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## RESEARCH ARTICLE

# Effect of organic farming on root microbiota, seed production and pathogen resistance in winter wheat fields

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**Societal Impact Statement**

Agricultural intensification is a major driver of biodiversity decline in agrosystems. For instance, it has been shown that conventional farming leads to a decline in soil microbial diversity and triggers a strong selection process, altering the functioning of the whole ecosystem. The present study shows that organic farming increases diversity and affects composition of crop plant microbiota, mostly as a response to field management and soil characteristics. Furthermore, crop plant microbiota influences crop production and resistance to pathogens. Therefore, agricultural practices affect plant performance through microorganism-mediated changes, which may be important pillars of future sustainable crop production.

**Summary**

- Agricultural intensification threatens biodiversity, but the effects of intensification on microorganisms are still overlooked despite their role in ecosystem functioning. Microorganisms associated with plants provide many services that affect plant growth and health. Organic farming is expected to strongly affect species composition, richness, and their interactions. We analyzed the effect of the farming system on endophytic microbial assemblages associated with winter wheat plants and plant performance in the field.
- We collected environmental data through farmer interviews, soil analyses, and plant inventories and analyzed root microbiota at vegetative and flowering stages.
- Organic farming increased fungal and bacterial diversity associated to wheat plants and affected species composition in most phyla. This effect was mostly due to soil characteristics and field management and a little to plant diversity in the field. Microbial responses were more pronounced at the late developmental stage, likely as a result of accumulative effect of management actions during plant development. Seed production and resistance to pathogens were related to specific phyla that are important for seed production and/or wheat resistance to septoriose.

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- This work advances our understanding of how agricultural practices affect plant performance through microorganism-mediated changes and supports the use of microorganisms as pillars of sustainable crop production.

**KEYWORDS**

agricultural intensification, agroecology, crop management, crop performance, organic farming, plant microbiota

## 1 | INTRODUCTION

Agricultural intensification has long been recognized as a major driver of biodiversity decline in agrosystems (Foley, 2005). Practices designed to improve plant productivity, such as massive use of mineral inputs, pesticides, and plant selection, are responsible for nutrient leaching, pollution, greenhouse gas emissions, and biodiversity loss (Foley, 2005; Stoate et al., 2009). The long-term effect of chemical fertilization regime in conventional farming negatively impacts the soil microbial reservoir and induces a strong selection processes on microbial community (Xu et al., 2020, 2024). Organic farming prohibits the use of chemical plant protection products and mineral inputs and is proposed as a more sustainable option than conventional farming because it takes advantage of the natural functioning of ecosystems. Organic farming is often associated with high soil quality and high biodiversity (McLaughlin & Mineau, 1995; Tuomisto et al., 2012; Winqvist et al., 2012). However, the impacts of conventional versus organic systems on biodiversity have mostly been compared based on macroorganisms (Hole et al., 2005; Gomiero et al., 2011; Tamburini et al., 2020; Tuck et al., 2014) and far less frequently on microorganisms (de Graaff et al., 2019; Verbruggen et al., 2010; Xu et al., 2020). Soil microorganisms are at the basis of ecosystem functioning (Coleman & Whitman, 2005). A fraction of the soil microorganism reservoir is recruited by plants to form their microbiota (Vandenkoornhuysen et al., 2015), and some of them form arbuscular mycorrhiza (i.e., Glomeromycotina). Plant microbiota strongly influences plant phenotype, growth, and health because of their role in plant nutrition, resistance to environmental stresses (Vannier et al., 2015; 2019) and overall health (Trivedi et al., 2020). Therefore, changes in plant microbiota depending on agricultural practices may affect plant productivity (Van Der Heijden et al., 1998).

Conventional and organic farming are characterized by a panel of practices that can influence soil microbial diversity and composition both directly by impacting their biological cycles and indirectly by modifying their environment (e.g., soil properties, available host plants). These practices can influence field management, soil characteristics, and plant composition in fields. Firstly, field management may disturb microbial community assembly and impact microorganism diversity (Hu et al., 2021; Wang et al., 2020). For instance, phytosanitary products can reduce plant mycorrhization and rhizobia performance or can delay the recruitment of rhizobia bacteria by crop plants (Hussain et al., 2009). Frequent tillage is detrimental for some fungi by disrupting hyphal networks (Manoharan et al., 2017). Secondly,

certain types of fertilizer can modify the physicochemical properties of soil over time, for example, by changing the soil pH (Liu et al., 2007; Patra et al., 2021), and can influence microbial activity, biomass, diversity, and composition (e.g., Bååth & Anderson, 2003; Chaudhry et al., 2012; Geisseler & Scow, 2014; Sun et al., 2021; Xu et al., 2020). Lastly, plant diversity can differ considerably in crop fields depending on the type of plant mixture sown (multispecies and/or multi-cultivar mixtures) and on any spontaneous vegetation. Because plants are preferentially associated with specific microorganisms at the genotype/cultivar level (e.g., Andreo-Jimenez et al., 2019) and at the species level (e.g., De Deyn et al., 2011; Vandenkoornhuysen et al., 2002), plant composition in fields may influence microbial composition. Given the importance of plant microbiota for crop productivity, disentangling the relative effects of field management, soil properties, and plant diversity on microorganism diversity and composition is a major challenge.

In the present study, we analyzed the effects of organic versus conventional farming systems on microorganisms associated with wheat roots to test the following hypotheses: (1) organic farming changes microbial community composition and species interactions and results in more diverse microbial assemblages than conventional farming (H1); (2) this effect is related to changes in management practices, soil properties, and/or plant diversity (H2); (3) changes in microbiota affect host plant reproduction and resistance to pathogens (H3). We tested these hypotheses using a large sampling design combining environmental data acquired through interviews with farmers, soil analyses, and plant surveys; sequencing microbiota associated with wheat roots; and measuring wheat phenotype and disease symptoms. We tested our hypotheses in two separate microbiota sampling campaigns corresponding to the early vegetative stage and then to the flowering stage to detect the stage at which responses are detectable. We expected responses to be more pronounced in May than in March due to the effects of the different farming systems during plant development that would increase divergences in microbiota composition (H4).

## 2 | METHODS

### 2.1 | Site selection

The study was conducted in 40 winter wheat fields in the Long-Term Socio-Ecological Research (LTSER) site “Zone Atelier Armorique,”

Rennes, north-western France (48°06'43"N 1°40'27"W). The 40 fields comprised 20 organic and 20 conventional fields. They are located in a bocage landscape, with a mosaic of agricultural fields interspersed with hedgerows. Agriculture in the study area is characterized by mixed crop-livestock farming.

## 2.2 | Environmental and agronomic data

### 2.2.1 | Survey of management practices

We collected information on the management practices applied to each field during the wheat cropping period through interviews with the farmers. We selected seven variables: ploughing (no ploughing/ploughing); The number of mechanical weeding actions after the crop is sown, total amount of nitrogen input (N unit/ha), and herbicide and fungicide treatment frequency index (TFI) (Table S1). The number of mechanical weeding actions varied from 0 to 4 (mean: 0.66 [SD: 1.1]). Nitrogen input varied from 0 to 287.5 N unit/ha (mean: 104.4 [SD:72.2]). The farmers applied no insecticides on their fields. We calculated total TFI (herbicides, fungicides) as an indicator of the reliance on plant protection products, which accounts for the number of treatments and the doses relative to the maximum authorized dose (Pingault, 2007). TFI varied from 0 to 28.1 (mean: 7.6 [SD: 8.4]). In terms of planning, wheat was sown from mid-October to mid-November depending on the field. Weeding (mostly mechanical) was carried out during winter (December to February). Fertilization (organic and mineral) was mostly done in February and March. Herbicides were spread in November and April and fungicides in April–May. We were not able to interview three farmers. Therefore, no information was available for three out of the 40 fields.

### 2.2.2 | Soil analysis

We collected information on the soil characteristics by analyzing soil samples for each field. Five soil subsamples (0–20 cm depth) were collected from each field (one in each corner and in the center) using a 5 cm Ø soil auger and then pooled them to obtain one composite sample per field. The soil samples were collected at the end of May, air-dried, gently crushed, and sifted through a 2-mm sieve. We measured the following soil physicochemical properties: grain size distribution ( $\text{g kg}^{-1}$ ; clay <2  $\mu\text{m}$ , silt 2–20  $\mu\text{m}$ , and coarse silt 20–50  $\mu\text{m}$ ; according to the standard French procedure NF X31 107), organic matter and nitrogen contents (in  $\text{g.kg}^{-1}$  by dry combustion; NF ISO 10694 or 14235, NF ISO 13878), pH (by water suspension, NF ISO 10390), and total phosphorus P2O5 (in  $\text{g.kg}^{-1}$ , Olsen method, by ICP-MS spectrometry; NF ISO 11263). Measures varied from 155 to 302  $\text{g.kg}^{-1}$  for clay (mean: 300.1 [SD: 35.5]), from 199 to 427  $\text{g.kg}^{-1}$  for fine silt (mean: 293.5 [SD: 36.4]), and from 111 to 441  $\text{g.kg}^{-1}$  for coarse silt (mean: 274.9 [SD: 91.9]), respectively. pH varied from 5.59 to 6.85 (mean: 6.3 [SD: 0.3]). OM and nitrogen contents varied from 21 to 49.9  $\text{g.kg}^{-1}$  (mean: 31.7 [SD:6.8]) and 1.39 to 2.62  $\text{g.kg}^{-1}$  (mean:

104.4 [SD: 72.2]), respectively. P2O5 varied from 0.017 to 0.244  $\text{g.kg}^{-1}$  (mean: 0.084 [SD: 0.38]). These analyses were conducted at the Soil Analysis Laboratory of the French National Research Institute for Agriculture, Food and Environment (LAS, INRAE; Arras, France).

### 2.2.3 | Plant survey and cultivar types

Floristic diversity is provided in field through wheat cultivar sowing and spontaneous plant growth. From farmers interviews, we got the information about wheat sowing density ( $\text{kg/ha}$ ) and wheat cultivar number. Wheat sowing varied from 80 to 300  $\text{kg/ha}$  (mean: 150.1 [SD: 39.8]). One to four wheat cultivars were sown per field (mean: 2.11 [SD: 1.2]). We were not able to take into account the cultivar identity because of the large set of cultivar types used over the study area. Each farmer had his own choice of cultivar identity, resulting in very low number of replicates per cultivar identity. We measured also the diversity of plants growing spontaneously in field and in field margins. Floristic surveys were conducted in each selected field, in the center of the field and in the field margins (two margins were sampled: one grassy margin and one hedgerow). The floristic surveys were conducted from mid-May to early June in ten 1 × 1 m quadrats in the field. The quadrats in the field were located at least 20 m from the margins to limit the edge effect and distributed equidistantly from each other in the field to account for overall diversity. In each margin, a 50 m-long transect consisting of ten 1 m<sup>2</sup> quadrats each separated by 4 m were surveyed. In each quadrat, we visually estimated the coverage of each vascular plant species as a percentage of the herbageous layer. From these surveys we calculated plant richness in the field and in the margins. Total plant richness in margins varied from 23 to 63 species (mean: 39 [SD: 12]) within the 10 m<sup>2</sup> sampled area, while total plant richness in field varied from two to 35 species (mean: 18 [SD: 11]) within the 10 m<sup>2</sup> sampled area.

Among variables characterizing management practices, soil properties, and plant diversity in fields, a sub-selection of variables was made to avoid strong correlations in each category of variables (Table S1). For each category of parameters (management practices, soil characteristics, plant diversity), we used multivariate analyses to aggregate the variables into a fewer number of synthetic variables (see statistical analyses).

## 2.3 | Microbiota analysis

### 2.3.1 | Sampling protocol

To analyze the bacterial and fungal microbiota associated with wheat, we sampled six individual wheat plants in each field (40 fields) at two dates (mid-March—vegetative stage and the end of May—early reproductive stage of wheat). These dates correspond also to two very different time points in the farmers' management schedule. The campaign in mid-March corresponds to wheat plants after most weeding and fertilization have been done, and the campaign in mid-May

corresponds to wheat plants after all treatments including pesticide applications have ended. Individual wheat plants were selected at 10-m intervals along two transects, starting at 10 m from the margin and ending 30 m from the margin (i.e., three individuals per transect) giving a total of 240 samples per sampling campaign ( $40 \times 2$  transects  $\times$  3 individual wheat plants per transect). Sampled individual plants were stored in plastic bags and processed within 12 h. To keep only endospheric microorganisms, the sampled roots were washed for  $\sim 5$  min with tap water and then placed for 10 min with a Triton X100 5% solution in a 20 mL sterile polypropylene tube. The roots were thoroughly rinsed with sterile 18 m $\Omega$  purified water (Vandenkoornhuysen et al., 2007). However, because we cannot exclude the possibility that some remaining rhizoplan microorganisms sticking to the roots, we talk about “root-associated microbiota” rather than “endosphere microbiota.” Small pieces of root (<1 cm) were sampled from different parts of the root system of each individual wheat plant, and 80 mg aliquots were stored in 1.5 mL Eppendorfs<sup>®</sup> tubes at  $-20^{\circ}\text{C}$  before DNA extraction.

### 2.3.2 | DNA extraction and Illumina sequencing

Frozen root samples in deep well plates were ground to powder in a bead beater for 2 min. DNA samples were extracted from roots at the Gentyane platform (<http://gentyane.clermont.inra.fr/v>, Clermont-Ferrand, France). DNA was extracted by magnetic beads (Sbeadex mini plant kit, LGC genomics) following a standard protocol using a robot (oKtopure robot, LGC Genomics); DNA concentrations were measured by fluorimetric quantification (Hoeschst). Concentrations in all the samples were normalized to 7 ng/ $\mu\text{L}$  (Bravo-Agilent<sup>®</sup>) of which 3  $\mu\text{L}$  was used for each PCR. We targeted a 16S rRNA (bacteria) and 18S rRNA-gene (fungi) fragments to analyze the wheat root-associated microbiota. Primers 799F (5'-AACMGATTA-GATACCCKG-3') and 1223R (5'-CCATTGTAGTACGTGTGA-3') (Vannier et al., 2018) were used to amplify the bacterial V5 to V7 16S rRNA gene region, leading to a 424 pb amplicon. Primers NS22b (5'-AATTAAGCAGACAAATCACT-3') and SSU817 (5'-TTAGCATG-GAATAATRAATAGGA-3') were used for specific amplifications of the fungal V4 and V5 18S rRNA gene region (Lê Van et al., 2017) leading to a 530pb amplicon. For multiplexing at their 3' region, all the primers contained the Illumina<sup>®</sup> adaptors. The PCRs were performed with Illustra PuReTaq Ready-to-go beads (GE Healthcare<sup>®</sup>). Bacteria PCRs started with a denaturation step at  $94^{\circ}\text{C}$  for 4 min followed by 35 cycles at  $94^{\circ}\text{C}$  for 30s,  $57.5^{\circ}\text{C}$  for 30s,  $72^{\circ}\text{C}$  for 1 min, and a final extension step at  $72^{\circ}\text{C}$  for 10 min. Fungal PCRs started with an initial denaturation step at  $95^{\circ}\text{C}$  for 4 min followed by 35 cycles of  $95^{\circ}\text{C}$  for 30s,  $53.5^{\circ}\text{C}$  for 30s,  $72^{\circ}\text{C}$  for 1 min, and a final extension step at  $72^{\circ}\text{C}$  for 10 min. All PCR products were then purified with AMPureXP magnetic beads (Agencourt<sup>®</sup>) using an automated liquid platform (Bravo-Agilent<sup>®</sup>) and quantified (Quant-iT PicoGreenTM dsDNA Assay Kit) to allow normalization at the same concentration by the “EcogenO” platform ([https://geh.univ-rennes.fr/?\[en\]](https://geh.univ-rennes.fr/?[en])). A second PCR was performed using the Smartchip-system (Takara) to achieve multiplex tagging

(up to 384 amplicons in one-step PCR). The tagged-amplicon pool was then purified (AMPureXP, Agencourt<sup>®</sup>) and quantified using Kapa Library Quantification Kit- Illumina<sup>®</sup> platforms (KAPA BIOSYSTEMS<sup>®</sup>) on a LC480 LightCycler qPCR instrument (Roche<sup>®</sup>). Pair-End  $2 \times 250$  cycles sequencing runs (MiSeq instrument, Illumina) and Pair-End  $2 \times 300$  cycles for bacterial and fungal sequencing libraries, respectively, were performed. The DNA concentration of each sample was normalized, the amplicon library constructed, and finally, sequencing was performed. The second PCR, purification, quantification, library construction, and sequencing step were done at the “EcogenO” platform ([https://ecogeno.univ-rennes.fr/?\[en\]](https://ecogeno.univ-rennes.fr/?[en])) (Rennes, France).

### 2.3.3 | Data trimming and taxonomic assignment

Data trimming consisted of removing primer and degenerated bases sequences (Cutadapt) ended with 8,082,468 and 9,110,420 reads for bacteria and 11,893,842 and 12,527,562 reads for fungi in March and May, respectively. Trimmed sequences were then analyzed using FROGS pipeline (Escudié et al., 2018). FROGS pipeline uses SWARM for cluster formation. SWARM is an adaptive sequence agglomeration based on aggregation parameters rather than on a global similarity threshold to produce “sequence clusters.” In comparison to the ASV approach, the main advantage of clustering in FROGS is avoiding overestimation of sequence diversity. Sequence clustering was combined with a rigorous chimera removal step. All clusters detected in less than three independent samples and with a threshold of 0.005% of total reads were removed. Silva132 (16S) (Quast et al., 2012) and PhymcoDB (Mahé et al., 2012) databases were used for the affiliation of bacteria and fungi, respectively. Sequence clusters were filtered using the quality of the affiliations with a threshold of at least 95% BLAST identity and 95% coverage. These sequence clusters can be somehow assimilated as “taxa.” Sequence data can be found in the European Nucleotide Archive under accession number PR-JEB43151.

Contingency matrices were normalized to 3986 and 4618 filtered bacteria reads and 7913 and 8000 sequences filtered fungal reads for March and May, respectively. Samples under these thresholds were deleted. By drawing rarefaction curves (Figure S1), we checked that this number of sequences per sample described both the root-associated bacterial and fungal assembly in sufficient depth (curve slopes asymptotically close to 0). Statistical analyses were performed on these four normalized contingency matrices. Sequence abundances were calculated at the field scale as the mean values of the wheat individuals of the field. Only fields composed of three or more available samples were taken into account. To keep the maximum of fields for a given sampling campaign, we preferred to analyze the March and May sequencing data separately.

### 2.3.4 | Diversity index

From the normalized contingency matrices, we calculated root-associated microbial species richness (hereafter sequence-cluster

richness), Shannon diversity and Pielou's evenness index at the field scale for the “all fungi” and “all bacteria” assemblages as well as for the phyla representing the most numerous sequence clusters. For each phylum and each campaign, we calculated Shannon diversity and evenness indices using the microbiome R package (Lahti & Shetty, 2017). The Shannon index was always strongly correlated with sequence-cluster richness or Pielou's evenness (Pearson correlation coefficient >0.7) and was not included in further analyses.

## 2.4 | Wheat fitness

Wheat fitness was measured using two indices: plant reproductive capacity and resistance to disease. To assess the plant reproductive capacity, we counted the number of seeds on the 240 individuals collected in May for microbiota analyses. The sampled individual wheat plants had all completed their reproductive phase. We then calculated the mean total number of seeds per field as the mean number of seeds of the six individual wheat plants.

Pathogen attacks were also evaluated synchronously on the six wheat plants sampled in each field. For each sampled plant, we counted the number of leaves and attributed each a leaf rank starting from the top of the canopy. F1 therefore corresponds to the uppermost leaf in the canopy. For each leaf, we visually estimated the fraction of the leaf that presented disease symptoms. Pathogen attacks by four species of pathogen were evaluated for: brown rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici*), yellow rust (*Puccinia coronata* Corda), powdery mildew (*Erysiphe graminis* [DC.] Speer), and septoria tritici blotch (*Zymoseptoria tritici* [Desm.] Quaedvlieg & Crous). Rusts and mildew, when present, rarely exceeded 3% of leaf surface while *Z. tritici* blotch was detected on every plant, often representing more than 20% of leaf surface. We thus concentrated our analysis on *Z. tritici* blotch. For each leaf, we calculated the percentage of diseased leaf surface (Garin et al., 2014; Robert et al., 2018). In March, leaves F1 and F2 were rarely attacked, while leaves F7 to F11, when present, were usually completely senescent and therefore difficult to analyze. Thus, the mean index of *Z. tritici* attack in March was calculated using F3 to F6 leaves. For similar reasons, F2 to F4 leaves were used in May.

## 2.5 | Statistical analyses

All the analyses were performed in 35 fields for fungi; in 34 fields for bacteria in March and in 29 fields for bacteria in May because information concerning agricultural management was lacking for three fields out of 40, and insufficient description of bacteria and fungi microbiota for one field out of 40 for bacteria in March; and for five fields out of 40 for bacteria in May. Consequently, only fields for which all variables were available were included in the analyses. Statistical analyses were performed per sampling campaign (in March and in May), and per taxonomic group (Bacteria and Fungi). All statistical analyses were performed using R software version 4.2.1.

### 2.5.1 | Effect of organic and conventional farming on microbiota composition, diversity, and species interaction

First, we tested the effect of the farming system (conventional versus organic) on wheat microbiota community assembly. We used a distance-based Redundancy Analysis (db-RDA) using farming system as explanatory variable. Monte Carlo tests were used to test for significance differences in composition between conventional and organic fields. db-RDA were performed using ade4 R package (Bougeard & Dray, 2018). In addition, we tested the effect of farming system on microbiota diversity using *t*-tests after checking for normality and homogeneity of variance of data distribution. When variance was not equal, Welch tests were performed. When neither of the requirements were met, we used nonparametric Mann–Whitney tests.

We also built correlation networks to describe the sequence-cluster interactions for the two farming systems. Microbial co-occurrence networks were constructed using Pearson's correlation method to identify pairwise associations of sequence cluster (Yuan et al., 2021). Sequence clusters with less than 0.01% relative abundance were removed. *p*-Values were corrected for multiple comparisons using the Benjamini–Hochberg false discovery rate (fdr). Robust correlations based on Pearson's correlation coefficients ( $\rho$ ) of  $>|0.8|$  and fdr corrected *p*-values  $<.05$  were used to construct networks (Jiao et al., 2022). The co-occurrence networks were visualized in Gephi. Network modules were detected using the *cluster\_fast\_greedy* function, and network modularity and network metrics (Karimi et al., 2017) were calculated using the modularity command in igraph R package (Csardi & Nepusz, 2006).

### 2.5.2 | Effect of environmental data on microbiota composition and diversity

To reduce the huge number of explanatory variables and to be able to disentangle the relative effect of the three categories of variables tested, we synthesized each set of variables (agricultural management practices, soil properties, and plant diversity) through three principal-component analyses (PCA) performed with the ade4 R package (Bougeard & Dray, 2018). PCA of management practices included four variables (Number of mechanical weeding actions, Tillage, Total IFT, Total nitrogen input); PCA of soil properties included eight variables (abundance of clay, fine-silt, coarse-silt, Organic matter, N-concentration, C/N, P205, and pH), and PCA of plant diversity included four variables (Wheat density, Cultivar number, Species richness in field margins, Species richness in field center). Each field was then characterized by its coordinate along Axes 1 and 2 for each of the three PCAs. All six variables were not correlated above  $r > 0.7$  except for the first axis of the “management practices” PCA and the first axis of the “plant diversity” PCA, which were correlated with a Pearson *r* coefficient of 0.75. We used multiple linear regression models to analyze the effect of environmental data (i.e., field values along the two first PCA axes for each of the three sets of variables;

six variables) on microorganism diversity. The models were ranked by their Akaike information criterion corrected for small sample sizes (AICcs). All the models with an AICc value included with the smallest AICc +2 were averaged (Full average, Burnham & Anderson, 2002). The models were tested using Anova II tests. The variance inflation factor (VIF) was calculated for each model to check for the absence of multicollinearity (VIF <3) (Dormann et al., 2013). We used the MuMIn (Bartoń, 2020) and car (Fox & Weisberg, 2019) R packages for multi-model inference. In addition, we used canonical correlation analyses (CCAs) to analyze the effect of environmental parameters (coordinates of each field on Axes 1 and 2 of each PCA) on microbiota composition and tested their significance with a Monte Carlo permutation test. CCAs were performed per phyla and per sampling campaign using vegan R package (Oksanen et al., 2019).

### 2.5.3 | Effect of microbial structure on wheat performance

We analyzed the effect of microorganism diversity (either richness or evenness for each taxonomic group—bacteria and fungi) on wheat performance (Number of seeds and Leaf attack) using multiple linear regression models and the same process as that explained above. The effect of microorganism diversity measured in March was tested on leaf attack in March, while the effect of microorganism diversity measured in May was tested on leaf attack in May.

## 3 | RESULTS

### 3.1 | Description of the wheat root-associated microbiota in March and May

The bacterial assemblage in the total pool (954 and 933 sequence clusters for March and May, respectively) was mainly composed of *Proteobacteria* and *Bacteroidetes*, while the fungal assemblage (291 and 282 sequence clusters for March and May, respectively) was mainly composed of *Ascomycota* (Figure 1). The richness and relative abundance of wheat root-associated microbiota differed slightly between March and May: for example, the relative abundance of sequence clusters of *Basidiomycota* increased by 80% (5% to 9%; 61 to 68 sequence clusters), while it was seven times lower (14% to 2%; 36 to 25 sequence clusters) for *Chytridiomycota*, and it decreased by 35% (2% to 0.7%; 18 to 11 sequence clusters) for *Glomeromycotina*.

### 3.2 | Effect of organic and conventional farming on microbiota composition, diversity, and network

#### 3.2.1 | Composition

The composition of microbial assemblages depended significantly on the farming system for all bacteria phyla except *Firmicutes* in March

and only for *Alphaproteobacteria* in May (Table 1) indicating early response in species composition for bacteria. The only significant effect on fungi was found in *Ascomycota* in March and in *Ascomycota* and *Zygomycota* in May.

#### 3.2.2 | Microbial diversity indices

In March, except for *Actinobacteria*, bacterial assemblage richness was not related to the farming system, while evenness was higher for *Actinobacteria* and lower for *Firmicutes* than in conventional farming. Conversely, in May, except for *Alphaproteobacteria*, all the phyla were significantly related to the farming system (Table 2), with higher sequence-cluster richness in organic fields. *Actinobacteria* and *Bacteroidetes* were more even in abundance in organic fields than in conventional fields, in contrast to *Firmicutes*. In March, except for *Glomeromycotina*, fungal richness was similar in the two farming systems. In May, all phyla displayed higher sequence-cluster richness in organic than in conventional fields (Table 2). Except for *Chytridiomycota* in March and *Ascomycota* in May, evenness of most phyla was not affected by the farming system.

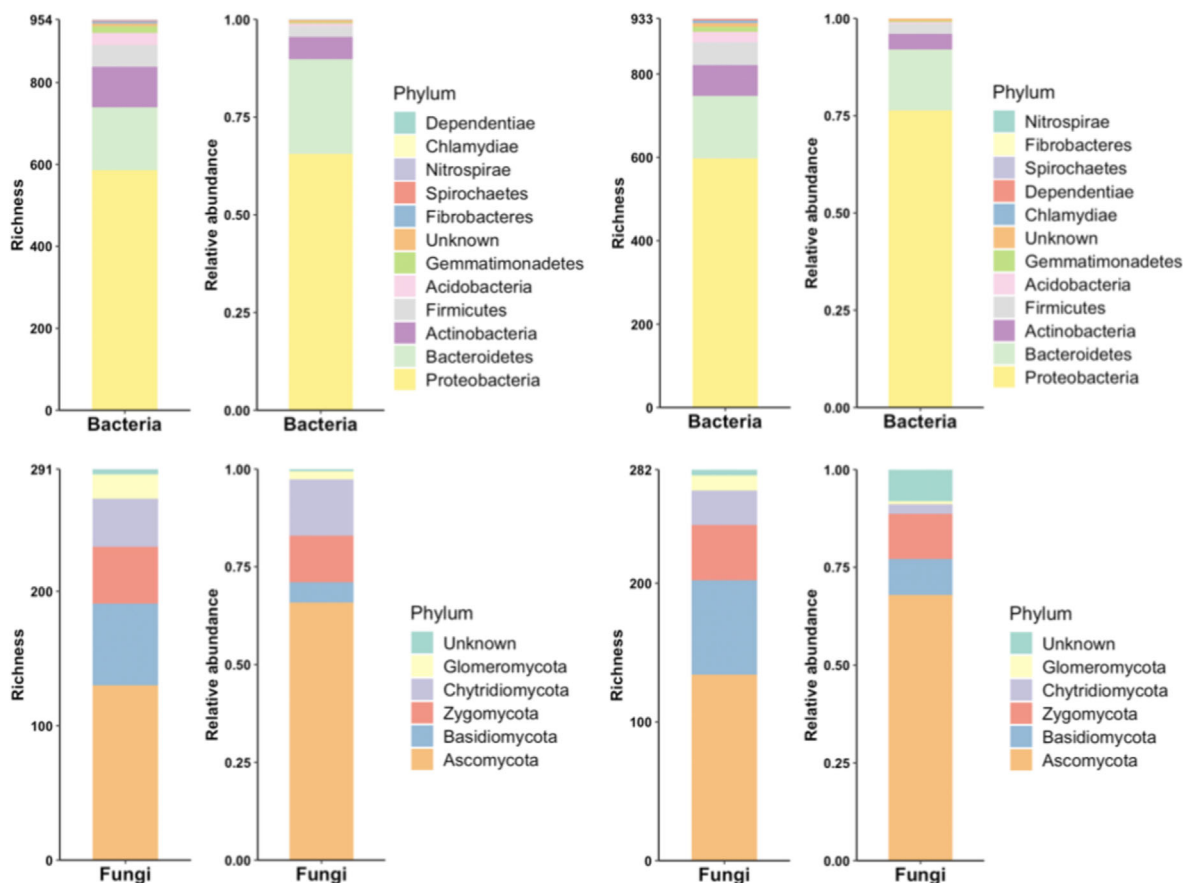
#### 3.2.3 | Network analysis

Co-occurrence networks were calculated for each farming system and each sampling campaign (Figure 2, Table S2). In March, the bacterial and to a less extent fungal networks had more nodes and edges in organic than in conventional farming system, while in May, the results were the reverse. Network modularity in bacterial networks (i.e., species specialization in different niches) was lower in March in organic (0.55) than in conventional farming (0.85), while the reverse was found in May (0.87 in organic and 0.71 in conventional farming, respectively). No obvious difference in fungi was found between the two farming systems. Connectance (i.e., the proportion of real links compared to potential links) was lower in organic farming for bacterial and fungal networks (0.009 and 0.026 in organic farming and 0.014 and 0.036 in conventional farming for bacteria and fungi, respectively, in May; 0.023 in organic farming and 0.030 in conventional farming for fungi in March) except for bacteria in March, where it was higher in organic farming (0.018 and 0.023 for bacteria and fungi, respectively) than in conventional farming for bacteria.

### 3.3 | Effect of agricultural management, soil properties, and plant diversity on bacteria and fungi assemblages associated with wheat roots

#### 3.3.1 | Categories of environmental variables

The total variation explained by the first two axes of the PCA related to agricultural management, soil properties, and plant diversity was



**FIGURE 1** Description of overall richness and relative abundance of bacterial and fungal assemblages in March (left) and May (right) for the total set of fields.

84.9%, 60.7%, and 83.0% of variation, respectively (Figure 3). All variables included in the PCAs represented a significant contribution on the first or second axes, except proportion of fine silt in the PCA based on soil properties. In the PCA based on management, positive values of Axis 1 corresponded to high inputs of nitrogen and pesticides. Axis 2 corresponded to soil management with positive values related to frequent mechanical weeding and negative values related to ploughing. In the PCA based on soil properties, Axis 1 was related to clay, total nitrogen, organic matter (positive values), and coarse silt (negative values), and Axis 2 to  $P_2O_5$  and pH (positive values) and C/N (negative values). In the PCA based on plant diversity, Axis 1 corresponded to plant richness in the field and in margins, and cultivar number (positive values), and Axis 2 to wheat density (positive values).

### 3.3.2 | Agricultural management

Effects on microbiota diversity were due both to the amounts of inputs in the field (“agricultural management” PCA Axis 1) and to soil management (ploughing vs. mechanical weeding, “agricultural management” PCA Axis 2) (Figure 4; Table S3). In bacteria, higher inputs in

the field (phytosanitary products and nitrogen) increased *Alphaproteobacteria* evenness in March but did not affect the other phyla. In addition, in May, higher inputs in the field reduced sequence-cluster richness in *Actinobacteria*, *Firmicutes*, *Deltaproteobacteria*, and *Gammaproteobacteria*. Soil management influenced only *Firmicutes* in March, with increased richness detected in fields with ploughing and less mechanical weeding. In May, ploughing instead of intense mechanical weeding increased *Alphaproteobacteria* and *Deltaproteobacteria* richness and *Acidobacteria* evenness. In fungi, higher inputs in the field increased *Chytridiomycota* evenness in the fields in March, and reduced *Ascomycota* evenness in May. Tillage and mechanical weeding had no effect on fungi phyla diversity whatever the campaign considered.

Agricultural management influenced bacterial and fungal composition in both March and May (Figure 4; Table S4). Nutrient and phytosanitary inputs affected *Bacteroidetes* and *Basidiomycota* composition in March but not the other phyla, whereas in May, the inputs affected *Actinobacteria*, *Ascomycota*, *Chytridiomycota*, and *Zygomycota* composition. Soil management through ploughing and mechanical weeding affected *Bacteroidetes* and *Basidiomycota* composition in March and *Acidobacteria*, *Gammaproteobacteria*, *Chytridiomycota*, and *Glomeromycotina* composition in May.

**TABLE 1** Distance-based redundancy analysis (db-RDA) of bacteria and fungi in both campaign (March and May).

	March		May	
	Permutation test	R <sup>2</sup>	Permutation test	R <sup>2</sup>
<b>Bacteria</b>				
All bacteria	<b>F = 2.08 (*)</b>	0.06	F = 1.36 n.s.	0.05
Acidobacteria	<b>F = 2.77 (*)</b>	0.08	F = 1.44 n.s.	0.05
Actinobacteria	<b>F = 2.72 (*)</b>	0.08	F = 1.83 n.s.	0.06
Bacteroidetes	<b>F = 2.08 (**)</b>	0.06	F = 1.40 n.s.	0.05
Firmicutes	F = 1.30 n.s.	0.04	F = 0.52 n.s.	0.02
Alphaproteobacteria	<b>F = 2.38 (*)</b>	0.07	<b>F = 1.80 (*)</b>	0.06
Deltaproteobacteria	<b>F = 2.31 (**)</b>	0.07	F = 1.17 n.s.	0.04
Gammaproteobacteria	<b>F = 1.83 (*)</b>	0.05	F = 1.15 n.s.	0.04
<b>Fungi</b>				
All fungi	<b>F = 1.54 (*)</b>	0.04	<b>F = 1.86 (**)</b>	0.05
Ascomycota	<b>F = 1.83 (**)</b>	0.05	<b>F = 1.70 (*)</b>	0.05
Basidiomycota	F = 1.21 n.s.	0.04	F = 1.49 n.s.	0.04
Chytridiomycota	F = 1.60 n.s.	0.05	F = 1.04 n.s.	0.03
Glomeromycotina	F = 1.97 n.s.	0.06	F = 1.08 n.s.	0.04
Zygomycota	F = 1.23 n.s.	0.04	<b>F = 2.88 (**)</b>	0.08

Note: Results of the permutation test (F-value) and related correlation ratio R<sup>2</sup> to test differences in microbiota composition associated with wheat roots in conventional and organic fields. Significant tests are highlighted in bold (*p*-value). The number of samples are listed for conventional farming (CF) and organic farming (OF) for each dataset: N = 18 CF and 17 OF for fungi; 18 CF and 16 OF for bacteria in March, 14 CF and 15 OF for bacteria in May. \**p* < .05, \*\**p* < .01, and \*\*\**p* < .001.

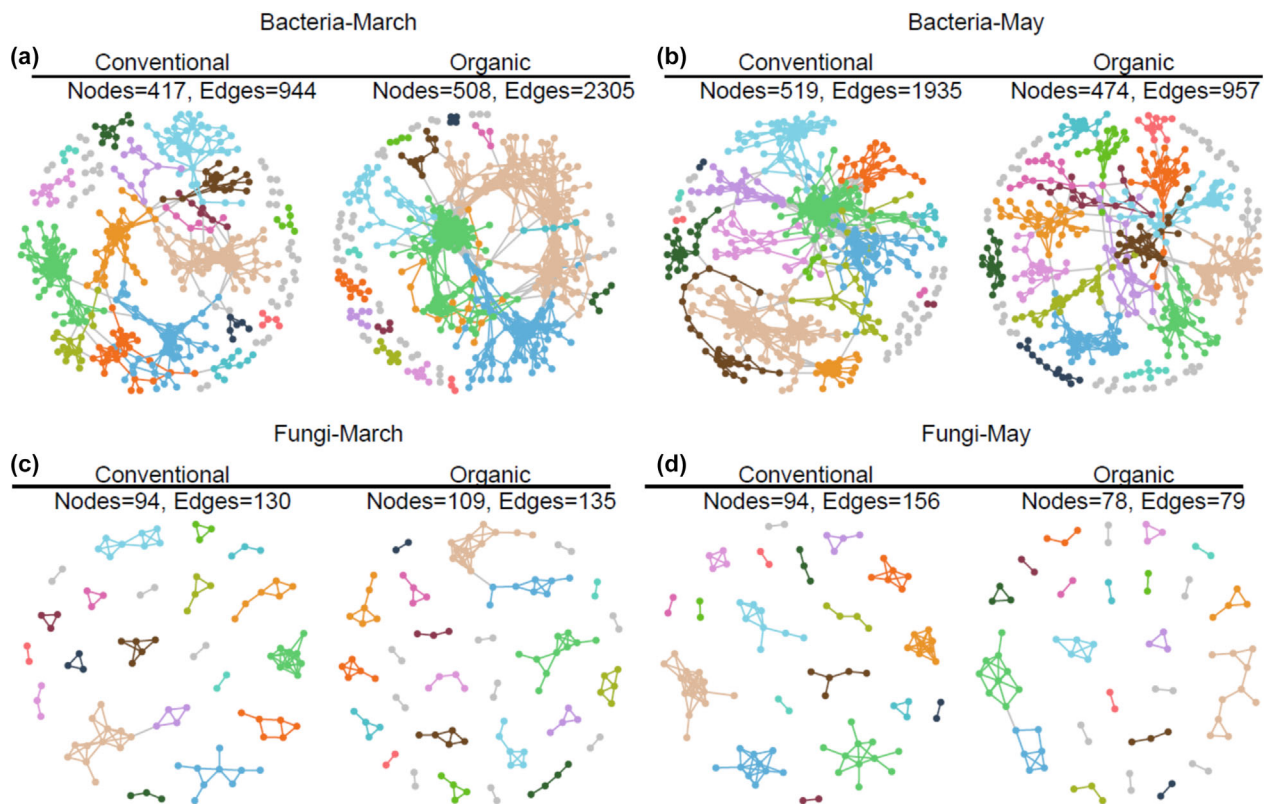
**TABLE 2** Results of the statistical tests.

	March		May				
	Richness	Evenness	Richness	Evenness			
<b>Bacteria</b>							
All bacteria	W = 191.5 ns	t = 0.89 ns	<b>W = 174**</b>	OF > CF	W = 101 ns		
Acidobacteria	t = 1.85 ns	t = -0.84 ns	<b>t = 2.85**</b>	OF > CF	W = 72 ns		
Actinobacteria	<b>t = 2.13*</b>	OF > CF	<b>W = 222**</b>	OF > CF	<b>W = 191***</b>	OF > CF	
Bacteroidetes	t = 2.02 (t)	t = 1.56 ns	<b>W = 181***</b>	OF > CF	<b>t = 2.91**</b>	OF > CF	
Firmicutes	t = 0.75 ns	<b>W = 76*</b>	CF > OF	<b>W = 170.5**</b>	OF > CF	<b>W = 25***</b>	CF > OF
Alphaproteobacteria	W = 172.5 ns	t = -1.04 ns	W = 132 ns		t = 0.30 ns		
Deltaproteobacteria	t = 1.33 ns	t = -0.79 ns	<b>W = 189.5***</b>	OF > CF	t = 1.87 ns		
Gammaproteobacteria	t = 1.59 ns	t = -0.52 ns	<b>W = 172**</b>	OF > CF	t = -0.40 ns		
<b>Fungi</b>							
All fungi	t = 0.33 ns	t = 0.10 ns	<b>t = 3.15**</b>	OF > CF	<b>t = 3.37**</b>	OF > CF	
Ascomycota	t = 0.66 ns	W = 192 ns	<b>t = 2.65*</b>	OF > CF	<b>W = 214*</b>	OF > CF	
Basidiomycota	W = 129 ns	W = 100 ns	<b>t = 3.90***</b>	OF > CF	W = 177 ns		
Chytridiomycota	W = 195 ns	<b>W = 72**</b>	CF > OF	<b>t = 2.15*</b>	OF > CF	t = -1.47 ns	
Glomeromycotina	<b>t = 3.19**</b>	OF > CF	W = 182 ns	<b>t = 2.80**</b>	OF > CF	W = 162 ns	
Zygomycota	t = 1.27 ns	W = 176 ns	<b>t = 3.43**</b>	OF > CF	t = 1.14 ns		

Note: A *t*-test (t), Welch test (W) or Mann-Whitney test (W) were performed depending on data distribution and variance. Significant results are in bold. Number of fields: N = 18 CF and 17 OF for fungi; 18 CF and 16 OF for bacteria in mid-March, 14 CF and 15 OF for bacteria in late May.

Abbreviations: CF, conventional field; OF, organic field.

\**p*-value < .05, \*\**p* < .01, and \*\*\**p* < .001.



**FIGURE 2** Correlation networks for bacterial and fungal assemblages sampled in March and May. The different colors correspond to the network modules (i.e., groups of nodes that are connected).

### 3.3.3 | Soil properties

Soil properties (PCA Axes 1 and 2) impacted bacterial and fungal assemblage diversity in both campaigns (Figure 4; Tables S2 and S3).

Regarding bacteria, wheat plants growing in soils with lower organic matter content, lower nitrogen concentration, and a smaller proportion of clay displayed significantly higher *Bacteroidetes* richness and a lower evenness of *Gammaproteobacteria* in March (Table 2). Plants growing in soils with lower C/N and higher pH and  $P_2O_5$  displayed assemblages with a higher *Bacteroidetes* richness, and higher *Firmicutes*, *Alphaproteobacteria*, and *Deltaproteobacteria* evenness. In May, plants growing in soils with lower organic matter content, lower nitrogen concentration, and a lower proportion of clay displayed significantly higher *Alphaproteobacteria* richness, while individuals grown in soils with a lower C/N ratio and higher pH and  $P_2O_5$  displayed higher evenness of *Acidobacteria* and *Alphaproteobacteria*.

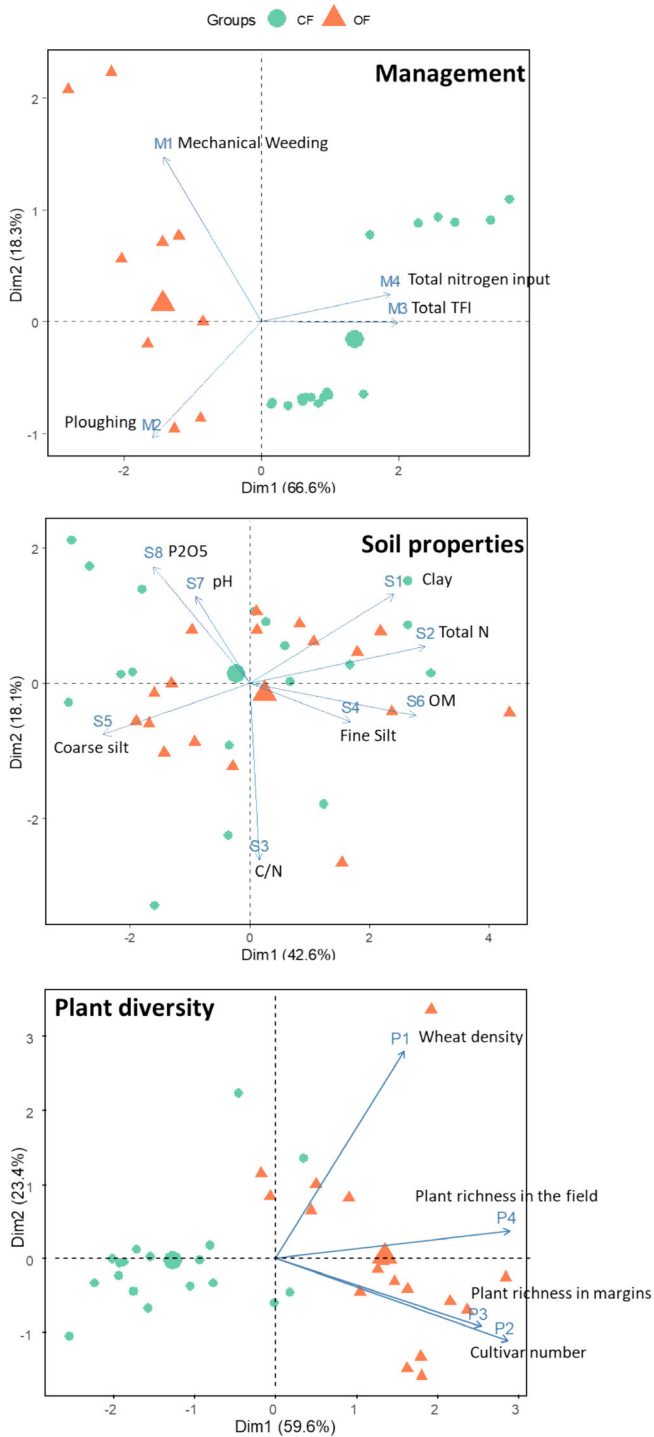
Regarding fungi, wheat plants growing in soils with a higher organic matter content, a higher nitrogen concentration, and a bigger clay proportion were associated with higher *Zygomycota* richness in March (Figure 4; Table S3). Individual wheat plants grown in soils with a higher C/N ratio and lower pH and  $P_2O_5$  displayed assemblages with higher richness of *Glomeromycotina* and *Zygomycota* and lower evenness of *Ascomycota* in March and lower richness of *Chytridiomycota* and a higher evenness of *Zygomycota* but a lower evenness of *Ascomycota* in May.

Soil properties influenced bacteria and fungi composition in both campaigns (Table S4). In March, composition depended on organic matter content, nitrogen concentration, and the proportion of clay and coarse silt for all bacteria phyla except *Deltaproteobacteria* and for *Chytridiomycota*. No effect of these parameters was detected in May. The C/N ratio, pH and  $P_2O_5$  affected all the bacteria phyla except *Actinobacteria* and all fungal phyla except *Ascomycota* and *Glomeromycotina* in March. These parameters affected all bacterial phyla except *Actinobacteria* and *Firmicutes* and all fungal phyla except *Chytridiomycota* and *Glomeromycotina* in May.

### 3.3.4 | Floristic diversity

Floristic diversity impacted one bacterial phylum and two fungal phyla in March, whereas no effect was detected in May (Figure 4; Table S3). A higher plant diversity in the field and in margins and a higher number of wheat cultivars were related to a higher *Glomeromycotina* richness and a less even *Firmicutes* and *Basidiomycota* assemblage in March. Increased sowing density was also related to higher *Glomeromycotina* richness.

Floristic diversity impacted the composition of both bacterial and fungal assemblages (Figure 4; Table S4). Plant diversity in the field and in margins and the number of wheat cultivars only affected *Chytridiomycota* composition in March and *Bacteroidetes* and



**FIGURE 3** Principal coordinate analysis (PCA) of agricultural management, soil properties, and plant diversity of the sampled wheat fields ( $N = 35$  fields). The green dots represent organic fields (OF), and the orange triangles represent conventional fields (CF). Dim1 and Dim2 correspond to the two first axis of the PCA. Management variables include ploughing occurrence, mechanical weeding, Total nitrogen input, Total treatment frequency index (TFI). Soil properties include grain size distribution (clay, fine silt, and coarse silt), organic matter (OM), total nitrogen content (N), carbon to nitrogen ratio (C/N), pH, phosphorus (P205). Plant diversity includes wheat density, cultivar number, and plant richness in the field and in margins.

*Glomeromycotina* composition in May. Wheat density only affected *Basidiomycota* phylum in March. In May, it induced changes in *Acidobacteria*, *Gammaproteobacteria*, *Chytridiomycota*, and *Glomeromycotina* composition.

### 3.4 | Effect of microbiota diversity on wheat fitness

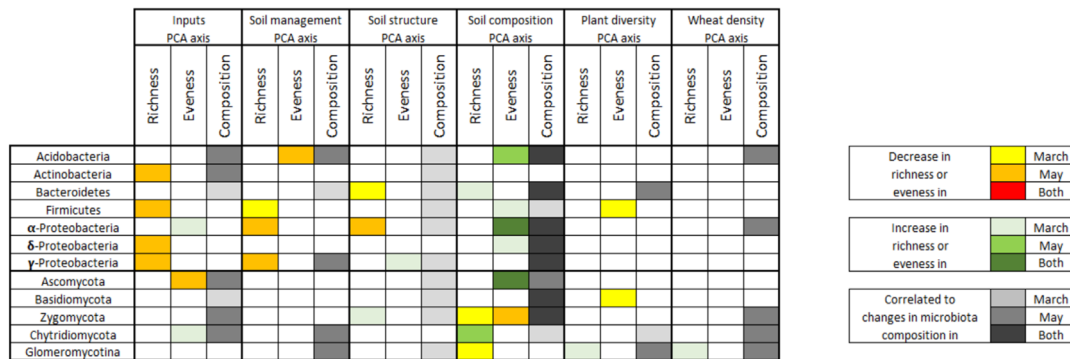
The number of seeds was correlated positively with the percentage of attacked leaf surface in March (Pearson correlation test:  $t = 2.75$ ,  $p$ -value = .01;  $r = 0.43$ ) but not in May (Pearson correlation test:  $t = 0.55$ ,  $p$ -value = .59). In addition, the percentage of attacked leaf surface in March and in May were not correlated (Pearson correlation test:  $t = -0.96$   $p$ -value = .34). This may be due to dry weather conditions in spring 2019, which were not favorable for *Z. tritici*. Wheat plants produced more seeds ( $t$ -test,  $t = -3.80^{***}$ ; CF:  $98 \pm 18$ ; OF:  $73 \pm 20$  seeds/plant) and had a higher proportion of leaf surface attacked by pathogens in March ( $t$ -test,  $t = -6.48^{***}$ ; CF:  $57.8 \pm 7.4$ ; OF:  $38.4 \pm 10.2$  leaf surface percentage) in conventional than in organic fields. However, no difference was detected between farming systems in May ( $t$ -test,  $t = 0.79$  ns; CF:  $17.4 \pm 11.1$ ; OF:  $20.0 \pm 8.7$  leaf surface percentage). The number of seeds and of leaf attacks were not related to either richness or evenness of bacteria assemblages in March whatever the phylum considered (Table 3). However, in May, the number of seeds increased with decreased *Actinobacteria* evenness, while the proportion of leaf surface attacked by pathogens decreased with an increase in the richness of *Alphaproteobacteria* and increased with an increase in the richness of *Firmicutes*.

When related to fungal assemblages, the number of seeds was related with sequence-cluster richness in both March and May, with lower values when there was an increase in the richness of *Chytridiomycota* (only in March) and in the richness of *Glomeromycotina* (in both campaigns). The proportion of leaf surface attacked by pathogens decreased with increased richness in *Glomeromycotina* in March (Table 3).

## 4 | DISCUSSION

### 4.1 | Organic versus conventional farming shapes microbial assemblages

As expected, farming practices affected both fungal and bacterial assemblages associated with wheat roots. From recent findings based on long-term fertilization regimes field experiment, chemical only fertilization leads to a carbon decline in soils. The decrease of soil carbon content leads to a strong decline in the soil microbial reservoir diversity in comparison to organic-only fertilization soil (Xu et al., 2020). It has been clearly shown that the plant microbiota originates from the soil microbial reservoir (Xiong et al., 2021). The plant microbiota is necessarily formed within the reservoir of microorganisms developing



**FIGURE 4** Synthesis of statistical significance found in tests analyzing the effect of field management, soil properties, and plant diversity on bacteria and fungi richness, evenness, and composition. Detailed results of the tests are presented in Tables S3 and S4.

**TABLE 3** Wheat performance (pathogen attacks, number of seeds) depending on richness and evenness of bacterial phyla in May and on richness of fungal phyla in March and in May.

	<i>Acidobacteria</i>	<i>Actinobacteria</i>	<i>Firmicutes</i>	Alphaproteo	Gammaproteo	$R^2$ (p-value)
<i>Richness in May</i>						
Leaf attack	12.31 (F = 0.06 ns)	0.47 (F = 2.15 ns)	0.91 (F = 13.72**)	-0.32 (F = 14.58***)		.45***
<i>Evenness in May</i>						
Number of seeds		-71.14 (F = 5.52*)	63.75 (F = 0.92 ns)		73.45 (F = 1.50 ns)	.29*
	<i>Ascomycota</i>	<i>Basidiomycota</i>	<i>Chytridiomycota</i>	<i>Glomeromycotina</i>	<i>Zygomycota</i>	$R^2$ (p-value)
<i>Richness in March</i>						
N° of seeds			-1.90 (F = 4.80*)	-1.93 (F = 4.40*)		.16*
Leaf attack	-0.07 (F = 0.06 ns)		-0.48 (F = 0.83 ns)	-1.61 (F = 7.84**)	-0.25 (F = 0.30 ns)	.20*
<i>Richness in May</i>						
N° of seeds	-0.46 (F = 0.73 ns)	-0.75 (F = 0.89 ns)	1.83 (F = 1.71 ns)	-4.41 (F = 6.07*)	0.43 (F = 0.12 ns)	.28*

Note: Only significant models are presented here (multiple linear regression models, Estimate, F-value, and p-value: (t):  $p < .1$ .  $R^2$  and p-values are given for the model). We removed *Bacteroidetes* and *Deltaproteobacteria* from the table because these variables were not retained in any models.

\* $p < .05$ . \*\* $p < .01$ . \*\*\* $p < .001$ .

in soil. Thus, organic farming makes better than conventional farming considering microorganisms' diversity. Shifts were detected in the sequence-cluster composition of bacteria, while higher richness was found in most fungal and bacterial phyla in organic farming systems, thereby validating our first hypothesis. Because there was no change in evenness in most phyla, this enrichment is likely related to the recruitment of rare taxa by wheat plants. Organic farming also strongly affected species interactions by increasing network complexity in March, as already demonstrated by Banerjee et al. (2019), likely because of strong local variability among plants in the recruitment of species; less heterogeneous assemblages were found in May, suggesting converging species distribution across the samples. The positive effect of organic farming on microbial diversity was more pronounced in May than in March for both fungi and bacteria, while an early effect on bacterial composition was detected in March. Such time-dependent responses to the farming system, which validated hypothesis 4, could be explained by the cumulative effect of phytosanitary products and nitrogen inputs during crop growth. Conventional farmers told us they applied fungicides in several steps from the seedling development stage to flowering and especially in April–May. Most

of the fungicides sprayed were succinate dehydrogenase inhibitors that can damage untargeted fungal and bacterial communities in the rhizosphere (Santísima-Trinidad et al., 2018). The richness of some phyla already responded to the farming system at the early stage of plant growth, likely because they are involved in plant nutrition (e.g., *Glomeromycotina*, Smith & Read, 2008) and stress resistance (e.g., *Actinobacteria*, Liu et al., 2017); and were probably recruited by host plants since the early growth. However, low  $r^2$  were detected using farming systems as explanatory variables of microbial composition suggesting the need to conduct more in-depth investigation of environmental variables linked with these farming systems to better understand the effect of the farming system on plant microbiota.

## 4.2 | Soil properties and agronomic inputs are more relevant than soil disturbance and floristic diversity to understand microbiota assembly

To better understand the factors that affect the wheat-root microbial assembly in organic versus conventional farming, we analyzed the

independent effects of management practices, soil properties and plant diversity on bacterial and fungal communities associated with wheat roots. Environmental parameters were measured a few weeks before wheat harvest (soil characteristics and plant composition at the end of May) or throughout the development of the wheat crop (for management parameters). We assumed that the soil characteristics we measured did not change during the development of the crop at the field level and that management practices and plant parameters affected wheat microbiota even at the early stage of wheat/plant growth.

In most phyla, the impact of the farming system (i.e., organic or conventional) on microbial assemblages associated with wheat roots was related to some of the environmental variables we studied, especially soil characteristics and field management. These changes may be related to changes in the soil reservoir in which wheat plant recruits and/or in the host plant's recruitment strategy that may differ depending on responses to local stresses. Soil parameters affected most bacteria and a few fungal phyla at the early stage of wheat growth, suggesting that soil properties only partly result from the farming system, whose effect was detected later. Indeed, even though the fields sampled were all located within a limited area, we cannot exclude slight differences in soil characteristics independent of the farming system, as suggested by the field distribution along the PCA axes (Figure 3). Soil parameters linked to both soil chemistry and physical structure shaped sequence-cluster composition and/or diversity in all phyla, with mostly positive effects on diversity resulting from higher concentrations of phosphorus, a lower C/N ratio, and higher pH. The predominant effect of soil on plant microbiota is well-known as it influences the soil microbiota and how a plant recruits its own microbiota from this pool to adapt to local soil conditions (for a review, see Custodio et al., 2022).

Among management practices, many bacteria and fungi phyla were impacted by inputs of nitrogen and plant protection products, as such variables are directly related to the farming system. Indeed, under organic farming, no phytosanitary products are used on wheat, and nitrogen inputs are lower despite the use of organic manure (no nitrogen inputs were added to eight of the 17 organic fields studied) (mean and standard deviation of nitrogen input for organic fields: 50.6 +/- 56.6 kg/ha; and for conventional fields: 140.4 +/- 34.4 kg/ha; data not shown). In all responding phyla, as expected, increasing nitrogen and phytosanitary inputs had a detrimental effect on species richness and shaped sequence-cluster composition (Liu et al., 2020). Soil disturbances also affected several fungal assemblages. The type and frequency of disturbance (for instance, ploughing or frequent mechanical weeding, which have opposite effects on composition) may select fungal species that are likely to recover after damage to their hyphae. The minor effect of soil management on sequence-cluster richness can probably be explained by the superficial tillage and moderate mechanical weeding used by farmers in this case study. Indeed, soil bacterial and fungal communities depend on ploughing depth with less effect of no-till and reduced ploughing (Sun et al., 2018). Only a few phyla were affected in the early stage of plant development, suggesting their sensitivity to practices applied at the very beginning of

crop management (i.e., ploughing, mechanical weeding, and early phytosanitary treatments). The late response of most phyla to management parameters may reflect the cumulative effects of agricultural practices over the growth period. Refining the variables, particularly the timing of a management action, and the type of fungicide used could better explain the response of microbiota over time.

The structure of the wheat microbiota was only slightly affected by floristic variables such as plant diversity and wheat density, which may indicate the predominance of the soil characteristics and field management in shaping microbial assemblages. However, *Glomeromycotina*, forming arbuscular mycorrhiza, which plays an important role in plant nutrition, was very sensitive to these parameters. The positive effect of plant diversity on *Glomeromycotina* richness has already been reported in several studies (De Deyn et al., 2011; Hiiesalu et al., 2014). A change in plant microbiota may result from the fingerprint of neighboring plants on the soil reservoir or transfer processes from neighboring plants to wheat (Hu et al., 2023).

### 4.3 | Microbiota variations have consequences for plant host fitness

Wheat performance linked to seed production and to pathogen resistance has been related to changes in microbial assemblage structure. For instance, the number of seeds has been related to changes in bacterial diversity (e.g., less even *Actinobacteria* assemblage) and fungi communities (e.g., less *Chytridiomycota* and less *Glomeromycotina*). In addition, resistance to pathogens increased with higher richness of *Glomeromycotina* in March, and higher richness of *Alphaproteobacteria*—but lower richness of Firmicutes at the end of May. All these phyla are known to be involved in plant defense mechanisms and resistance to pathogen stress (e.g., Stratton et al., 2022 for *Glomeromycotina*). These results suggest that environmental drivers affect wheat performance through indirect effects that lead to changes in microbiota, but these results should be interpreted with caution considering the empirical character of this study.

### 4.4 | Plant-associated microbiota as a major driver of sustainable agriculture

The present study provides a better understanding of the effect of organic farming on plant-associated microbiota and stresses the importance of soil characteristics and management in shaping microbiota composition and diversity. It also highlights the fact that plant seed production and resistance to pathogens are related with particular microbial assemblages. More specifically, *Alphaproteobacteria* and *Glomeromycotina* were seen to be key phyla in mediating wheat fitness, while also responding to environmental parameters. To provide more specific guidelines, we first need to test the indirect effect of environmental parameters on wheat fitness mediated by changes in microbial phyla, for instance, through structural equation modelling. This would require more samples and integrating other variables of

importance. For instance, past management practices or crop rotation should be taken into account as they can affect the soil microbial reservoir (Town et al., 2022). Another important parameter to be taken into account is wheat genotype. Wheat cultivars have been shown to be preferentially associated with different microbial assemblages (Latz et al., 2021; Quiza et al., 2023), likely resulting from genotypic differences in defense mechanisms but also in plant root morphology (Iannucci et al., 2021; Spor et al., 2020). However, it is difficult to control for this genotype effect in field studies due to wide range of cultivars used by farmers even at the level of one farm. Because organic farmers do not use the same genotypes as those used by conventional ones (Murphy et al., 2007), we acknowledge that this may partially changes in microbiota and plant performance between organic and conventional farming systems. The global demand for wheat is expected to increase due to population growth and shifting patterns of consumption (Lobell et al., 2009). Maintaining and even increasing productivity in a sustainable way, that is, while limiting harmful effects on the environment, is a key challenge in the future (Foley et al., 2011). The present study suggests that changes in agricultural practices can affect crop performance by modifying environmental factors and microbiota characteristics, highlighting the importance of microbiota in agroecology.

#### AUTHOR CONTRIBUTIONS

Claire Ricono, Cendrine Mony, Audrey Alignier, Colette Bertrand, and Philippe Vandenkoornhuys conceived the project, and all the authors contributed to the methodology of the study. Claire Ricono, Audrey Alignier, Pierre-Antoine Precigout, Corinne Robert, Colette Bertrand, Cendrine Mony, and Philippe Vandenkoornhuys collected the data. Claire Ricono performed the sequence analyses. Claire Ricono, Audrey Alignier, and Cendrine Mony did the floristic survey. Pierre-Antoine Precigout, Corinne Robert, and Colette Bertrand did the pathogen survey and soil analysis. Stéphanie Aviron handled the farmer network and provided the methodology for farmer interviews and helped Claire Ricono calculate the variables. Claire Ricono, Cendrine Mony, and Ting-Ting Wang performed statistical analysis of the data. Claire Ricono, Cendrine Mony, and Philippe Vandenkoornhuys wrote the manuscript. All the authors contributed critically to the interpretations of results and to the drafts and gave their final approval for publication.

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#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

#### DATA AVAILABILITY STATEMENT

Sequence data were deposited in the European Nucleotide Archive under accession number PR-JEB43151. Datasets are publicly available on Figshare ([10.6084/m9.figshare.25657026](https://doi.org/10.6084/m9.figshare.25657026)).

#### ETHICS STATEMENT

This study adhered to the general ethical guidelines of the LTSEr site “ZA Armorique.” All farmers were asked for permission to access their property for sample collection. They were informed about the study's objectives, potential outcomes, and methodology. Written consent was also obtained from each farmer to use the information gathered from the interviews and from their fields for research purposes. The collection locations were not disclosed, and the farmers' personal data were anonymized in the datasets.

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