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RESEARCH ARTICLE

An *in-situ* characterization of *Triticum aestivum* sheds light on the role of wheat genotype and agricultural practices on the below-ground microbiota

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Claudia Bartoli[1], Enguerrand Burel[2], Sébastien Carrère[1], Renaud Rincent[3], Benoit Lefebvre[1]

[1]Laboratory of Plant-Microbe-Environment Interactions (LIPME), Université de Toulouse, INRAE, CNRS, Castanet-Tolosan, 31326, France
[2]CREABio, Centre de Recherche et d'Expérimentation en Agriculture Biologique, Auch, 32000, France
[3]Génétique Quantitative et Évolution - Le Moulon, Université Paris-Saclay, INRAE, CNRS, AgroParisTech, Genetique Quantitative et Evolution Le Moulon, Gif-sur-Yvette, 91190, France

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Abstract

The below-ground microbiota plays a pivotal role in enhancing plant resilience to environmental stresses. Investigating microbial diversity within native agricultural systems is essential for understanding the potential of microbiota in sustaining crop health and productivity. Agricultural practices, including the selection of plant genotypes, are key determinants in shaping plant-microbe interactions. However, the role of plant genetic diversity in structuring below-ground microbiota has been only partially explored. Furthermore, many studies examining crop-microbiota interactions have been conducted under controlled conditions. In this study, we performed a two-year *in situ* survey of root and rhizosphere microbiota in 35 wheat (*Triticum aestivum*) genotypes grown in open fields under different organic agricultural practices, including unfertilized conditions, organic fertilization, and intercropping with faba bean. We characterized both bacterial and fungal communities and found that plant genotype, along with organic fertilization, were significant factors influencing the diversity and composition of root and rhizosphere microbiota. In addition, we showed that landraces varieties harbor a more diversified fungal microbiota when compared to modern varieties. Our study underscores the importance of incorporating wheat genetic diversity into agricultural strategies to enhance microbiota diversity.

Open Peer Review

Approval Status *AWAITING PEER REVIEW*

Any reports and responses or comments on the article can be found at the end of the article.

Keywords
wheat, organic agriculture, genetic diversity, root microbiota, metabarcoding.

This article is included in the COST Actions gateway.

Corresponding authors: Claudia Bartoli (claudia.bartoli-kautsky@inrae.fr), Benoit Lefebvre (benoit.lefebvre@inrae.fr)

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Introduction

The concept of a plant holobiont refers to a broader understanding of a plant as a complex ecological unit, consisting of the plant itself and the diverse microbial communities inhabiting it (Baedke et al., 2020). The holobiont concept recognizes that organisms often coexist in intricate symbiotic relationships with the microbial complexes (or microbiomes) that have a direct impact on plant evolution and health (Rosenberg & Rosenberg, 2018). The recruitment of beneficial microbiota is a promising trait in plant breeding strategies to develop crop varieties that are resistant to biotic and abiotic stresses (Spooren et al., 2024). Plant-associated beneficial microbes can enhance plant fitness through nutrient solubilization (Hayat et al., 2010), phytohormone production, modification of plant architectural features (Harting et al., 2021), plant immunity priming (Hacquard et al., 2017) and/or by competing for pathogen ecological niches (Gu et al., 2020). The use of microbiomes is thus a promising avenue to reduce chemical inputs in agriculture while minimizing production loss (Compant et al., 2024). Several microorganisms are commercialized to prevent/reduce disease or enhance plant production, but integrative approaches taking into account the whole microbial diversity, plant genetic variability, agricultural practices and environmental constraints are still missing. One of the main limits to fully exploit the potential of plant-associated microbes is the lack of information about the effect of crop genotypes and agricultural practices on the plant-associated microbiota. Indeed, few are the studies describing the plant microbiota in natural and realistic conditions. In our study, we studied the effect of crop genetic variability and agricultural practices on the below-ground microbiota of bread wheat (*Triticum aestivum*).

Bread wheat is a globally significant cereal crop, central to human nutrition. Its domestication occurred approximately 10,000 years ago in the Fertile Crescent, and since then, selective breeding has shaped its development (Faris, 2014). By the 19th century, wheat cultivation predominantly relied on landraces—locally adapted varieties with high genetic diversity, conferring resilience to environmental fluctuations (Venske et al., 2019). In the 20th century, these genetically diverse landraces became critical reservoirs for breeding modern wheat cultivars. However, intensive breeding programs led to the widespread replacement of landraces by modern, high-yield varieties, which significantly reduced wheat's genetic diversity (Faris, 2014). This reduction poses challenges for the crop's future adaptability to environmental stresses, including its interaction with microbial communities essential for plant resilience (Bordes et al., 2008). While understanding the interaction between wheat genetic diversity and microbiota selection is essential for enhancing wheat resistance to stress, to date, relatively few studies have explored the influence of wheat genotypes on below-ground microbial communities. One of the major limiting factors is the inherent complexity of the wheat genome (Guan et al., 2020), which complicates efforts to unravel how genetic variation impacts the composition and function of soil-associated microbiota. Latz et al. (2021) found that wheat variety explains approximately 25% of the variation in microbial communities observed in leaves and roots, with a significant effect on fungal microbiome diversity as measured by the Shannon index. However, Simonin et al. (2020) reported that wheat genotype accounted for only 2% of the variance in fungal and bacterial communities in the rhizosphere (the soil fraction under the influence of plant roots, often sampled as root-adhering soil), with no significant differences in microbial species richness or community structure across genotypes. Interestingly, Donn et al. (2015) observed a genotype-specific effect on rhizosphere community structure in mature wheat plants, but only after two years of experimentation. Similarly, Rossmann et al. (2020) found no differences in rhizosphere α-diversity among wheat genotypes but reported significant variation in rhizosphere microbiota composition between landraces and modern cultivars. This was further supported by a higher percentage of unique operational taxonomic units (OTUs) for both fungi and bacteria in landraces compared to modern cultivars. In a more recent study, Quiza et al. (2023) analyzed bacterial and fungal communities in 10 wheat genotypes, sampling at different stages over four years. Their findings highlight that microbial community variations are primarily dependent on time and on the plant below-ground compartment (with a stronger effect on roots than on the rhizosphere, but not consistent across all periods). Therefore, by analyzing the literature, no consensus on the impact of wheat genotype on both fungal and bacterial microbiota in the rhizosphere and roots is clearly demonstrated. However, most of the previous studies were conducted in greenhouse conditions, few are the investigations carried out in open fields.

To advance our understanding about the influence of genotypic diversity and agricultural practices on wheat-associated microbiota, we conducted an *in-situ* characterization of both bacterial and fungal communities in the rhizosphere and root of 35 wheat genotypes grown in a French experimental station under various organic agricultural practices. Our findings revealed that, when assessing the wheat microbiota under ecologically realistic conditions, wheat genotypic diversity emerges as a driver of microbiota composition.

Material & methods

The *in-situ* wheat plant experimental design

In order to characterize root and rhizosphere microbiota of a large range of wheat genotypes under several agricultural managements, we took advantage of field experiments of the La Hourre farm belonging to the CREABio experimental

station located in Auch, south-west of France. The CREABio fields are under organic farming since 1999. Two-year surveys were conducted in this study.

In 2016–2017 (thereafter referred as 2017) two field trials were conducted. Firstly, in order to test the impact of wheat genotype, we performed a first genetic trial with 20 wheat varieties sown in November 2016 and harvested in July 2017. Secondly, to test the effect of wheat-legume intercropping, we carried out a faba bean association trial where 5 wheat varieties were sown in association or in the absence of the faba bean AXEL variety in November 2016; harvesting was performed in July 2017. The 20 wheat genotypes and faba bean association trials were carried out in the LH7 and LH6B field plots respectively. These field plots showed a similar soil physical structure (Figure S1).

In 2017–2018 (thereafter referred as 2018) three field trials were carried out. Firstly, we conducted a second genetic trial with 19 wheat genotypes sown in October 2017 and harvested in July 2018; wheat varieties between the 2017 and 2018 genetic trials were not strictly the same. Secondly, a second faba bean association trial was also performed as described above (sown in October 2017 and harvested in July 2018). As for the wheat genetic trial, wheat varieties in association with the faba bean were not strictly the same of those tested in 2017. Thirdly, to test the fertilization impact, we performed a trial with 5 wheat landraces sown in October 2017, fertilized or not with 100 kg/ha of organic fertilizer constituted by transformed animal proteins, and harvested in July 2018. The fertilizer composition corresponded to 5.61 kg of N/ ha, 1.46 kg P_2O_5/ ha, 0.61 kg K_2O / ha and 9.74 kg CaO/ha, with a C/N of 4.6. The faba bean association trial was carried out in the LH6B plot, the 20-wheat genotype trial and the fertilization trial were performed on the LH8 plot showing a similar physical structure (Figure S1).

A list of wheat genotypes collected by year is reported in Table S1. All trials were conducted in a split-plot design constituted by 3 spatial blocks, in each block wheat varieties were randomized. For the faba bean association trials, 3 spatial blocks consisted of wheat genotypes in association with faba bean and 3 supplementary blocks with wheat genotypes grown in absence of the legume. The experimental design summary is reported in Figure 1. Wheat phenotypic traits (Thousand-Grain Weight (TGW), yield and % of protein in the wheat grain) were also estimated for all the experimental fields as described previously (Burel et al., 2022, Burel & Escalier, 2019).

Sampling and generation of *gyrB* and ITS2 amplicons

For each trial, samples were collected in Spring (within March 2017 and May 2018) by digging root systems to a depth of about 10–15 cm. For each condition, 2 pools of 6 plants per each of the 3 plots were harvested (Figure 1). Plants were placed in a cold box to prevent microbiota changes and transported to the laboratory with their root systems and adherent soil. For each plant pool, roots were separated from the rhizosphere and washed with sterilized water. Root and rhizosphere compartments were analyzed separately except for the 2017 wheat genotype trial where only roots were harvested. Each root sample was excised in small fragments with a sterilized scalpel and placed in 96-well S-block plates containing three 3 mm metallic beads. Rhizospheres were also placed in the S-block 96-well plates containing three 3 mm metallic beads. Plates were then immerged in liquid nitrogen and homogenized for 1 min at 35 Hz with the mixer mill

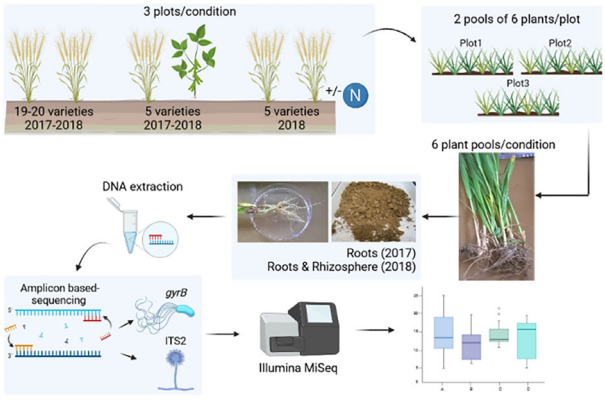

Figure 1. Experimental field design and microbiota characterization procedures. Three trials were conducted over two years (2017–2018) except for the fertilization trial performed in 2018 only. Each trial consisted of 3 blocks. In each block, we collected 2 pools of 6 plants (N = 6 independent pools/condition). Root and rhizosphere compartments were separated in the laboratory. The 6 pools were independently analyzed for fungal (ITS) and bacterial (*gyrB*) communities through amplicon sequencing with the Illumina MiSeq technology. Sequences were used to estimate microbial diversity and composition descriptors.

RETSCH MM 400. DNAs from the homogenized samples were extracted with the ZymBIOMICS™ 96 DNA kit by following manufacturer's instructions. DNA samples were used for metabarcoding (Figure 6.1). Specifically, bacterial communities were characterized through amplification of a fraction of *gyrB* gene encoding for the bacterial gyrase *β*-subunit by using primers with internal TAGS for multiplexing as described in Bartoli et al., (2018). Fungal communities were characterized by amplifying the ITS2 region of the rRNA through a nested PCR. For this a first PCR was performed to increase PCR specificity (and avoid plant DNA amplification) with the following primers: rcAMDGR 5′ - ATGATTAATAGGGATAGTTGGG - 3′ and ITS4ngs 5′ - TCCTSCGCTTATTGATATGC - 3′. PCR program consisted of: initial denaturation at 95 °C for 5 min, 40 cycles at 95 °C for 5 sec, 62 °C for 30 sec, 72 °C for 1 min and a final elongation at 72 °C for 10 min. One µl of the first PCR was used for the second PCR by using primer pairs including Illumina adaptors and internal TAGS (Table S2) and the following PCR program: initial denaturation at 95 °C for 5 min, 40 cycles at 95 °C for 30 sec, 55 °C for 30 sec, 72 °C for 1 min and a final elongation at 72 °C for 10 min. All PCRs were performed in a final volume of 50 µl with the MTP™ Taq DNA Polymerase. PCR amplicons for both *gyrB* and ITS2 fragments were purified by using Agencourt®AMPure® magnetic beads following the manufacturer's instructions. Purified amplicons were quantified by spectrometry and appropriately diluted to obtain an equimolar concentration. Two microliters of equimolar PCR-purified products were used for an additional PCR containing 288 Illumina indexes. The PCR amplicons were then purified and quantified as described above to obtain a unique equimolar pool that was quantified by real-time quantitative reverse transcriptase-polymerase chain reaction and sequenced with Illumina MiSeq 2 × 250 v3 (Illumina Inc., San Diego, CA, USA) in the GeT-PlaGe Platform (Toulouse, France).

Bioinformatics analysis and data filtering

Raw reads were demultiplexed by considering the Illumina indexes and the three internal TAGS. For the fungal communities, the average number of complete pairs sequences per sample was 10,083 ± 7,869. For the bacterial data set we retrieved 31,607 ± 8,724 sequences per each sample. For both *gyrB* and ITS2 datasets we removed samples with less than 1,000 reads. Taxonomic affiliation of ITS2 and *gyrB* sequences was performed by using a Bayesian classifier implemented in the classify.seqs command of MOTHUR (Wang et al., 2007) against the UNITE ITS database v 8.2 (Nilsson et al., 2018; fungi-only) and an in-house *gyrB* database (Bartoli et al., 2018) with an 80% bootstrap confidence score. Clustering of sequences into OTUs was performed with Swarm (Mahé et al., 2015) by using a clustering threshold (d) = 1. Only OTUs with a minimum of five sequences across all samples were kept. After data filtering, for the ITS2 we obtained 772 samples and 4,582 OTUs and for *gyrB* 771 samples and 50,161 OTUs. On these datasets, we further applied two filters: i) first we estimated a Good's coverage score (Schloss et al., 2009) for each sample. Based on the distribution of the Good's coverage score, only samples with a score more than 0.9 were kept ii) second, only OTUs showing a minimum relative abundance of 1% in at least one sample were selected. The final datasets corresponded to a matrix of 645 samples/591 OTUs for the ITS2 and 648 samples/1485 OTUs for the *gyrB*.

Analysis of microbiota descriptors

The final OTU matrices obtained after data filtering were used to estimate microbiota α-diversity descriptors (Shannon index, species richness and Simpson index) with *summary.single* function in mothur (Schloss et al., 2009). Microbiota composition (β-diversity) was inferred and analyzed with two complementary approaches. Firstly, by considering Bartoli *et al*., (2018), a Hellinger transformation was performed by using the vegan R package (Oksanen et al., 2009) and the relative Hellinger distance was inferred with the *decostand* command in the vegan R package prior β-diversity analyses. For both ITS2 and *gyrB* datasets, the Hellinger distance matrices were reduced by running a Principal Coordinates Analysis (PCoA) with the ape R package (Paradis et al., 2004). Secondly, we applied constrained ordination (tb-RDA) on the OTU matrices. For this, we first estimated the relative abundance of each bacterial and fungal OTU and selected OTUs with a frequency ≥ 1%. The trimmed OTU count table was then normalized and tb-RDA inferred with the *rda* function in vegan R package. To control for plant compartment, tb-RDA was independently calculated on rhizosphere and root samples. Natural variation for microbiota descriptors (species richness, Shannon index, first and second PCoA axes) for both fungal and bacterial communities was used to perform liner mixed models with the *lmer* function in the lsmeans R package and lm4 (Bates et al., 2015) by considering the below-ground compartment, genotype and treatment (i.e. with or without faba bean and with or without fertilization) as fixed factors in the model. *P*-values obtained by linear-mixed analysis were corrected for the FDR. As both fungal and bacterial communities were associated to the plant below-ground compartment, linear mixed models were inferred separately on rhizosphere and root samples. To test the impact of the wheat microbiota on the plant phenotypic traits (TWG, yield and % of protein content in the grain), we first estimated lsmeans with the lsmeans R package on the wheat root microbiota descriptors corresponding to the wheat genotype trials performed in 2017 and 2018. The rhizosphere samples were not included in this analysis as not collected in 2017. We then estimated lsmeans on the wheat phenotypic traits. A matrix combining lsmeans obtained from microbiota descriptors and wheat phenotypic traits was used to perform a sparse partial least-squares regression (sPLSR) (Carrascal et al., 2009; Lê Cao et al., 2008) that maximize the covariance between linear combinations of wheat phenotypic traits (matrix X) and

linear combinations of microbiota descriptors (matrix Y). sPLSR was run with the *regression* mode in the mixOmics R package (Rohart et al., 2017).

Analyses of the effect of wheat genetic diversity on microbiota descriptors

To explore the wheat variety effect on wheat microbiota descriptors, broad-sense heritability, i.e. the proportion of phenotypic variance explained by wheat genetics, was inferred on microbiota descriptors of datasets obtained from the wheat genotype trials 2017 and 2018. Rhizosphere samples were not included in this analysis as not collected in 2017. For this, variance of random and fixed effects was calculated for each microbiota descriptors with *lmer* function in lm4 R package. In the model, wheat varieties were considered as random effects. Broad-sense heritability was then estimated as following: $H^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_E^2/r)$ Where σ_G^2: Genotypic variance (i.e. variance of the genotype effect), σ_E^2: Residual variance (i.e. environmental or error variance), and r: Number of replicates. To test the relationship between the genetic wheat distances and the microbiota descriptors, the total 33 wheat varieties considered in the study were genotyped with a 35 k (Ben-Sadoun et al., 2020) or a 280 k SNP array (Rimbert et al. 2018). After trimming for markers with a heterozygosity above 8%, or missing rate above 10%, and monomorphic markers, we obtained 21,145 polymorphic SNPs. The genotypic matrix was coded in 1/0.5/0, corresponding to the homozygous for the reference allele, the heterozygous, and the homozygous for the alternative allele, respectively. Missing values were imputed as the mean allele frequency of the reference allele. These SNPs were used to estimate distance between the wheat varieties as one minus the kinship coefficient estimated as in VanRaden (2008). A hierarchical clustering was implemented using the Ward method using R function hclust from package stats. A PCoA was then applied on the genetic distance matrix to extract the 10 most important PCoA axes for the 33 wheat varieties, representing the most important genetic trends in this panel.

Adjusted phenotypes (best linear unbiased prediction, BLUPs) for microbiota descriptors on the 33 varieties were estimated by pooling all datasets obtained from the different trials carried out within 2017 and 2018. The analysis was performed on root samples only. For this we used *lmer* function in lm4 R package to estimate variances of the following mixed-effect model:

$$Y_{ijk} = \mu + G_i + E_j + R_k + \varepsilon_{ijk},$$

in which μ is the intercept, G_i is the random genetic effect of wheat variety i, E_j is the random trial effect, R_k is the random replicate effect, and ε_{ijk} is the error. All random effects follow a normal distribution with an identity matrix as covariance matrix, and were considered independent.

An sPLSR analysis between the microbiota descriptors (matrix X) and the first ten wheat PCoA axes (matrix Y) was then performed. To validate the linear combinations obtained by sPLSR we additionally performed regressions on the same dataset with the *lm* R function. Regression-derived *P*-values were corrected for multiple comparisons using the FDR method, with a threshold of 5%.

Results

Faba bean association exerts subtle impacts on wheat rhizosphere and root microbiota

To assess the impact of wheat-legume intercropping on wheat-associated microbiota, we analyzed rhizosphere and root samples from wheat grown with or without faba bean (Figure 1). Experiments were conducted over two years (2017 and 2018) and across five wheat genotypes that differed between years (Table S1).

In 2017, variance analyses indicate that faba bean association partially affected fungal descriptors in either the rhizosphere or roots (Table 1, Figure 2a, Figure S2). We observed weak shifts in the fungal microbiota composition as observed along root PCoA axis 1 ($P = 0.021$) and rhizosphere PCoA axis 2 ($P = 0.043$). A nested faba bean association × wheat genotype interaction was detected for root fungal species richness ($P = 0.048$) and root fungal PCoA axis 1 ($P = 0.023$), but not in the rhizosphere. For bacterial communities in 2017, faba bean association influenced root PCoA axis 2 ($P = 0.046$), with stronger effects when nested within the wheat genotype factor (Table 2, Figure 2b). In 2018, we confirmed most of these trends. Root fungal communities showed no overall effect of faba bean association, except for PCoA axes 1 ($P = 0.043$) and 2 ($P = 0.003$). Rhizosphere fungal descriptors were unaffected by the faba bean association (Table 1, Figure 2a). In contrast, bacterial root composition was influenced by the interaction between intercropping and wheat genotype, although α-diversity remained unchanged (Table 2, Figure 2b). Similarly, rhizosphere bacterial composition (PCoA) was affected by the faba bean association × plant genotype interaction ($P = 2.66e\text{-}06$), without significant changes in α-diversity. Overall, these results suggest that faba bean association only weakly influences wheat-associated microbiota at the time of sampling. When effects were detected, they were more statistically significant for bacterial than fungal communities, and mainly concerned community composition rather than diversity. In addition, responses were often genotype-dependent.

Table 1. Analysis of deviance table (Type II Wald chi-square tests) for the fungal α- and β-diversity indices in the root or rhizosphere compartment of wheat genotypes grown under different agricultural practices. Fungal diversity was inferred by using ITS2 marker region. Analyses were independently run on root or rhizosphere data sets. Bold *P*-values represent statistically (<0.05) significant results after False Discovery Rate (FDR) correction.

		Factors	Shannon index			Species richness			PCo1			PCo2		
			Chisq	df	*P*-value	Chisq	df	*P*-value	Chisq	df	*P*-value	Chisq	df	*P*-value
ROOTS	Pure 2017	genotype	61.26	19	**6.27e-05**	44.34	19	**9.39e-03**	26.87	19	3.91e-01	188.68	19	**3.23e-15**
	Pure 2018	genotype	34.22	15	**9.39e-03**	46.27	15	**2.12e-04**	54.89	15	**1.36e-05**	23.41	15	1.39e-01
	Faba bean association 2017	treatment	0.03	1	6.50e-01	0.34	1	9.33e-01	5.32	1	**2.11e-02**	0.60	1	4.38e-01
		genotype	1.86	4	7.62e-01	2.05	4	7.54e-01	3.16	4	1.20e-01	6.59	4	1.59e-01
		treatment: genotype	8.01	2	9.11e-02	9.60	2	**4.78e-02**	11.31	2	**2.33e-02**	1.73	2	7.85e-01
	Faba bean association 2018	treatment	0.41	1	5.23e-01	0.10	1	7.53e-01	4.11	1	**4.26e-02**	6.92	1	**8.51e-03**
		genotype	5.95	4	2.03e-01	1.26	4	8.68e-01	0.39	4	9.83e-01	2.45	4	6.53e-01
		treatment: genotype	3.08	4	2.14e-01	0.90	4	6.39e-01	16.47	4	**2.66e-04**	12.73	4	**1.72e-03**
	Fertilization	treatment	5.04	1	**9.10e-03**	4.43	1	**7.22e-02**	0.11	1	8.31e-01	77.20	1	**3.23e-15**
		genotype	10.41	4	**9.39e-03**	10.27	4	**7.22e-02**	3.39	4	7.09e-01	2.63	4	7.82e-01
		treatment: genotype	10.46	4	**9.30e-03**	15.30	4	**1.06e-02**	2.22	4	8.06e-01	2.87	4	7.54e-01
RHIZOSPHERE	Pure 2018	genotype	11.68	18	9.99e-01	16.32	18	9.99e-01	21.22	18	7.03e-01	5.56	18	9.99e-01
	Faba bean association 2017	treatment	0.64	1	4.24e-01	0.44	1	5.09e-01	1.25	1	2.65e-01	4.09	1	**4.31e-02**
		genotype	0.27	4	9.92e-01	1.35	4	8.52e-01	5.87	4	2.09e-01	2.03	4	7.31e-01
		treatment: genotype	0.09	4	9.99e-01	0.54	4	9.70e-01	3.58	2	4.66e-01	5.00	4	2.87e-01
	Faba bean association 2018	treatment	0.35	1	5.54e-01	0.41	1	5.22e-01	0.28	1	5.96e-01	0.72	1	3.95e-01
		genotype	0.60	4	9.63e-01	0.62	4	9.61e-01	15.00	4	**4.71e-03**	2.53	4	6.39e-01
		treatment: genotype	3.63	4	4.59e-01	2.05	4	7.26e-01	2.27	4	6.87e-01	11.28	4	2.36e-02
	Fertilization	treatment	3.04	1	4.02e-01	1.91	1	5.91e-01	2.63	1	4.53e-01	9.81	1	**1.10e-02**
		genotype	1.37	4	9.99e-01	1.27	4	9.99e-01	6.82	4	5.68e-01	4.45	4	7.55e-01
		treatment: genotype	1.00	4	9.99e-01	1.09	4	9.99e-01	23.94	4	**6.42e-04**	5.17	4	7.03e-01

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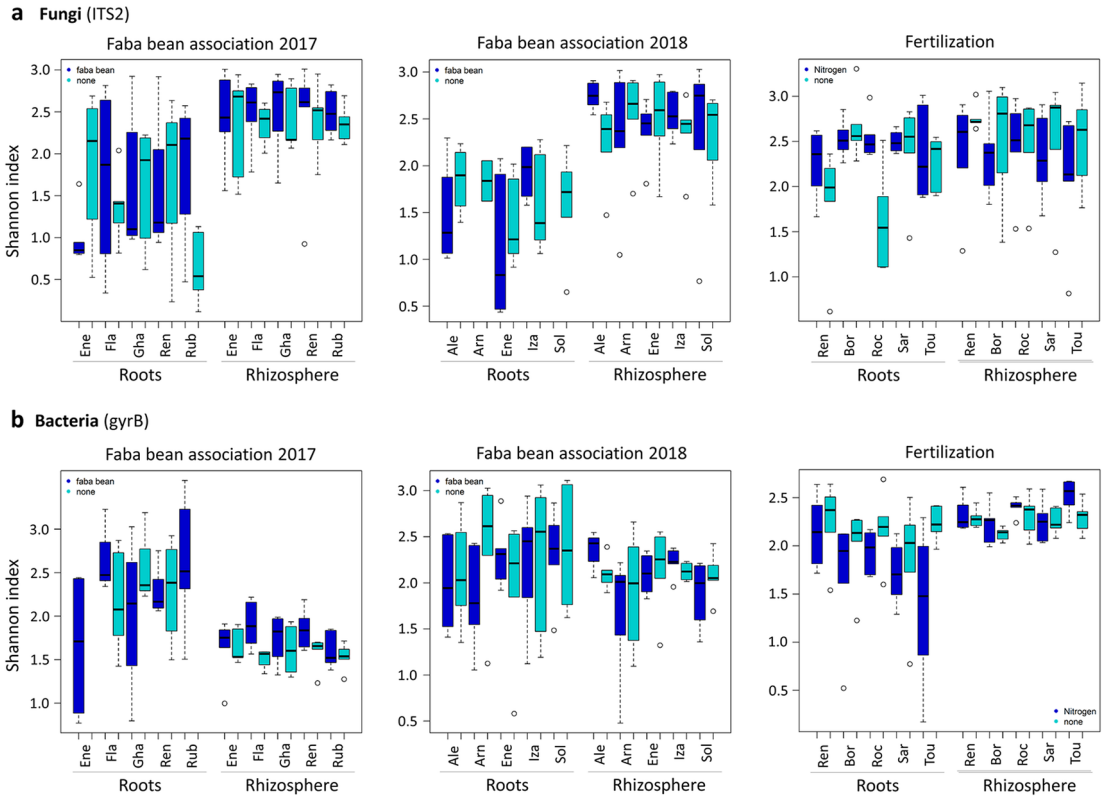

Figure 2. Fungal and bacterial α-diversity estimated for the faba bean association and fertilization trials. Box plots representing the microbiota the α-diversity inferred as Shannon's index for (**a**) fungal communities estimated by sequencing an ITS fragment and (**b**) bacterial communities estimated by sequencing a *gyrB* fragment in roots of wheat from the faba bean association trials (2017–2018) and fertilization trial (2018). *y*-axes represent the Shannon's index and *x*-axes the wheat genotypes. Root and rhizosphere samples are represented. Dark blue represents plants under the treatment (faba bean association or fertilization) and light blue represents the control plants., wheat genotype short names are indicated and full names are available in Table S1.

Organic fertilization and plant genotype are correlated with variation in wheat microbiota descriptors

To evaluate the influence of organic fertilization, we analyzed root and rhizosphere samples from five wheat varieties or populations grown with or without fertilization in 2018 (Figure 2a, Table S1). Most of the tested genotypes were landraces (Table 1).

For fungal communities, fertilization significantly affected both α- and β-diversity in the roots (Figure 2a, Figure S2, Table 1), but not in the rhizosphere. However, the responses to the fertilization were genotype-specific. For instance, i) genotype significantly influenced Shannon diversity (P = 9.39e-03) and fungal species richness (P = 0.072) ii) nested genotype × fertilization interactions were significant for both Shannon index (P = 9.30e-03) and species richness (P = 0.011) iii) individual wheat populations exhibited contrasting responses to fertilization. For bacterial communities, we observed that: i) in roots, only wheat genotype influenced α-diversity (P = 0.012, Table 2), while fertilization significantly altered community composition (P = 0.024, Table 2), ii) in rhizosphere samples, fertilization significantly affected both α- and β-diversity (Figure 2b, Table 2). As for fungi, the bacterial response to fertilization varied across wheat genotypes, with some showing increased diversity and others reduced diversity. Altogether, these findings indicate that organic fertilization has a stronger and more consistent effect than faba bean association, particularly on root-associated fungi and rhizospheric bacteria. However, the magnitude and direction of these responses depend strongly on wheat genotype.

Microbiota diversity and composition are affected by wheat genotype and variable across plant below-ground compartments and microbial kingdoms

To further investigate the possible role of wheat genotype on the below-ground microbiota, we studied 20 and 19 wheat varieties sampled in 2017 and 2018 respectively (Figure 1). In 2017, only wheat roots were collected, while in 2018 both rhizosphere and root samples were considered (Figure 1, Table S1). After data trimming and quality control, samples for

Table 2. Analysis of deviance table (Type II Wald chi-square tests) for the bacterial α- and β-diversity indices in the root or rhizosphere compartment of wheat genotypes grown under different agricultural practices. Bacterial diversity was inferred by using a portion of the *gyrB* gene. Analyses were independently performed on root or rhizosphere data sets. Bold *P*-values represent statistically (<0.05) significant results after False Discovery Rate (FDR) correction.

		Factors	Shannon index			Species richness			PCo1			PCo2		
			Chisq	df	*P*-value	Chisq	df	*P*-value	Chisq	df	*P*-value	Chisq	df	*P*-value
ROOTS	Pure 2017	genotype	29.26	19	1.45e-01	58.33	19	**9.22e-05**	41.47	19	**1.00e-02**	102.20	19	**9.82e-12**
	Pure 2018	genotype	32.79	17	**3.80e-02**	46.00	17	**1.31e-03**	23.96	17	2.32e-01	69.49	17	**8.01e-07**
	Faba bean association 2017	treatment	0.02	1	9.79e-01	0.03	1	9.77e-01	2.43	1	2.32e-01	3.00	1	1.76e-01
		genotype	5.70	4	3.81e-01	3.40	4	6.79e-01	8.20	4	1.76e-01	12.41	4	**4.60e-02**
		treatment: genotype	2.89	2	3.90e-01	0.78	2	8.35e-01	7.38	2	7.30e-02	27.95	2	**1.55e-05**
	Faba bean association 2018	treatment	0.66	1	6.12e-01	0.39	1	6.79e-01	4.05	1	1.11e-01	12.80	1	**2.00e-03**
		genotype	1.85	4	9.14e-01	1.69	4	9.36e-01	26.53	4	**2.50e-04**	10.70	4	8.30e-02
		treatment: genotype	3.21	4	6.79e-01	7.47	4	2.29e-01	19.80	4	**4.00e-03**	12.92	4	**3.80e-02**
	Fertilization	treatment	8.18	4	1.76e-01	8.59	4	1.64e-01	4.95	4	4.68e-01	16.65	4	**1.00e-02**
		genotype	8.89	1	**1.20e-02**	16.62	1	**4.16e-04**	1.82	1	3.16e-01	15.1	1	**1.00e-03**
		treatment: genotype	5.64	4	3.84e-01	3.25	4	6.79e-01	0.52	4	9.87e-01	14.40	4	**2.40e-02**
RHIZOSPHERE	Pure 2018	genotype	39.92	18	**1.00e-02**	42.45	18	**5.00e-03**	30.83	18	8.30e-02	19.67	18	5.34e-01
	Faba bean association 2017	treatment	7.21	1	**2.50e-02**	10.95	1	**5.00e-03**	6.53	1	**3.70e-02**	8.88	1	**1.20e-02**
		genotype	3.53	4	6.64e-01	2.00	4	8.92e-01	8.20	4	1.76e-01	3.63	4	6.53e-01
		treatment: genotype	4.50	2	5.27e-01	3.75	2	6.37e-01	0.88	2	9.87e-01	5.22	2	4.31e-01
	Faba bean association 2018	treatment	0.00	1	9.89e-01	0.00	1	9.87e-01	0.03	1	9.77e-01	0.01	1	9.87e-01
		genotype	10.59	4	8.60e-02	6.98	4	2.54e-01	2.73	4	7.53e-01	32.26	4	**2.57e-05**
		treatment: genotype	3.26	4	6.79e-01	7.06	4	2.52e-01	2.98	4	9.77e-01	37.91	4	**2.65e-06**
	Fertilization	treatment	19.21	4	**4.00e-03**	9.31	4	1.29e-01	14.09	4	**2.50e-02**	13.40	4	**3.30e-02**
		genotype	4.31	1	9.90e-02	0.99	1	5.02e-01	0.04	1	7.53e-01	1.79	1	3.17e-01
		treatment: genotype	3.95	4	6.12e-01	6.79	4	2.68e-01	0.55	4	9.87e-01	1.27	4	9.77e-01

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20, 15 and 19 wheat varieties passed quality threshold for root samples collected in 2017 and root and rhizosphere samples collected in 2018 respectively. Linear-mixed models on microbiota descriptors (i.e. species richness, Shannon index, PCoA axes 1 and 2) on samples collected in 2018 showed a strong plant below-ground compartment effect on both fungal and bacterial communities (Table S11). Consequently, statistical analyses were independently performed on root and rhizosphere samples.

When considering root samples collected in 2017 (20 wheat varieties) we observed a strong plant genotype effect on both fungal (P = 6.27e^{-05} for Shannon index, P = 9.39e^{-03} for species richness and P = 3.23e^{-15} for PCoA axis 2) and bacterial (P = 9.22e^{-05} for species richness, P = 1.00e^{-02} for PCoA axis 1 and P = 9.82e^{-12} for PCoA axis 2) microbiota α- and β-diversity descriptors (Table 1, Table 2, Figure 3a, b, Figure S3). In accordance, tb-RDA analysis, reflecting the microbiota composition, showed that the wheat genotype was a significant factor explaining the variance of the root microbiota composition for both fungal (P = 0.001) and bacterial (P = 0.001) communities (Table 3, Figure 4a, b, Table S12 and S13). For the fungal communities, the Arnold, Descartes and Renan varieties were those with the most differentiated (based on the Hellinger spatial distance matrix) root microbiota composition when comparing to other wheat varieties (Figure 3a). For the bacterial communities, the tb-RDA showed a more diversified pattern, however, as for the fungal communities, Descartes and Renan, in addition to Activus and Metropolis, were also the most differentiated varieties, in terms of root microbiota composition (Figure 3b).

For rhizosphere samples collected in 2018 (19 wheat varieties), we did not observe a significant effect of the wheat genotype on the fungal microbiota α-descriptors (Table 1, Figure 3a). On the other hand, an effect on the fungal microbiota composition was observed on the tb-RDA analysis (P = 0.001, Table 3, Figure 3a Table S12). When considering the bacterial microbiota, a significant effect of the wheat genotype was found on the Shannon's index (P = 1.00e^{-02}, Table 2) and species richness (P = 5.00e^{-03}, Table 2), however the genotype effect was not significant on

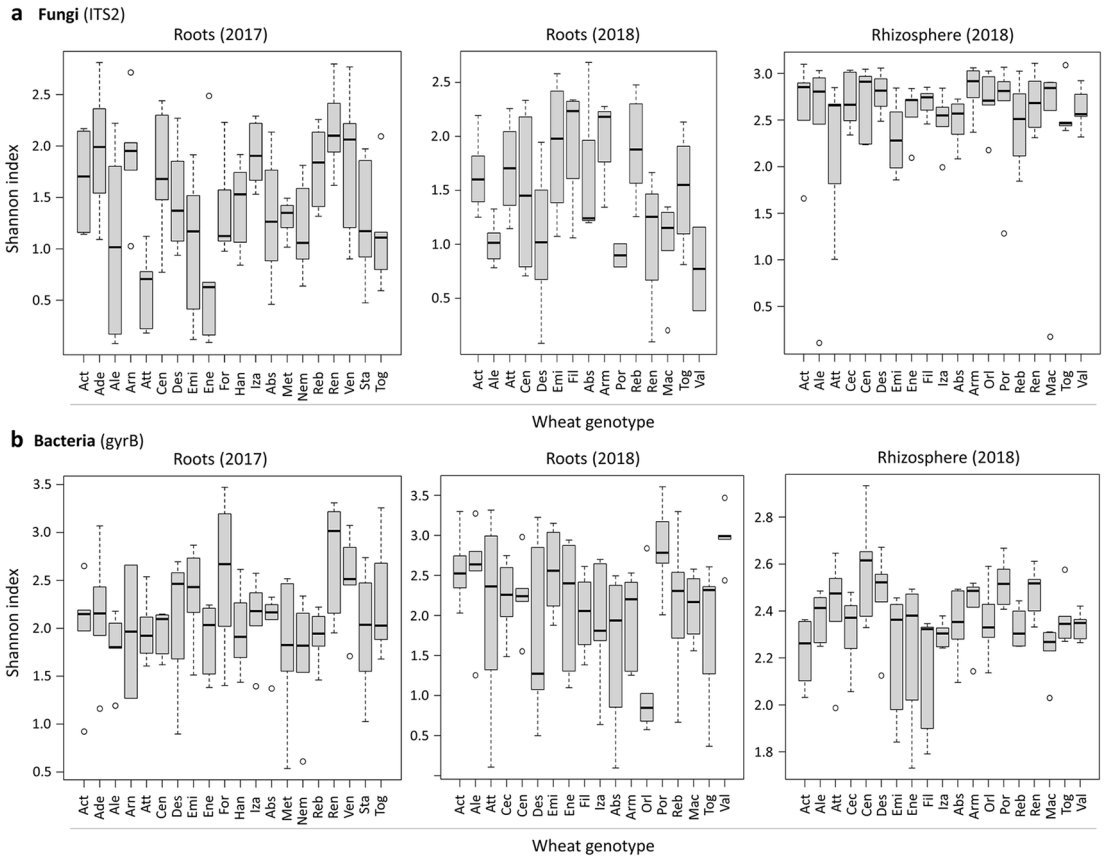

Figure 3. Fungal and bacterial α-diversity estimated for the wheat genotype trial. Box plots representing the microbiota α-diversity inferred as Shannon's index for (**a**) fungal communities estimated by sequencing an ITS fragment and (**b**) bacterial communities estimated by sequencing a *gyrB* fragment in the root wheat samples in 2017 and 2018 and rhizosphere samples in 2018. *y*-axes represent the Shannon's index and *x*-axes the wheat genotypes. Root and rhizosphere samples are represented in separated boxes.

Table 3. tb-RDA on microbial communities performed independently on the two compartments (root and rhizosphere). Bold *P*-values represent statistically (<0.05) significant results after False Discovery Rate (FDR) correction.

		Roots				Rhizospheres			
		Variance	**df**	***F***	***P*-value**	**Variance**	**df**	***F***	***P*-value**
FUNGI	wheat genotype 2017	0.178	19	1.3444	**1e-03**				
	wheat genotype 2018	0.209	15	1.3364	**1e-03**	0.110	18	1.5504	**1e-03**
BACTERIA	wheat genotype 2017	0.180	19	1.3507	**1e-03**				
	wheat genotype 2018	0.179	18	1.3988	**1e-03**	0.118	18	1.648	**1e-03**

the PCoA axes. tb-RDA analysis on the rhizosphere bacterial communities showed that wheat genotype affected the bacterial composition ($P = 0.001$, Table 3, Table S13), and Activus, Alessio and Attlass were the most differentiated wheat varieties (Figure 3b). Altogether, in accordance with results obtained from roots collected in 2017, we observed a significant effect of wheat genotype on both fungal and bacterial α- and β-diversity descriptors (Figure 3a, 3b, Figure 4a, 4b, Table 1, Table 2, Table 3).

Root microbiota descriptors are strongly influenced by wheat varieties and wheat landraces display a more diversified fungal microbiota

As microbiota descriptors in the root compartment were highly influenced by the wheat genotype, we investigated the possible correlation between root microbiota and the genetic differentiation of the wheat varieties. For this, we estimated

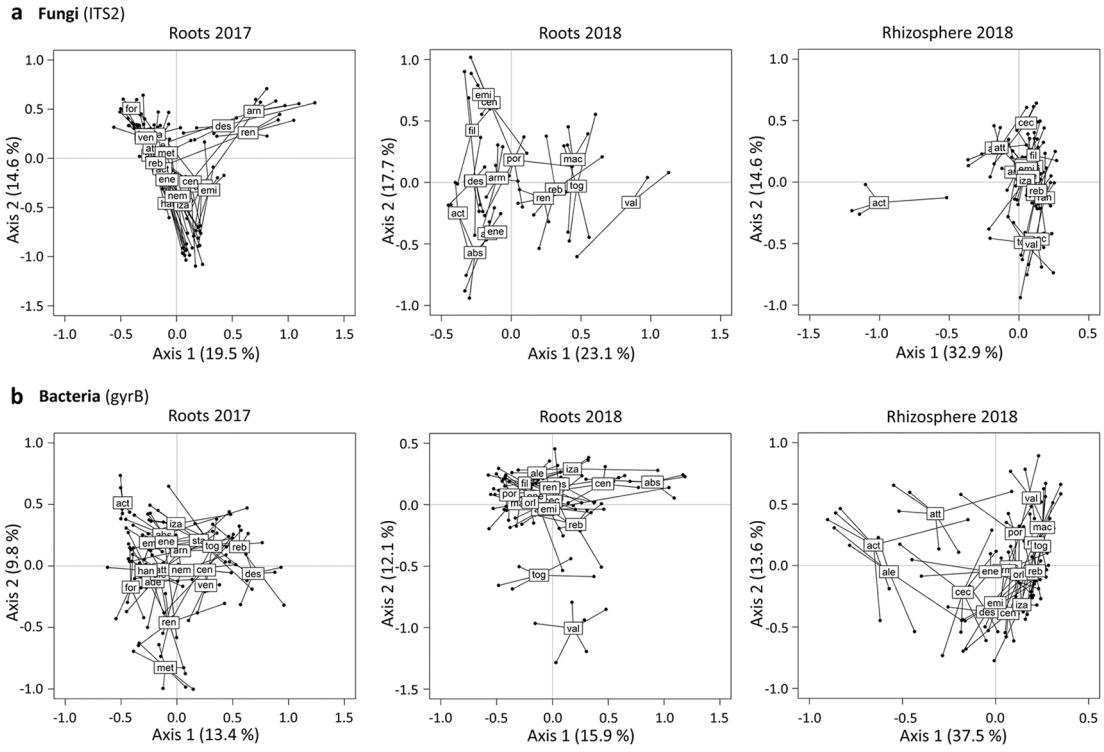

Figure 4. Fungal and bacterial β-diversity estimated for the wheat genotype trial. Transformation based-Redundancy Analysis (tbRDA) representing (**a**) the variation in the fungal community composition within the wheat genotypes collected in 2017 (for the root samples) and 2018 (for the root and rhizosphere samples) and (**b**) the variation in the bacterial community composition within the wheat genotypes collected in 2017 (for the root samples) and 2018 (for the root and rhizosphere samples).

corrected means (BLUPs) of microbiota descriptors in all wheat varieties collected over 2017 and 2018. We also included control plants of the faba bean and fertilization trials. In total, we succeeded to obtain BLUPs for 33 wheat genotypes (Table S14 for fungal communities, Table S15 for bacterial communities). These genotypes were characterized for 21,145 SNPs showing a genetic differentiation among the wheat varieties. As expected, the landrace populations clustered together and were clearly differentiated from most of the elite varieties, as shown by the PCoA (Figure 5a, Table S16). BLUPs estimated on the microbiota descriptors were used to perform a Sparse Partial Least-Squares Regression (sPLSR) with the PCoA axes of the wheat genetic distance matrix (thereafter, wheat PCoA axes). Wheat PCoA axis 7 and 10 were correlated with most of the microbiota descriptors (Figure 5b, Table S17). In particular, both Axis 7 and 10 significantly explained the fungal diversity descriptors (i.e. species richness and Shannon and Simpsons's indexes) ($P \leq 0.01$, Figure 5c) and the bacterial diversity descriptors ($P \leq 0.01$) and composition (PCoA analysis) ($P \leq 0.1$, Figure 5c). In addition, linear regressions showed that wheat PCoA axis 1 was significantly correlated with fungal species richness ($P \leq 0.1$) and fungal and bacterial composition ($P \leq 0.01$, Figure 5c, Table S18). PCoA axis 7 was correlated with fungal and bacterial species richness ($P \leq 0.01$, $P \leq 0.01$ respectively) and to bacterial composition ($P \leq 0.01$, Figure 5c) and PCoA axis 10 was significantly correlated with fungal species richness and composition ($P \leq 0.01$, Figure 5c) but not with bacterial microbiota descriptors. The significant regressions were negative or positive (Figure S4) although as made on PCoA, this should not be interpreted as a biological positive or negative relationship. Overall, both sPLSR and linear regressions claimed that host genetic differentiation is a driver of both fungal and bacterial diversity in the root compartment (Figure 5).

As previous studies suggested an effect of ancient wheat varieties on the rhizosphere microbiota (Rossmann et al. 2020), we additionally tested whether there were significant differences in plant below-ground microbiota between modern wheat varieties and landraces. To test it, we performed ANOVAs on the fungal and bacterial microbiota descriptors, using the wheat factor (modern *vs.* landrace) as a fixed effect. For the fungal communities, we found a strong effect of the wheat

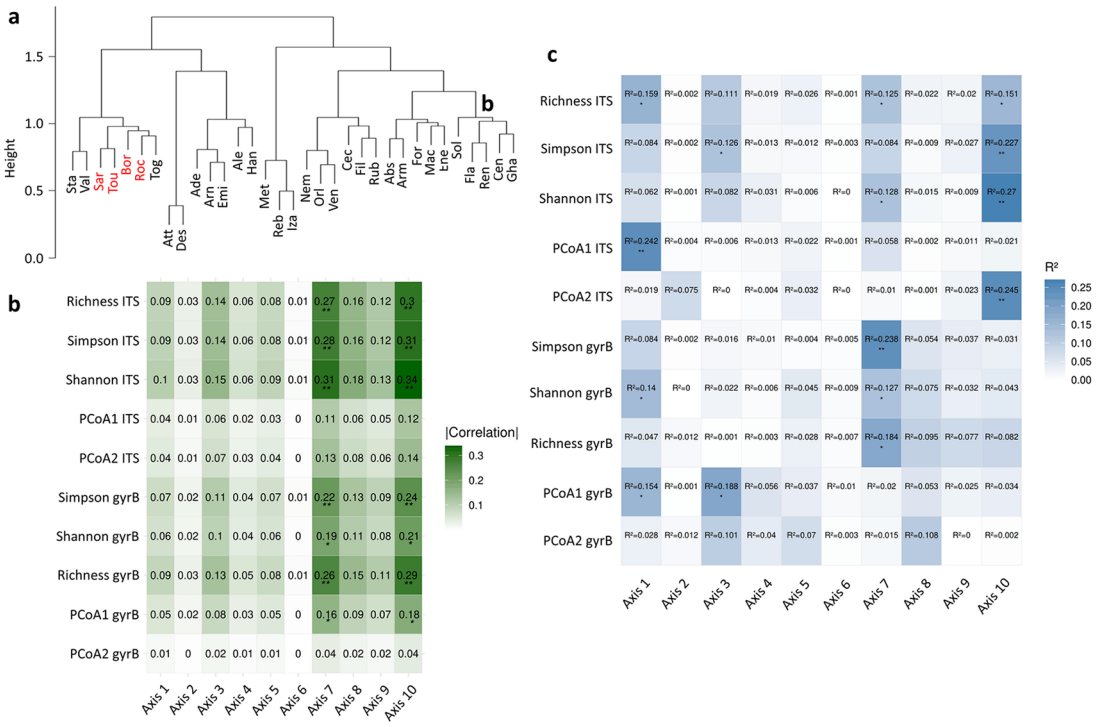

Figure 5. Relationship between wheat genetic differentiation and the microbiota diversity descriptors. (**a**) Hierarchical clustering based on the Ward method applied to the wheat genetic distance matrix estimated using 21,145 polymorphic SNPs. Red names indicate the landrace populations. (**b**) Relationships between microbiota α- and β-diversity fungal and bacterial descriptors and the wheat PCoA axes estimated on the 21,145 polymorphic SNPs based on a sparse partial least-squares regression (sPLSR). Only microbiota descriptors with a loading value above 0.2 are considered significant. *P*-value significance from a permutation test is indicated as: $P < 0.005$ = ** and $P < 0.05$ = *. The correlation intensity is represented by the color scale ranging from white (weak) to dark green (strong). (**c**) Linear regression between microbiota α- and β-diversity descriptors and the wheat PCoA axes estimated on the 21,145 polymorphic SNPs. *P*-value significance is indicated as: $P < 0.005$ = ** and $P < 0.05$ = *. The regression strength is represented by a color scale ranging from white (low) to dark blue (high).

Table 4. Summary of One-Way ANOVA testing the effect of modern *vs.* landrace wheat varieties on fungal and bacterial microbiota descriptors. Bold *P*-values represent statistically (<0.05) significant results after False Discovery Rate (FDR) correction.

Fungal communities									
Richness ITS		**Simpson ITS**		**Shannon ITS**		**PCoA1 ITS**		**PCoA2 ITS**	
F	***P* value**	***F***	***P*-value**	***F***	***P*-value**	***F***	***P*-value**	***F***	***P*-value**
32.14	**3.15e-06**	22.27	**4.80e-05**	16.69	**2.88e-04**	2.144	1.53e-01	2.723	1.09e-01
Bacterial communities									
Richness *gyrB*		**Simpson *gyrB***		**Shannon *gyrB***		**PCoA1 *gyrB***		**PCoA2 *gyrB***	
F	***P* value**	***F***	***P*-value**	***F***	***P*-value**	***F***	***P*-value**	***F***	***P*-value**
2.136	1.54e-01	0.038	8.46e-01	0.001	9.76e-01	22.88	**3.73e-05**	2.505	1.23e-01

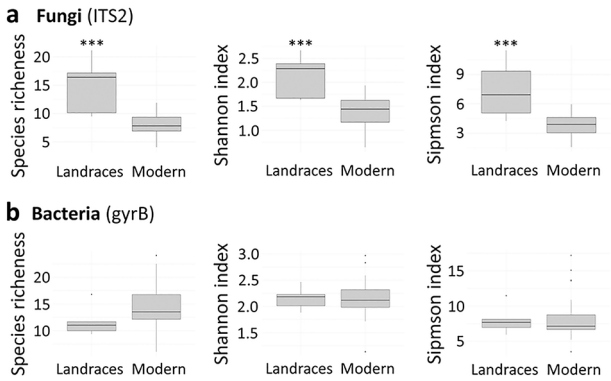

Figure 6. Box plot representing microbiota diversity descriptors in landrace and modern wheat. Box plots representing the microbiota variation of the α-diversity inferred as species richness (**a**) Shannon's index (**b**) and Simpson index (**c**) for fungal communities. *P*-values estimated by a 2-way ANOVA after FDR correction are indicated in the plots as $P < 0.0005$ = ***. Box plots representing the microbiota variation of the α-diversity inferred as species richness (**d**) Shannon's index (**e**) and Simpson index (**f**) for bacterial communities.

factor on fungal α-diversity descriptors (*P-values* ≤ 0.0001, Table 4), but not on fungal community composition (Table 4). Specifically, landraces harbored a more diverse fungal microbiota than modern varieties ($P \leq 0.0001$, Figure 6a–c). In contrast, we did not observe significant differences in bacterial microbiota descriptors, except for PCoA axis 1 ($P \leq 0.0001$, Table 4, Figure 6d–f). Altogether, our results show that landraces are associated with more diverse fungal communities, while their effect on bacterial communities is limited to compositional differences.

Wheat yield traits are influenced by plant genotype and faintly correlated with bacterial root microbiota descriptors

In addition to understanding the role of host genetic variability in modulating microbiota diversity, it is essential from an agronomical point of view, to test whether a link exists between microbiota descriptors and crop yield traits. To investigate whether microbiota descriptors were correlated with wheat agronomically important traits, we characterized the protein content, the TGW (Thousand Grain Weight) and grain yield on all wheat trials (Table S19). We first observed that the wheat genotype factor influenced significantly all the traits and, in all trials, (Table 5) with the exception of grain yield in the faba bean association trial performed in 2017. We also observed that faba bean association influenced the protein content, while fertilization influenced significantly all the traits. To explore the association between microbiota descriptors and wheat traits, we performed correlation analysis between the root microbiota descriptors and the plant traits for both the 2017 and 2018 datasets. For root samples collected in 2017, we observed a significant positive correlation between the bacterial α-diversity descriptors (Shannon index and species richness) and the TGW ($P < 0.05$, Figure 7a). On root samples collected in 2018 we only observed a significant negative correlation between the bacterial PCoA axis 2 and TGW ($P < 0.05$, Figure 7b). In both 2017 and 2018 datasets, fungal microbiota descriptors were not significantly correlated with wheat phenotypic traits. Overall, our results suggest that wheat bacterial root microbiota α- and β-diversity only partially correlate with wheat agronomic traits in these field assays.

Table 5. Analysis of deviance table (Type II Wald chi-square tests) for wheat agronomic traits: protein content (protein), Thousand-Grain Weight (TGW), and grain yield (yield). Bold *P*-values represent statistically (<0.05) significant results after False Discovery Rate (FDR) correction.

	Factors	Protein			TGW			Yield		
		Chisq	df	*P*-value	Chisq	df	*P*-value	Chisq	df	*P*-value
Wheat genotypes 2017	Genotype	216.73	15	**2e-16**	191.16	15	**2e-16**	161.74	15	**2e-16**
Wheat genotypes 2018	Genotype	202.93	20	**2e-16**	51.23	20	**1.e-04**	38.30	20	**8e-03**
Faba bean association 2017	Treatment	28.66	1	**9e-08**	2.81	1	0.0934	0.128	1	0.721
	Genotype	280.02	4	**2e-16**	36.50	4	**2e-07**	4.50	4	0.342
	Treatment: Genotype	14.79	4	**5e-03**	2.92	4	0.572	1.74	4	0.784
Faba bean association 2018	Treatment	20.89	1	**5e-06**	0.530	1	0.467	2.19	1	0.139
	Genotype	137.51	4	**2e-16**	27.95	4	**1e-05**	37.73	4	**1e-07**
	Treatment: Genotype	8.39	4	0.078	15.24	4	**4e-03**	23.75	4	**9e-05**
Fertilization	Treatment	19.97	1	**8e-06**	70.33	1	**2e-16**	404.10	1	**2e-16**
	Genotype	103.07	4	**2e-16**	43.16	4	**1e-08**	10.31	4	0.036
	Treatment: Genotype	0.623	4	0.961	0.946	4	0.918	3.19	4	0.526

Discussion

Agricultural management, including crop rotation, intercropping, selection of crop genotypes carrying a particular plant trait, fertilization, utilization of pesticides and tillage, has been described to significantly influence plant-associated and soil microbes (Hartman et al., 2018). The growing interest in sustainable agricultural practices has driven research into how these practices impact the structure and function of the crop microbiota, with the ultimate goal of reducing chemical inputs and improving resilience to stress (Rebouh et al., 2023). Fertilization, particularly the use of nitrogen (N) fertilizers, is one of the most studied agricultural practices concerning its impact on the host and soil microbiota. In wheat, N fertilization alters the diversity and composition of both fungal and bacterial communities (Lori et al., 2023) and fungal communities seem to be more sensitive to N availability (Bai et al., 2022). Studies have shown that increased N availability tends to reduce microbial diversity, favoring fast-growing bacterial species that can rapidly assimilate nitrogen and diminishing the prevalence of slower-growing species involved in N-cycling (Paes da Costa et al., 2024). Notably, inorganic N application reduces the abundance and prevalence of Plant-Growth-Promoting Rhizobacteria (PGPR) in wheat plants grown in controlled conditions (Reid et al., 2021). On the other hands, organic N sources have been reported to promote more diverse microbial communities compared to synthetic fertilizers, which tend to select for specific microbial groups (Lazcano et al., 2021). In our study we investigated the role of organic fertilization on wheat landraces and found that organic fertilization was a non-negligent factor in modulating bacterial and fungal wheat belowground communities. In our one-year field experimental trial, fertilization had a notable impact on fungal communities in the root compartment, where both α- and β-diversity were influenced. Interestingly, the response to fertilization was highly variable among wheat genotypes, with some varieties/populations showing increased microbial diversity (e.g., Renan and Rouge du Roc, showing an increase of fungal diversity), while others (e.g., Saragnet and Touselle) showed a decrease in microbial species richness. This suggests that the response of microbial communities to organic fertilization is genotype-dependent, which could be tied to different N uptake efficiencies or root architectures among genotypes. This variability underscores the importance of considering genetic diversity when studying plant-microbe interactions under different agricultural practices. However, the interaction between organic fertilization and wheat genotype needs to be followed over several seasons to appreciate the long-term impact on the microbial communities. In addition, our study does not explore the nested effects of wheat developmental stage and fertilization on host-associated microbial communities. Previous research by Chen et al. (2019) demonstrated in a single wheat variety that the developmental stage of the host is a crucial factor in shaping the influence of N fertilization on bacterial communities. Furthermore, the effect of N on root microbial communities is context-dependent, varying with environmental conditions. For example, Usyskin-Tonne et al. (2021) investigated the combined effects of three different N levels and CO_2 concentrations on one

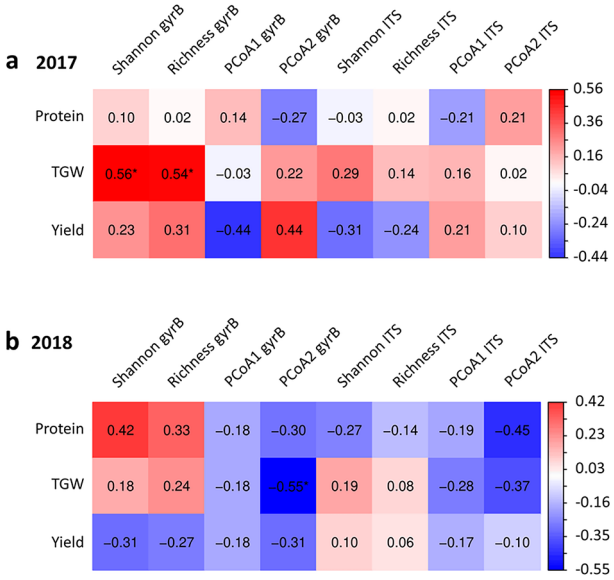

Figure 7. Correlation between microbiota descriptors and wheat phenotypes. Correlation between microbiota α- and β-diversity fungal and bacterial descriptors and the wheat phenotypes: protein content (protein), Thousand Grain Weight (TGW), and grain yield (yield) estimated on the entire wheat trials on all genotypes for 2017 trials (a) and 2018 trials (b). Positive correlations are indicated with red color while negative correlations with blue color. Significant correlation indicated by * corresponds to $P < 0.05$.

wheat genotype under controlled conditions. Their findings revealed that high nitrate levels only increased bacterial abundance when wheat was grown in elevated CO_2 environments.

A more sustainable approach to increase N availability in soils is the integration of leguminous crops capable of nitrogen fixation through symbiosis with beneficial bacteria called rhizobia (Jensen et al., 2010; Peoples et al., 1992) through either crop rotation or intercropping with wheat. When wheat is grown in rotation with legumes, such as faba bean, changes in the rhizosphere and root microbiomes have been observed, including an enrichment of nitrogen-fixing bacteria and other beneficial microbes (Siczek et al., 2024). A field study in Poland showed that faba bean generally supports higher microbial diversity compared to wheat, contributing to soil N-fertility (Siczek et al., 2024), underlining the importance of crop succession in plant-microbe interactions. Wheat/faba bean intercropping has shown to increase wheat production after two cropping seasons with an impact on mycorrhizal fungi (Wahbi et al., 2016). To extend the understanding of the wheat/faba bean intercropping, we tested this interaction within 5 wheat genotypes and over 2 years. The results of our study demonstrate that the association of faba bean with wheat exerts a relatively weak influence on the wheat microbiota, particularly in terms of fungal and bacterial diversity. While some effects were observed on the microbial composition, particularly in the root compartment, the overall impact of faba bean association on the wheat microbiota was minimal. This suggests that while legume-wheat intercropping may have potential in influencing microbial communities, the effects may be genotype-specific, indirect (through enriching soil with organic N) or context-dependent, requiring further investigation across different environmental and genetic conditions. Indeed, some of the observed effects varied among the five wheat genotypes, reinforcing the idea that host genetic diversity is a crucial factor to consider in conjunction with other agricultural management practices.

Table 6. Heritability estimated on the microbial diversity descriptors in wheat roots.

	Fungal diversity descriptors		***Bacterial diversity descriptors***	
Descriptors	**2017**	**2018**	**2017**	**2018**
Shannon's index	**0.68**	**0.63**	0.38	**0.5**
Species richness	**0.56**	**0.71**	**0.69**	**0.65**
PCoA axis1	0.3	0.032	**0.55**	0.33
PCoA axis2	0.098	0.088	**0.82**	**0.93**

We further investigated the role of wheat genetic variability in influencing the diversity and composition of below-ground bacterial and fungal communities in varietal assays under field-realistic conditions. Recent literature shows that studies rarely focused on the role of wheat genotype in modulating microbiota diversity. Moreover, most of these studies on wheat below-ground microbiota were conducted under controlled conditions that do not fully reflect the microbial diversity of agricultural fields. However, even with a limited number of wheat genotypes and in non-realistic conditions, the majority of these studies have shown that wheat genotype influences fungal and bacterial root and rhizosphere diversity (Azarbad et al., 2020), with the variety factor explaining up to 25% of fungal variation in wheat roots (Latz et al., 2021) or the relative abundance of bacterial order Rhizobiales, with an estimated heritability of 25% (Spor et al., 2020). On the other hand, Simonin et al. (2020), in a controlled experiment with eight wheat genotypes, did not identify a significant role of wheat genotype in modulating bacterial and fungal species richness and composition. A large field study on 95 winter varieties in China (Zheng et al., 2023) pointed out that: i) a core set of root-associated prokaryotic species was consistently found across different wheat varieties; ii) the composition and abundance of certain bacterial groups in the wheat rhizosphere were linked to specific plant traits; and iii) significant variation in root-associated prokaryotic communities was observed depending on the wheat genotype, suggesting that plant genetic differences shape the microbiome, which in turn can impact important agricultural traits. In agreement with Zheng et al., 2023, the influence of wheat genotype on the below-ground microbiota was one of the most influencing factors across both experimental years. Indeed, heritability on both fungal and bacterial microbiota descriptors, clearly shows a significant effect of the wheat variety factor on the root microbiota (Table 6). In fact, heritability values were highly significant for both 2017 and 2018 trials when considering both fungal and bacterial diversity and composition descriptors. In particular, our study provides compelling evidence that wheat genetic differentiation significantly shapes root-associated microbial communities, with distinct impacts on both bacterial and fungal diversity. Through sPLS and linear regressions, we found that wheat host genetics correlate with microbial species richness and community composition, particularly for fungi. This confirms that host plant identity plays a central role in structuring the root microbiome as observed in other plant species (Bulgarelli et al., 2012; Edwards et al., 2015). Importantly, our findings suggest that species richness is the microbiota descriptor most sensitive to host genetic differentiation, as supported by both multivariate modeling and linear regressions. This is particularly evident for fungal communities, which showed consistent and significant correlations with several wheat PCoA axes representing the genetic distance between the genotypes (notably axes 1, 7, and 10). The type of these correlations (positive or negative, Figure S4) suggests that distinct plant genotypes may either favor or restrict microbial diversity, potentially reflecting genotype-specific differences in root exudation patterns, immune signaling, or root morphology. One intriguing outcome of our study is the differential effect of the wheat genetic background on fungal *vs* bacterial communities and depending on the plant below-ground compartment. Indeed, we observed that in the root compartment, the effect of wheat genotype was stronger on fungal communities, whereas in the rhizosphere, plant genotype had a greater influence on bacterial communities. These results underscore the asymmetric relationship between wheat genotype and the two microbial kingdoms, possibly due to the tighter co-evolutionary history of fungi with plants compared to more generalist bacterial colonizers. Similar kingdom and compartment specific effects were observed with fertilization. Together, these results suggest that niche utilization and plant-microbe interactions differ between kingdoms, highlighting the importance of considering all microbiota members when studying holobionts.

When considering the contrast between landraces and modern varieties, our findings emphasize the potential of landraces to support richer fungal microbiota. This is a prominent result, revealing a strong effect of landrace *vs* modern wheat on fungal species richness ($P \leq 0.0001$), but not on fungal community composition or most bacterial descriptors. Our result is in agreement with a previous study on wild barley and bean, demonstrating how ancient accessions support a more diversified microbiota (Escudero-Martinez et al., 2022; Pérez-Jaramillo et al., 2017). The higher fungal diversity in landraces observed in our study could be a consequence of their higher within-population heterogeneity (when comparing with modern wheats), reflecting the inherent genetic diversity in these ancient populations. Unlike modern cultivars, which are often genetically homogeneous, landraces are genetically diverse populations, each harboring a mosaic of allelic combinations. This intra-population variability may create a broader spectrum of root traits and exudate profiles, offering a more diverse niche landscape for microbial colonization. From an ecological and evolutionary standpoint, this result aligns with the hypothesis that genetic diversity within a plant population enhances microbiome diversity by increasing the number of available microbial niches. In turn, this could confer greater functional resilience and adaptability to environmental stressors. In contrast, we did not find any significant historical trends among the modern genotypes.

An essential agronomic consideration is the role of wheat genetic diversity in shaping phenotypic traits associated with productivity. A recent study conducted on 150 elite bread wheat genotypes in Ethiopia (Mulugeta et al., 2024) demonstrated significant divergence among wheat genotypes in terms of quantitative traits related to yield and plant health. Genome-Wide Association (GWA) studies across multiple wheat varieties have identified numerous loci and up to 28 candidate genes associated with key agronomic phenotypic traits (Jung et al., 2021). However, apart from the study

by Zheng et al. (2023), the relationship between wheat phenotypic traits and microbiota, particularly across a broad panel of genotypes under ecologically relevant conditions, remains largely unexplored. In our study, we characterized three agronomic traits - protein content, thousand-grain weight (TGW), and grain yield - across multiple trials over two years. By regressing these phenotypic traits against bacterial and fungal descriptors, we observed a positive correlation between root bacterial microbiota diversity and TGW ($P < 0.01$). However, fungal microbiota descriptors were not correlated with the economically important wheat traits that are protein content and grain yield. Our results suggest that microbiota members may influence plant traits related to fitness more strongly than those related to growth and production. As variations in the below-ground microbiota diversity descriptors between wheat genotypes do not correlate with variations in their agronomic performance, further studies may focus on the microbiota ecosystem functions rather than microbiota diversity to determine whether the wheat below-ground microbiota can enhance agricultural production. Alternatively, a broader range of environmental conditions and agricultural practices may be investigated to unravel whether below-ground microbiota diversity descriptors correlate with agronomic performance in particular stress condition. In any case, our results suggest that integrating the genetic diversity of wheat for both recruitment of microbial communities and response to these microbial communities is crucial for developing effective biostimulants or optimizing recruitment of endogenous soil microorganisms.

Author roles

Bartoli C: Conceptualization, Data Curation, Formal Analysis, Investigation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Burel E:** Conceptualization, Investigation, Resources; **Carrère S:** Data Curation, Formal Analysis; **Rincent R:** Data Curation, Validation, Formal Analysis, Writing – Review & Editing; **Lefebvre B:** Conceptualization, Funding Acquisition, Project Administration, Supervision, Validation, Visualization, Writing – Review & Editing.

Ethics and consents

Ethical approval and consent were not required.

Data availability

The raw FastQ reads were deposited in the Sequence Read Archive (SRA) of NCBI https://trace.ncbi.nlm.nih.gov/Traces/index.html?view=study under the study number SRP587060 for the fungal reads and SRP587039 for the bacterial reads.

Supplementary Data of the paper: An in-situ characterization of *Triticum aestivum* sheds light on the role of wheat genotype and agricultural practices on the below-ground microbiota. In Zenodo https://doi.org/10.5281/zenodo.19595901. This project includes the following data:

Figure S1. **Soil texture of the three plots where the wheat assays were conducted in 2016–2017 and 2017–2018.** The LH7 plot was used for the 20 wheat genotypes trial and the LH6B plot for the faba bean association trial, in 2016–2017. The LH8 plot was used for the 19 wheat genotypes assays and the fertliziation trial, and the LH6B plot for the faba bean association trial, in 2017–2018.

Figure S2. **Fungal and bacterial α-diversity estimated for the faba bean association and fertilization trials**. Box plots representing the microbiota variation of the α-diversity inferred as Species Richeness for (**a**) fungal communities estimated by sequencing an ITS fragment and (**b**) bacterial communities estimated by sequencing a *gyrB* fragment in roots of wheat from the faba bean association trials (2017–2018) and fertilization trial (2018). *y*-axes represent the Species Richeness index and *x*-axes the wheat genotypes. Root and rhizosphere samples are represented. Dark blue represents plants under the treatment (faba bean association or fertilization) and light blue represents the control plants.

Figure S3. Box plots representing the wheat genotype microbiota variation of the α-diversity inferred as species richennes for (**a**) fungal communities estimated by sequencing an ITS fragment and (**b**) bacterial communities estimated by sequencing a *gyrB* fragment in wheat roots and rhizospheres collected in 2017 and 2018 on the wheat genotype assay. *y*-axes represent the species Species Richeness index and *x*-axes the wheat genotypes.

Figure S4. Grid plot for the significant linear regressions found by regressing fungal and bacterial microbiota descriptors (*x* axis), and wheat genetic distance PCoA axes. R2 and the corresponding *P*-values corrected for FDR are illustrated for each plot.

Table S1. Genotype abbreviated names.

Table S2. Primer pairs used for ITS amplification. Red bases indicate the Illumina adaptors, blue bases indicate the internal TAGS used for sample multiplexing, blue bases indicate the ITS2 primer.

Table S3. TAGS for demultiplexing the fungal reads in the study SRP587060. r = roots, rh = rhizosphere, fert = fertilized, nfert = control in the fertilization trial, f = faba bean association, nf = control in the faba bean association trial, a = 2017, b = 2018. Other conditions were not used in this study.

Table S3. TAGS for demultiplexing the bacterial reads in the study SRP587039. r = roots, rh = rhizosphere, fert = fertilized, nfert = control in the fertilization trial, f = faba bean association, nf = control in the faba bean association trial, a = 2017, b = 2018. Other conditions were not used in this study.

Table S5. Fungal OTU affiliation.

Table S6. Fungal OTU count table. r = roots, rh = rhizosphere, fert = fertilized, nfert = control in the fertilization trial, f = faba bean association, nf = control in the faba bean association trial, a = 2017, b = 2018. Other conditions were not used in this study.

Table S7. Bacterial OTU affiliation.

Table S8. Bacterial OTU count table. r = roots, rh = rhizosphere, fert = fertilized, nfert = control in the fertilization trial, f = faba bean association, nf = control in the faba bean association trial, a = 2017, b = 2018. Other conditions were not used in this study.

Table S9. Raw values for both α-diversity and β-diversity indices estimated on the ITS and used for statistical analysis.

Table S10. Raw values for both α-diversity and β-diversity indices estimated on the *gyrB* used for statistical analysis.

Table S11. Analysis of deviance table (Type II Wald chisquare tests) for the α-diversity and β-diversity indices in the root and rhizosphere compartments of wheat genotypes grown under different agricultural practices. Bacterial diversity was inferred by using a portion of the *gyrB* gene and fungal diversity was inferred by using ITS2 marker region.

Data Set S12. Pairwise comparisons for the ITS data set using factor fitting to an ordination for the wheat genotype 2017 dataset (A) and genotype 2018 root compartment dataset (B). P-values have been corrected with FDR method.

Table S13. Pairwise comparisons for the *gyrB* Table using factor fitting to an ordination for the wheat genotype 2017 dataset (A) and genotype 2018 root compartment dataset (B). P-values have been corrected with FDR method.

Table S14. BLUPs for the ITS Table.

Table S15. BLUPs for the *gyrB* Table.

Table S16. PCoA Axis for plant genotypes.

Table S17. sPLSR between axes of the PCoA of the SNPs and microbiota descriptors.

Table S18. Linear regressions between axes of the PCoA of the SNPs and microbiota descriptors.

Table S19. Wheat phenotypic values. Protein content (protein), Thousand-grain weight (TGW), and Grain yield (yield).

Data are available under the terms Creative Commons Zero v1.0 Universal (CC-BY 4.0).

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