimental site. A community profiling approach revealed high variation in community composition across generations and between individuals, suggesting an importance of diversification, dispersal and ecological drift in the assembly of seed-associated microbiota. To assess the relative importance of these neutral processes, we compared the observed distribution of microbial taxa to an estimated distribution predicted with a neutral community model. According to these predictions, seed-associated microbial communities were composed of few dominant taxa that were selected by the host or the environment, as well as multiple rare taxa whose distributions could be explained by neutral processes.

Material & methods

Seed collection

Experiments were carried out for 3 consecutive years (2013–2015) on the same experiment plot located at the

FNAMS experimental station (47°28′12.42″ N, 0°23′ 44.30″ W, Brain-sur-l'Authion, France). Daily rainfall and temperature and were recorded (Fig. S1). Radish seeds (*Raphanus sativus* var. Flamboyant5) were sown in March at a density of 10 seeds per linear meter with 0.8 m between rows. At the end of August, some plants were hand-collected and placed in individual paper bags. To limit border effect, plants located at the border of the experimental plot were not sampled. Nine, 32 and 32 plants were hand-collected in 2013, 2014 and 2015. Paper bags were stored for one week at 9 °C at a relative humidity of 60%. Mature pods were removed from each plant, crushed and placed on three cleaning sieves with different mesh sizes, resulting in 500 to 1000 seeds per plant. The remaining seeds in the experimental plot were mechanically-harvested with a thresher. Seed samples that were mechanically-harvested were stored at 9 °C at a relative humidity of 60% for 6 months. Mechanically-harvested seeds were used for sowing in the following season.

DNA extraction was performed on 73 hand-harvested seed samples and 3 subsamples of 1000 seeds for each mechanically-harvested seed sample, resulting in 82 samples. Seeds were soaked in 25 mL of phosphate buffer saline (PBS) supplemented with 0.05% (*v*/v) of Tween® 20 during 2 h and 30 min at room temperature under constant agitation (140 rpm). Suspensions were collected and centrifuged (6000×*g*, 10 min, 4 °C). Pellets were suspended in approximately 2 ml of supernatant and transferred in PowerBeads tubes of the Power Soil DNA Kit (MoBio Laboratories). DNA extractions were performed with the Power Soil DNA kit using the manufacturer's protocol with the following modifications. PowerBeads tubes were placed in mixer (Retsch – MM301) and shook twice (30 Hz, 2 min, room temperature). DNA elution was performed with 60 μl of C6 solution of the Power Soil DNA kit.

Libraries construction and sequencing

Amplifications of one bacterial marker (*gyrB*) and one fungal marker (ITS1) were performed with the primer sets gyrB_aF64/gyrB_aR353 (Barret et al. 2015) and ITS1F/ITS2 (Buée et al. 2009) following procedures described earlier (Barret et al. 2015). Amplicon libraries were mixed with 5% PhiX control according to Illumina's protocols. A total of three sequencing runs was performed for this study with MiSeq Reagent Kits v3 (600 cycles).

Data analysis

The choice of *gyrB* as a bacterial molecular marker was dictated by its lowest taxonomic resolution (species-level) in comparison to classic molecular markers based on the hypervariable regions of the 16S rRNA gene (Barret et al. 2015). The *gyrB* database is composed of 30,525 sequences retrieved from 30,175 genomic sequences available in the IMG database v4 (Markowitz et al. 2012). According to the gANI cliques defined previously by Varghese and collaborators (Varghese et al. 2015), the *gyrB* marker has the best precision (0.964) and sensitivity (0.955) at a genetic distance of 0.02 (Barret et al. 2015).

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

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

Observed richness (number of OTUs) and evenness (estimated with Simpson's reciprocal index) were calculated with the R package phyloseq (McMurdie and Holmes 2013) on OTU tables rarefied to 5000 sequences per sample. Differences in richness and evenness between variables were assessed by one-way ANOVA with post-hoc Tukey's HSD test.

Differences in community membership and composition between plant generations were assessed with Jaccard and Bray-Curtis resemblance, respectively.

Jaccard and Bray-Curtis resemblance were calculated on OTU tables transformed to even sampling depth (OTU count divided by the number of reads per sample and multiply by 1000,000). Principal coordinate analysis (PCoA) was used for ordination of Jaccard and Bray-Curtis resemblances. Permutated multivariate analysis of variance (PERMANOVA; Anderson 2001, implemented with the adonis function in the vegan package in R) was used to assess the importance of the plant generation on seed-associated microbial community profiles. To quantify the contribution of the plant generation in microbial community profiles, canonical analysis of principal coordinates was performed with the function capscale of the R package vegan 2.4.2 (Oksanen et al. 2017) followed by PERMANOVA. Changes in relative abundance of OTUs between the different plant generations (2013, 2014 and 2015) were assessed with likelihood ratio test (LRT) with the R package DESeq2 1.14.1 (Anders and Huber 2010). OTU with a corrected p-value <0.01 and a log2FC > |2| were considered as differentially abundant between the different plant generations.

Testing the importance of neutral processes in community assembly

To investigate the relative role of dispersal and ecological drift in shaping seed-associated microbial community, we used a conceptual framework based on neutral theory. In this conceptual framework, a seed sample harvested from an individual plant represents a local community that is part of a larger metacommunity. The metacommunity includes all seed samples collected from different plants of the same generation. As the neutral hypothesis assumes that community members are ecological equivalents, changes in structure of the local community is attributed to the death of an individual that can either be replaced by an immigrant from the metacommunity or by local reproduction.

We assessed the fit of the Sloan neutral community model to estimate expectations in OTU abundances and frequencies. This model estimates the rate of immigration (m) into the local community by comparing OTU frequency across multiple samples (Sloan et al. 2007). The Sloan model predicts that abundant entities in the large community are widespread across samples because their dispersal could occur by chance, while rare members are more likely to be lost by ecological drift in the local community. We used a custom R script written by Burns and collaborators for fitting the Sloan neutral community model to OTU distribution (Burns et al. 2016). Goodness of fit of was assessed with root mean squared errors (RMSE) and compared with the fit of a binomial distribution model using the Akaike information criterion (AIC). The binomial distribution model is used for assessing the importance of random sampling on community structure in absence of drift and dispersal limitation (Sloan et al. 2007). Calculations of 95% confidence intervals for the Sloan neutral community model were used to detect OTUs that were more and less frequent than expected.

The validity of neutral models predictions for sequencing datasets has been recently assessed by Sommeria-Klein and collaborators (Sommeria-Klein et al. 2016). According to this study, neutral-model inference is affected by the number of reads per sample, which should be smaller than the number of individuals observed (Sommeria-Klein et al. 2016). This limitation can be easily tested by subsampling the number of reads and assessing whether the model immigration terms are unchanged. We rarefied the number of sequences per sample either to 1000 or 5000 counts before fitting the Sloan neutral community model. Because both rarefaction procedures did not significantly impact the estimated migration term, the largest dataset (5000 counts per sample) was used in subsequent analyses.

Results

The assembly of radish seed-associated microbial communities was monitored across three plant generations (2013, 2014 and 2015) through a community profiling approach. Bacterial and fungal assemblage profiles were estimated after assignment of *gyrB* and ITS1 sequences to operational taxonomic units (OTUs). Overall, 43,102 bacterial OTUs and 25,618 fungal OTUs were detected across all seed samples. While the number of observed bacterial OTUs was not significantly different between hand- and mechanically-harvested seed samples, fungal richness was significantly higher in mechanically-harvested seed samples ($P < 0.01$, one-way ANOVA with post hoc Tukey's HSD test, Fig. S2). However, higher fungal richness did not impact evenness (as

assessed with Simpson's reciprocal index, Fig. S2). Because alpha-diversity of the mechanically-harvested seed samples used for sowing was comparable to that of seed samples collected from individual plants, we concluded that the harvesting method was negligible for explaining the variation of microbial community composition across years. For consistency, we considered hand-harvested seed samples in the subsequent analyses.

Assembly of the seed microbiota over successive plant generations

The assembly patterns of radish seed microbiota was monitored from hand-harvested samples in 2013, 2014 and 2015. First, we compared microbial richness and evenness over the three plant generations (Fig. 1). A significant increase in bacterial and fungal richness (Fig. 1a and b) was observed for samples collected in 2015 vs. those collected the previous years ($P < 0.01$, one-way ANOVA with post hoc Tukey's HSD test). However, this increase in microbial richness was not associated with differences in microbial evenness over the successive plant generations (Fig. 1c and d).

Similarity in community membership and community composition was estimated through calculation of Jaccard and Bray-Curtis similarities, respectively. Ordination of Jaccard and Bray-Curtis similarity with principal coordinate analysis (PCoA) revealed a clustering of seed-associated fungal communities according to the plant generation (PERMANOVA, $P < 0.001$; Fig. 2a and b). In contrast, seed-associated bacterial communities were only significantly clustered for the

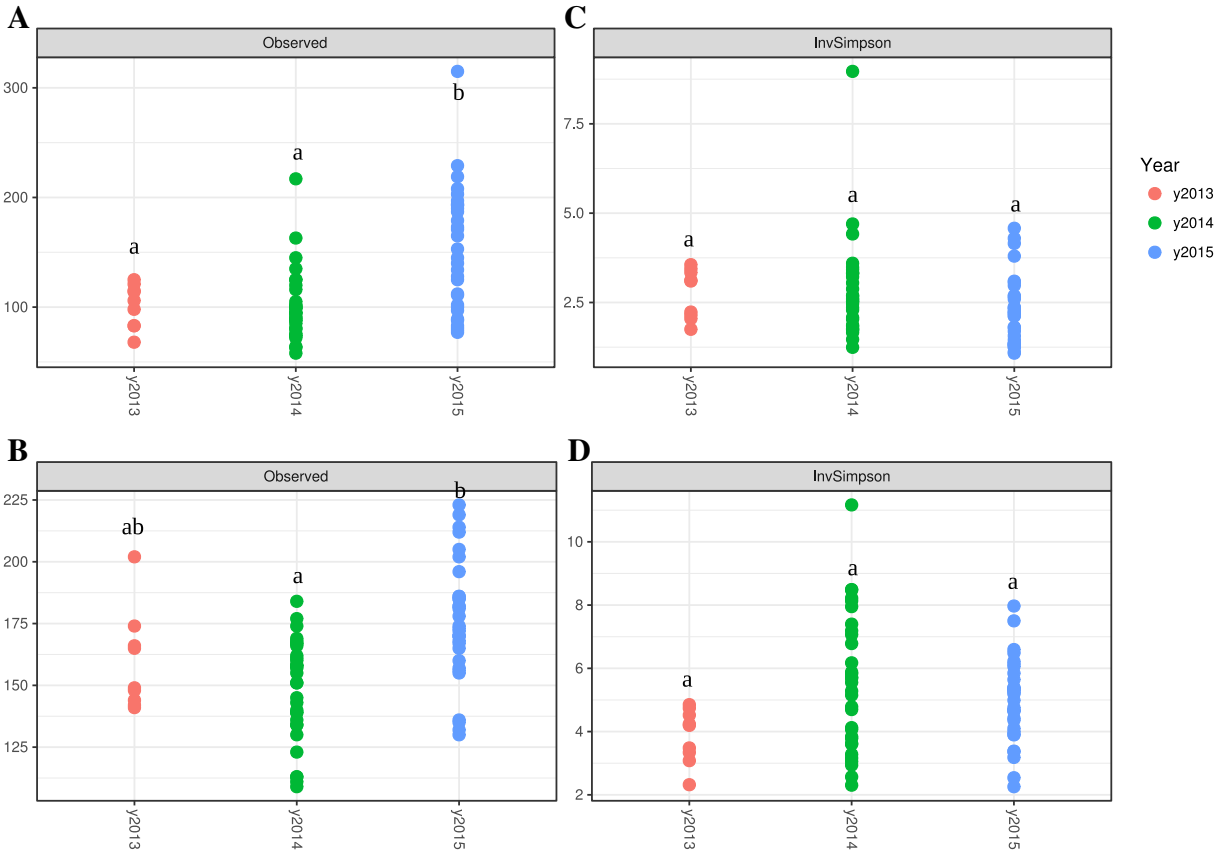

Fig. 1 Diversity of seed-associated microbial communities. Richness ("Observed", **a**, **b**) and diversity ("InvSimpson", **c**, **d**) were estimated for bacterial (**a**, **c**) and fungal communities (**b**, **d**) with *gyrB* and ITS1 sequences, respectively. Richness and evenness were assessed with the number of OTUs rarefied to 5000 sequences per sample and Simpson's reciprocal index. Each dot corresponds to a seed sample collected in 2013 (red), 2014 (green) or 2015 (blue). Letters "a" and "b" denote significant differences between conditions considered at a *p*-value ≤0.01 as assessed by ANOVA with post hoc Tukey's HSD test

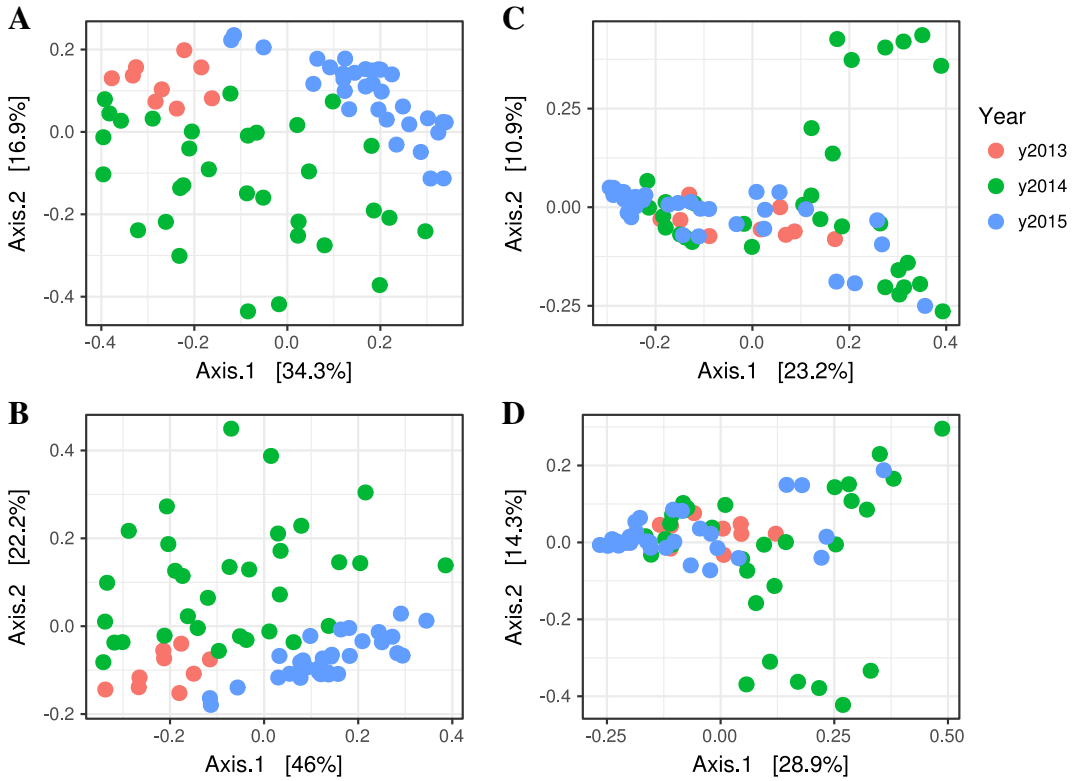

Fig. 2 Similarities in microbial community membership and composition between seed samples harvested from three different plant generations. Similarities in community membership and composition were assessed with Jaccard and Bray-Curtis indices, respectively. Principal coordinate analysis (PCoA) was used for ordination of Jaccard (**a** and **c**) and Bray-Curtis (**b** and **d**) indices calculated with ITS1 (**a** and **b**) and *gyrB* sequences (**c** and **d**). Each dot represents a microbial community associated with seeds harvested in 2013 (red), 2014 (green) and 2015 (blue)

third plant generation ($P < 0.001$; Fig. 2c and d). Jaccard ordinations had higher explanatory value and more discrete clustering by year than Bray-Curtis, suggesting that inter-annual differences in community structure can be attributed to patterns in the presence and absence of taxa (e.g. many taxa observed exclusively in one year). The relative contribution of the plant generation in community profiles was further investigated through canonical analysis of principal coordinates (CAP) followed by PERMANOVA. According to CAP, the plant generation explained 22% and 32% of variation in bacterial and fungal community membership and 27% and 39% of bacterial and fungal community composition (PERMANOVA, $P < 0.001$).

To determine if some OTUs were specifically associated with one particular generation, we performed likelihood ratio tests (LRT) with DESeq2 on OTU table. LRT revealed that the relative abundances of 25 bacterial OTUs were significantly different (p-value ≤0.01 and log2FC ≥ |2|) between the first and second plant generations (Fig. S3A). The number of differentially abundant bacterial OTUs with differences was 62 between the second and third plant generation (Fig. S3E). Changes in relative abundances of 69 and 125 fungal OTUs were also detected between 2013 vs 2014 and 2014 vs 2015, respectively (Fig. S3B and Fig. S3F). In conclusion, differences in richness, diversity, community membership, community composition and OTU relative abundances' indicated a contribution of plant generation and/or harvest year on the structure of seed-associated microbial communities.

Assembly of seed-associated microbial communities among individual plants within the same harvest year

Moving forward from our results that plant generation and/or harvest year are important drivers of the structure of the seed microbiota, we wanted to understand the variation in richness (Fig. 1) and community

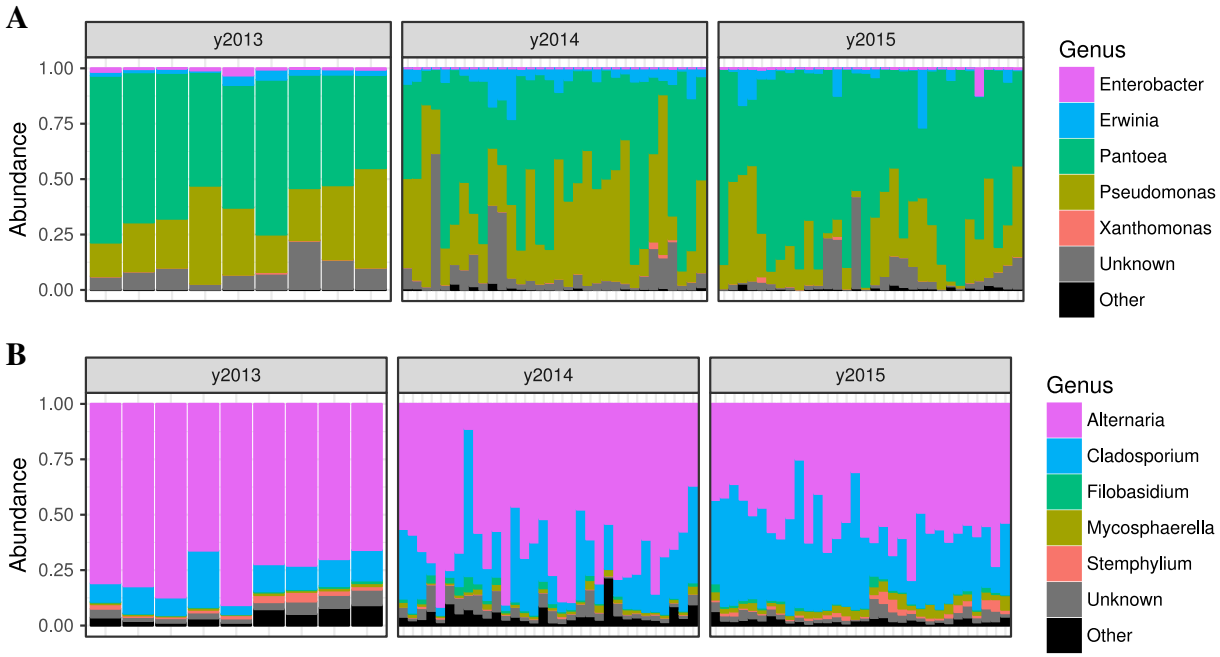

Fig. 3 Variation in relative abundance of microbial communities. Relative abundance of the most prevalent 5 bacterial and fungal genera within seed samples collected from individual plants across 3 successive generations (2013, 2014 and 2015) according to *gyrB* (**a**) and ITS1 (**b**) sequences. Taxonomic affiliation of bacterial and fungal OTUs was performed with an in-house *gyrB* database and the UNITE database, respectively. Unknown taxa represent OTUs that could not be assigned taxonomy at the genus level

composition (Fig. 3) of the radish seed microbiota collected within the same year but on distinct individual plants. Overall, the radish seed microbiota is mainly composed of 2 bacterial genera, namely *Pantoea* and *Pseudomonas* (Fig. 3a), and 2 fungal genera: *Alternaria* and *Cladosporium* (Fig. 3b). These genera were consistently detected across individual plants, but had variable relative abundances (Fig. 3a and b).

To further evaluate variation in community membership and composition of the radish seed microbiota, we counted the number of OTUs that were conserved among all individual plants (core microbiota, Shade and Handelsman 2012). Among the 43,102 bacterial OTUs detected in the *gyrB* dataset, only three were observed in all seed communities. These three bacterial OTUs are affiliated to *Pantoea agglomerans*, *Pseudomonas viridiflava* and *Erwinia tasmaniensis* (Table 1). For the fungal communities, nineteen fungal OTUs among 25,618 OTUs detected were conserved in all seed samples. Among these 19 fungal OTUs, 14 were affiliated to the *Alternaria* genus (Table 1).

Although the core fraction of the radish seed microbiota had few OTUs, these taxa were highly abundant and represented an average of 70% and 87% of all bacterial and fungal reads, respectively. Despite this strong conservation in detection across individuals, the relative abundance of the core members of the radish seed microbiota varied greatly between seed samples (Fig. S4). For instance the relative abundance of bacterial OTU00001, which is affiliated to *P. agglomerans*, ranged from 7 to 82% within seed samples collected in 2014 (Fig. S4A). There were also shifts in the relative abundances of fungal OTUs between seed samples, with variation ranging from 4 to 43% for OTU00002 (*Cladosporium*) in 2014 (Fig. S4B). The high variability of seed community composition collected from the same plant genotype and grown on the same experimental plot suggested that non-selective processes such as dispersal, drift and diversification may be involved in community assembly of the seed microbiota.

Neutral processes are involved in intra-annual community assembly of the seed microbiota

The fit of the Sloan neutral model to the observed OTU distribution was used to assess the relative importance of dispersal limitation and ecological drift. Based on RMSE values, frequency of bacterial and fungal OTUs

Table 1 Relative abundance of the core members of the seed microbiota

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

Bacterial and fungal OTUs that occurred in all seed samples were defined as core members of the radish seed microbiota

occurrence within local community was adequately described by the neutral model for seed collected in 2014 and 2015 (Fig. 4). Larger differences between observed OTUs occurrence and predicted OTUs occurrence were reported for microbial communities associated to seed harvested in 2013 (RMSE = 0.12 and 0.14 for bacterial and fungal OTU datasets, respectively). This increase in RMSE values could be explained by the limited number of samples employed (n = 9) in 2013. Overall, the estimated migration rate (m) was more important for seed fungal communities in comparison to seed bacterial communities (Table 2).

To estimate the effect of dispersal and ecological drift on local microbial community structure, we compared the fit of the Sloan model to the fit of a binomial distribution model that is used for assessing the importance of random sampling on community structure (Sloan et al. 2007). According to AIC, both models performed equally well (Table 2), therefore suggesting that dispersal, ecological drift and random sampling were involved in local assembly of seed-associated bacterial communities.

Although the distribution of the vast majority of the OTUs were adequately predicted by the Sloan model, some OTUs were outside the 95% confidence interval of the model (Fig. 4). These OTUs that were found more and less frequently than estimated, and could represent microbial taxa that were positively and negatively selected by the host or the environment. Interestingly, only a subset of microbial taxa consistently diverged from neutral expectations across the three plant generation (Table 3). A total of three bacterial OTUs related to the *Pseudomonas fluorescens* group were less frequently observed than expected (Table 3), and could represent taxa that are under negative selection. In contrast, one bacterial and five fungal OTUs had a higher observed frequency for each plant generation. These putative positively-selected taxa corresponded to one bacterial endophyte affiliated to *Plantibacter flavus* and 5 OTUs related to the *Alternaria* genus (Table 3). These taxa that were not determined to be part of the radish seed core microbiota.

Discussion

We investigated the structure of microbial communities associated to several seed samples collected on different plant individuals during three successive generations. Based on the molecular markers employed in this work, seed microbiota composition and structure were distinct between plant generations. Overall, only three bacterial OTUs and 19 fungal OTUs were detected in all seed samples. These core OTUs were affiliated to bacterial (e.g. *Pantoea agglomerans* or *Pseudomonas viridiflava*) and fungal taxa (*Alternaria* or *Cladosporium*) that are frequently associated with seeds of various plant species (Barret et al. 2015; Hodgson et al. 2014; Links et al. 2014; Rezki et al. 2016; Truyens et al. 2015). Altogether, these results suggest a low heritability of the seed microbiota across plant generations. Limited heritability was observed previously for bacterial communities associated with seeds and rhizosphere of maize (Johnston-Monje and Raizada 2011 and Peiffer et al. 2013, respectively), and was attributed to site-specific variation. In

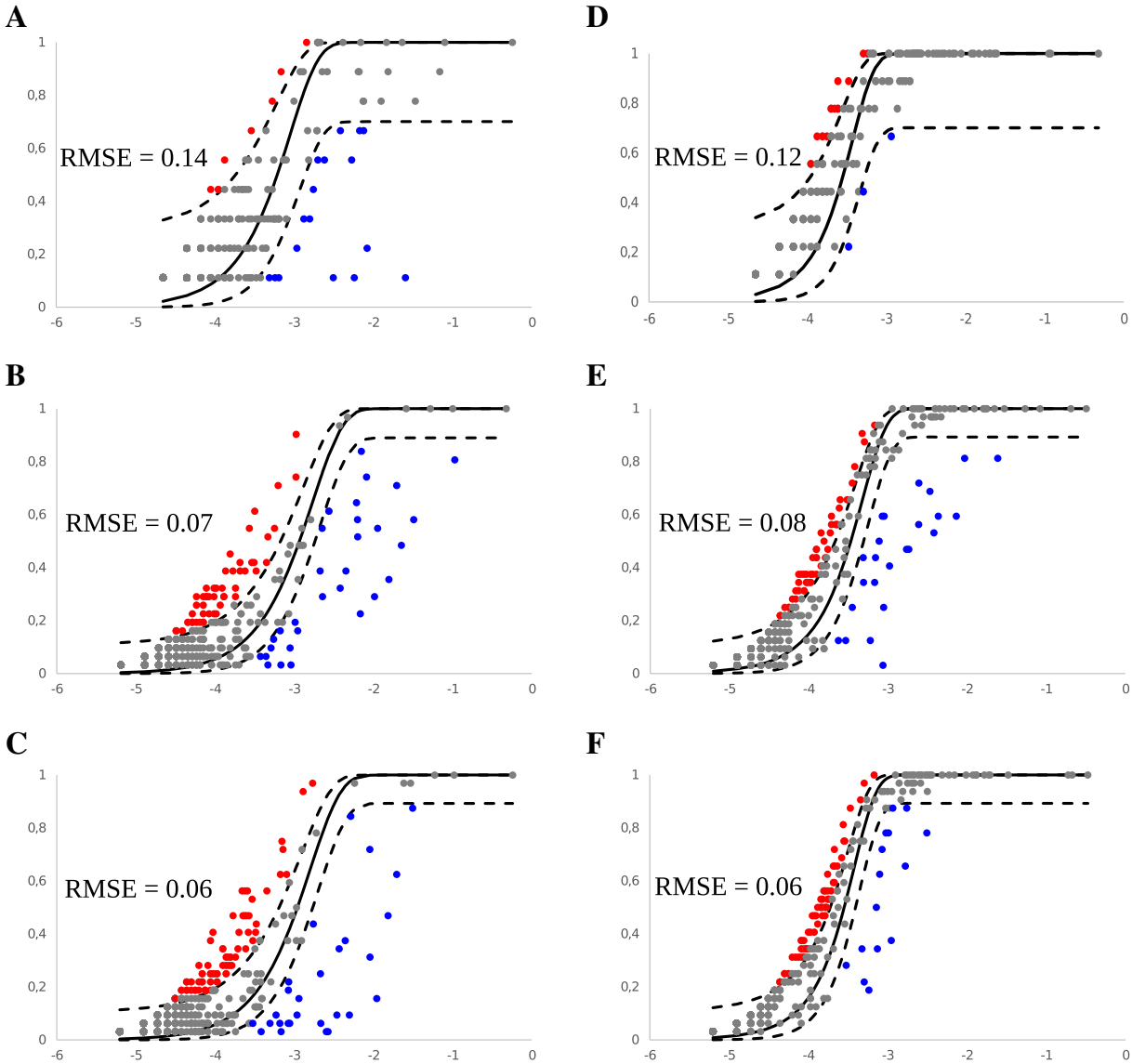

Fig. 4 Fit of the Sloan neutral model. Frequency and abundance of bacterial (**a**, **b**, **c**) and fungal (**d**, **e**, **f**) OTUs in seed samples collected in 2013 (**a**, **d**), 2014 (**b**, **e**) and 2015 (**c**, **f**). Goodness of fit of the Sloan model was estimated with root mean squared errors (RMSE). Dashed lines represent 95% confidence intervals around the neutral community model prediction. OTUs that occurred more and less frequently than predicted by the neutral model are displayed in red and blue, respectively

the present work, geographic distance was not involved in the observed variability of community composition, as all plants were grown in the same experimental plot. However, annual fluctuations in local weather conditions (Fig. S1) could contribute to differences in community structure across successive plant generations. Indeed, it is well accepted that climatic changes influence the composition of soil and plant-associated microbial communities (Compant et al. 2010; Classen et al. 2015). Other variables such as the type of cultivated plants that surrounded the experimental plots could also contribute to observed variation in seed community composition. Recent work indicates that emigration of epiphytic bacteria and fungi from plants surfaces are important local sources of airborne micro-organisms (Lymperopoulou et al. 2016). Although the same experimental plot was employed over the course of this study, other cultivated plant species in the immediate vicinity of the radish plants were not controlled between the different years.

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

Datasets	Samples	N	OTUs	*m*	RMSE Sloan	RMSE binomial	AIC Sloan	AIC binomial
ITS1_2013_1000	9	1000	236	0.83 ± 0.17	0.13	0.08	−292	−508
ITS1_2013_5000	9	5000	712	0.78 + 0.10	0.12	0.07	−977	−1840
ITS1_2014_1000	32	1000	501	0.47 + 0.08	0.09	0.09	−954	−974
ITS1_2014_5000	32	5000	1434	0.41 + 0.05	0.08	0.09	−3136	−3071
ITS1_2015_1000	32	1000	650	0.79 + 0.09	0.07	0.05	−1696	−1982
ITS1_2015_5000	32	5000	2169	0.75 + 0.07	0.06	0.05	−5801	−6942
gyrB_2013_1000	9	1000	232	0.18 + 0.07	0.17	0.20	−169	−141
gyrB_2013_5000	9	5000	640	0.12 + 0.02	0.14	0.20	−666	−384
gyrB_2014_1000	32	1000	633	0.03 + 0.01	0.08	0.14	−1342	−780
gyrB_2014_5000	31	5000	1906	0.04 + 0.01	0.07	0.13	−4440	−2575
gyrB_2015_1000	32	1000	1044	0.02 + 0.01	0.07	0.12	−2664	−1545
gyrB_2015_5000	32	5000	3813	0.04 + 0.01	0.06	0.10	−10,118	−7147

Fungal (ITS1) and bacterial (*gyrB*) OTU tables were rarefied to 1000 or 5000 sequences per sample. The number of manually-harvested seed samples ("Samples"), sequences ("N") and OTUs are shown for each dataset. In addition the migration rate (*m*), RMSE and AIC values from the Sloan and binomial model are also indicated

While generation-specific variation was observed within seed-associated microbiota, the highest variation was found between seed samples collected within the same year but on different individual plants. As most diversity surveys of seed-associated microbial communities are performed on seed samples consisting of multiple plant entities (Barret et al. 2015; Links et al. 2014: Klaedtke et al. 2016), this source of variation is usually ignored. The fluctuations in microbial community composition observed between seed samples collected on different individual plants were partly explained by neutral processes. The Sloan neutral model predicted the distribution of microbial taxa across seed-associated microbial communities, suggesting that ecological drift and dispersal explained a significant amount of diversity. Given the small value of estimated migration rate for seed-associated bacterial communities (m = 0.04), ecological drift was deduced as an important driver of bacterial community assembly. The importance of ecological drift in community assembly has been previously reported in communities (i) under weak host selection and (ii) where the vast majority of taxa are in low relative abundances (Nemergut et al. 2013). These two features are frequently encountered in seed-associated bacterial communities. Indeed, the overall composition of seed-associated bacterial communities is not shaped by the host genotype (Barret et al. 2015; Klaedtke et al. 2016). Moreover, seed-associated microbial communities are composed of few abundant entities and a multitude of low-abundance members (Rezki et al. 2016) that may be prone to local extinction.

Table 3 OTUs that deviated from the Sloan model

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

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

In contrast to the seed bacterial communities, high migration rates (m > 0.4) were estimated for fungal communities at each harvesting year. Hence, loss of a fungal entity within local community may be replaced by immigration of an individual from the regional metacommunity. Fungal communities usually display strong biogeographical patterns as a result of dispersal limitation at regional scale (Peay et al. 2016). For instance, it has been shown that the geographic location of the production region shapes the structure of seed fungal

communities (Klaedtke et al. 2016). However, passive dispersal of fungal entities occurred frequently at short-distances via aerial movement of spores. Indeed, most fungal spores can disperse from centimeters to meters (Norros et al. 2012). Taken together, the predicted importance of dispersal for seed fungal community assembly and the observed differences in fungal diversity and composition within the same experimental plot are indicative of historical contingency (Fukami 2015). In historical contingency, the order of species arrival impacts the final composition of the community. Hence the first fungal taxa that colonize the seed may have a competitive advantage over fungi that require the same resources but arrive subsequently.

Though most microbial OTUs followed the expectation of the Sloan neutral model, some OTUs deviated clearly. The taxa that were more widespread than expected could represent microbial entities that are positively selected by the host or the environment. Positive-selection of these microbial taxa by the host would suggest that they possess properties beneficial to plant. Among those taxa, one bacterial OTU and 5 fungal OTUs were consistently overrepresented in every plant generation. The bacterial OTU was related to *Plantibacter flavus*, a bacterial endophyte belonging to several plant families including *Asteraceae*, *Fabaceae* and *Poaceae* (Lumactud et al. 2016) that possesses 1-aminocyclopropane-1 carboxylate activity (Lumactud et al. 2017), which reduces stress ethylene production in plant. All fungal OTUs were related to the *Alternaria* genus, more precisely to the section *Alternata* and *Infectoria* (Woudenberg et al. 2013). Although these fungal taxa are usually described as plant pathogens (Woudenberg et al. 2013), their prevalence in symptomless seeds (Links et al. 2014; Barret et al. 2015) and their potential selection by the host plant may indicate plant-beneficial traits.

In conclusion, the present work shows a low heritability of the seed microbiota during successive plant generations. This low level of heritability is explained in part by the importance of neutral based processes in community assembly. Neutral processes related to ecological drift are important for the structure of seed-associated bacterial communities, while dispersal was involved in assembly of the fungal fraction of the seed microbiota.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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