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## RESEARCH ARTICLE

# Neighbourhood effect of weeds on wheat root endospheric mycobiota

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## Abstract

1. Micro-organisms associated with plants provide essential functions to their hosts, and therefore affect ecosystem productivity. Agricultural intensification has modified microbial diversity in the soil reservoir and may affect plant-microbial recruitment. Weeds develop spontaneously in crop fields, and could influence micro-organisms associated with crop plants through a neighbourhood effect. We explore the effect of weed species on crop plant microbiota as potentially auxiliary plants that affect agricultural productivity.
2. We combined field and controlled laboratory studies to analyse the neighbourhood effect of weeds on wheat root endospheric mycobiota (i.e. fungi within roots) and growth. First, we analysed the effect of weed species diversity and identity recorded in the neighbourhood of individual wheat plants on soil and wheat root mycobiota in the field. Second, we used a plant-matrix design in laboratory conditions to test the effect of weed identity (nine weed treatments) and their ability to transmit root mycobiota to wheat roots, and the resulting impact on wheat growth.
3. In contrast to soil mycobiota, we demonstrated that wheat root endospheric mycobiota was influenced by the diversity and identity of weeds developing in their 1 m<sup>2</sup> neighbourhood. Wheat root endospheric microbiota strongly differs in terms of richness and composition depending on the neighbouring weed plant species. Weed species transmitted from 13% to 74% of their root microbiota to wheat roots depending on weed identity in controlled conditions.
4. *Synthesis.* Weed neighbours modified wheat plant performance, possibly as a result of competitive interactions and changes in microbiota. Our findings suggest that crop root mycobiota was variable and was modulated by their weed neighbourhood. Synergistic effects between mycobiota of crops and weeds could therefore contribute to soil biodiversity and sustainable agriculture.

## KEYWORDS

biodiversity conservation, microbiota transmission, neighbourhood effect, plant-plant interaction, plant-soil interactions, root endospheric mycobiota, spontaneous flora

Jie Hu and Claire Ricono contributed equally.

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## 1 | INTRODUCTION

Plants harbour diverse micro-organisms in and on their tissues, forming their associated microbiota (Berg et al., 2016). Plant-associated microbiota fulfil essential functions for plant nutrition (Hardoim et al., 2015), plant protection against abiotic stress (Lenoir et al., 2016) and plant immune system (Hacquard et al., 2017). Maintaining or even engineering plant-associated microbiota can therefore help boost plant yields in a sustainable way (Busby et al., 2016). However, today's intensive agricultural systems have led to a microbial diversity crisis, caused, for example, by agrochemical application, mechanical management, crop rotation reduction and monospecific plant assemblages leading to global loss of biodiversity in agroecosystems (Creamer et al., 2016; Hartman et al., 2018). This microbial diversity crisis may affect plant fitness and productivity through detrimental recruitment of its microbiota, especially that of plant endophytic microbiota.

Plants recruit their microbiota in the local soil reservoir (Vandenkoornhuysen et al., 2015), and recruitment is in part deterministic (Guo et al., 2021; Wipfel et al., 2021). Environmental factors and the dispersal capacity of micro-organisms shape the microbial reservoir in ecosystems (Fierer, 2017; Martiny et al., 2006). Plants recruit micro-organisms in soil reservoir through a filtering process related to plant morphological, chemical and biological traits such as root type (Saleem et al., 2018), root exudate profile (Haichar et al., 2008) and plant immunity (Dodds & Rathjen, 2010). In addition, some rewarding processes that promote root colonisation by specific fungi that are the most cooperative for the plants (Kiers et al., 2011). These active and passive filtering processes have led to a certain level of host preference which can be observed both at the species and genotypic level. For instance, (Xiong et al., 2021) showed that crop identity (maize, rice or wheat) mainly determined microbiota recruitment rather than the field location or fertilisation management. Distinct root-associated microbial communities have been reported in phylogenetically distant plants, including maize, sorghum and wheat (Bouffaud et al., 2014), among close plant relatives such as *Arabidopsis* and *Cardamine hirsuta* (Schlaeppli et al., 2014), and even different cultivars such as rice (Andreo-Jimenez et al., 2019). Interactions between individual plants and their associated micro-organisms are well described (Hardoim et al., 2015). However, in situ plant-microbe interactions occur in a more complex biotic context where monospecific plant assemblages are the exception, and multi-species assemblages or spontaneous flora developing together with crop plants are the norm. Consequently, little is known about how plant-plant interactions in multispecies assemblages affect plant-microbe interactions, particularly their associated microbiota.

Recent studies suggest a plant neighbourhood effect on a focal plant endospheric microbiota (i.e. micro-organism community within roots) in grassland mesocosms (Bittebiere et al., 2020; Mony et al., 2020). The identity of plants growing within a few centimetres of the focal plant was shown to affect the richness and composition of the root endospheric mycobiota associated with *Brachypodium pinnatum*. This neighbourhood effect could be caused indirectly by root exudate production that can modify local soil microbiota (Saunders

et al., 2010) via favouring or rejecting specific micro-organisms. Specific mixtures of root exudates were reported to modify soil microbial composition (Steinauer et al., 2016), and the chemical class of root exudates accurately predicted changes in microbial composition and diversity (Gu et al., 2020). Neighbouring plants can also directly transfer part of their microbiota to focal plants (Mony et al., 2021). This transmission can be achieved through root contact or small-scale dispersal (Enkhtuya et al., 2005; Smith & Read, 2008). Such exchange is increased when neighbours share similar micro-organisms, for instance N-fixing microbes for leguminous species, or arbuscular mycorrhizal fungi (AMF). How and to what extent the identity and diversity of neighbouring plants and their associated microbiota can affect the microbiota and its consequences on the fitness of the plants developing in this neighbourhood need to be investigated more thoroughly.

In agrosystems, cultivated crop plants are usually spontaneously surrounded by weed plants. Agricultural fields harbour a large seedstock of weed plants that contribute to a varied population of neighbouring plants for crops, especially under organic management (Armengot et al., 2013). Weeds are thus likely to influence the microbiota of crop plants through direct contact or indirect modification of the soil microbial reservoir. Weed species vary in their ability to recruit microbiota for themselves and may also shape the diversity and abundance of micro-organisms in the soil. Furthermore, weeds may influence the productivity of crop plants through changes in their functional microbiota. For instance, experimental removal of particular weed species in fields, which resulted in modifications in the AMF composition associated with crops, led to a reduction of their beneficial effects on plant productivity in the field (Feldmann & Boyle, 1999; Kabir & Koide, 2000). A study combining soil-plant bioassay and comparative metatranscriptomics showed that alteration of root microbiome by neighbouring plants may regulate basic plant physiological processes via modulation of molecular functions in the root microbiome (Liao et al., 2021). The potential positive role of weeds led to a debate with farmers that endorsed the paradigm that weed species compete with crops for resources, reduce crop yields and have to be removed, in addition to their emerging resistance to herbicides (Llewellyn et al., 2004). Moreover, it has been proposed that we need to better understand the relationship between weeds and crops in agrosystem functioning and agricultural management (Carlos et al., 2014). In an agricultural context, the importance of weeds for the microbial compartment has been overlooked up to now.

In this study, we analysed how the mycobiota associated with a crop plant can be influenced by weeds. We focused on the influence of weed diversity and identity on soil mycobiota and on wheat root endospheric mycobiota in fields under organic management. First, we analysed the effect of composition and richness of neighbouring weeds on soil mycobiota and wheat root endospheric mycobiota in a set of organic fields by sampling individual wheat plants surrounded by different local weed plant neighbourhoods. Second, we conducted an experiment in controlled laboratory conditions on nine weed species selected based on field data to analyse how weed root endospheric mycobiota affects wheat performance via the transmission of weed root mycobiota to crop roots.

We hypothesised that (1) in field conditions: (i) weed species diversity shapes the composition and elevates the richness of mycobiota in the soil and in that associated with crop roots; (ii) the identity of weed species in the neighbourhood plays a specific role in shaping soil and wheat root-associated mycobiota; (2) in controlled conditions: (i) the composition and diversity of the mycobiota associated with the roots of weed species affect their ability to transmit mycobiota to individual wheat plants; (ii) when the mycobiota of weed plant species is transmitted to wheat plants, there is a change in the composition and an increase in the diversity of the wheat root mycobiota; (3) transmitted mycobiota compensate for growth reduction of wheat plants due to plant–plant interspecific and intraspecific competition, especially when there is no or only a limited microbial reservoir.

## 2 | MATERIALS AND METHODS

### 2.1 | Field study

We selected 15 organic winter wheat fields in the Long-Term Socio-ecological Research (LTSER) site ‘Zone Atelier Armorique’, located in north-western France (48°06′43″N 1°40′27″W). The 15 fields are located in an agricultural-dominated landscape composed of grasslands and crops, partly surrounded by hedgerows. Wheat fields were managed using tillage and mechanical weeding and no plant protection products or chemical fertilisers were used for field and hedgerow management (Ricono et al., 2022). Eight fields were not treated with any fertilisers and seven fields were gained only organic fertilisers. Fourteen of 15 fields were tilled before sowing the crops. Six, five and four fields have been mechanically deseeded zero, one and two times since crop has been sown respectively. In each field, we selected four sampling points located at least 10 m from the edge of the field to avoid edge effects. At each sampling point, we collected soil and wheat roots when individual wheat plants were at the reproductive stage. Wheat plants with their rooting system were sampled directly in the fields (depth ~20 cm) and placed in plastic bags. Once arriving in the laboratory, a soil aliquot from each sample wheat plant was stored at –20°C in a 15 mL polypropylene tube (Falcon) until total DNA extraction. Common weed species shared by more than half of 15 fields. At each location where the samples of soil and wheat were collected, we performed floristic surveys in 1 × 1 m quadrats to identify the floristic neighbourhood of each individual wheat plant. In each quadrat, we visually estimated the percentage cover of each weed species. From these data, we identified the composition and abundance of the weed community. We did not need permission for our fieldwork.

### 2.2 | Controlled experiment

We analysed the influence of weed neighbour species on wheat root endospheric mycobiota in a controlled experiment using a plant-matrix design. We used 3 L pots (diameter 19 cm, height 15 cm) filled

with sterile vermiculite substrate where individual wheat plants were planted in a matrix of four individuals of the same weed species (Figure S1, Table S1). We used the winter wheat variety Attlass and focused on weed species (i) that were frequent in wheat fields, (ii) that had sufficient root biomass to enable molecular analysis, (iii) that were representative of different plant families and (iv) of which wild seeds were available without domestication by breeders. We selected nine weed species as a subsample of the weed species pool found in the field. Ten replicates of each treatment were performed using a neighbour of a single weed species (i.e. nine treatments (i) *Galium aparine*, (ii) *Lamium purpureum*, (iii) *Matricaria sp.*, (iv) *Papaver rhoeas*, (v) *Poa annua*, (vi) *Poa trivialis*, (vii) *Trifolium repens*, (viii) *Veronica persica* and (ix) *Vicia sativa*), with two additional control treatments (i.e. a single wheat plant grown alone, and an individual wheat plant surrounded by four sterile wheat plants). These two controls help to provide results about plant performance when wheat is without competitors or with strong intraspecific competition. For both controls, wheat individuals are grown on sterile substrate. For each replicate, the roots of the focal plant and of the neighbouring individual plants were sampled to characterise the associated endospheric mycobiota. Wheat and weed above-ground dry biomass were also measured as a proxy of wheat fitness. More details about experimental design were included in Supplementary Materials.

### 2.3 | Soil and root mycobiota analysis

#### 2.3.1 | Sample preparation

A homogenised aliquot of soil was sieved to 4 mm and 50 g of soil were sent to the Genosol platform for lyophilisation or stored at –40°C before DNA extraction. From each individual plant sample, 80 mg of roots were washed in tap water for 5 mins, then placed in a 20-mL sterile polypropylene tube with a 5% Triton X100 solution for 10 mins. Finally, the roots were thoroughly rinsed with sterile 18 mΩ purified water. Small pieces of root (<1 cm) were sampled randomly from different parts of the root system of each individual wheat plant, and 80-mg aliquots of roots were stored in 1.5 mL Eppendorfs® tubes at –20°C before DNA extraction along with samples taken from subsequent controlled experiments.

#### 2.3.2 | DNA extraction, 18S rRNA amplicon sequencing and bioinformatics

DNA from soil samples was extracted at the GenoSol Platform. DNA was extracted from the sample roots of all the weed and wheat plants from both the field study and controlled experiments at the Gentyane platform. We used general fungal primers for 18S rRNA to analyse the root endospheric mycobiota of the wheat and weed plants. Primers NS22b (5′-AATTAAGCAGACAAATCACT-3′) and SSU817 (5′-TTAGCATGGAATAATRRATAGGA-3′) were used for specific amplification of the fungal V4 and V5 18S rRNA gene region

(Borneman & Hartin, 2000; Lê Van et al., 2017) leading to a ~550 pb amplicon including sequencing adaptors. All PCR products were purified with AMPureXP magnetic beads (Agencourt®) using an automated liquid platform (Bravo-Agilent®) and quantified (Quant-iT PicoGreen™ dsDNA Assay Kit) to allow DNA normalisation at the same concentration, and a second round of PCR, purification, quantification, library construction and sequencing step was performed at the 'EcogenO' platform.

Data trimming consisted of removing primer and degenerated base sequences (Cutadapt). Trimmed sequences were then analysed using the FROGS pipeline (Escudé et al., 2018). Within FROGS, the tool to assemble the reads 1 and 2 (pair-end sequencing) was set at 0% of mismatch in the overlapped region. Despite this stringency, we did not notice a loss of sequences. Merged sequences were then clustered using SWARM allowing to limit the overestimation of sequence diversity which conversely might be the case when using ASVs. As recommended in the FROGS guidelines, affiliation was done by blastn+ for one representative sequence of each sequence cluster using herein PhymycoDB (Mahé et al., 2012) as a reference database (threshold of at least 95% BLAST identity and 95% coverage applied). In the particular case of uncertainty in the taxonomic affiliation (e.g. identical blastn+ scores for a given representative sequence to affiliate), the last convergent taxonomic rank was kept. Based on the rarefaction curves drawn for each dataset (Figure S2), contingency matrices were normalised to 21,743 reads for soil mycobiota, 14,530 reads for wheat root endospheric mycobiota for the field study, and 4203 for wheat and weed root endospheric mycobiota for the controlled experiment. Samples under these thresholds were removed. More details about DNA extraction, 18S rRNA amplicon sequencing and bioinformatics were included in Supplementary Materials.

### 2.3.3 | Mycobiota parameter calculation

In both studies, the number of sequences per sample made it possible to describe the root endospheric fungal assembly in sufficient depth (curve slopes asymptotically close to 0). A total of 60 soil mycobiota samples were analysed, 60 wheat root endospheric mycobiota samples (15×4 sampling points) in the field study; and 93 wheat and 84 weed root mycobiota samples were analysed in the controlled experiment (seven wheat root samples and six weed root samples were discarded due to low quality or quantity of DNA or PCR products). All statistical analyses were performed on these normalised contingency matrices.

We calculated the diversity of the soil and wheat root endospheric fungal communities based on the normalised contingency matrices in the field study, including diversity (hereafter sequence cluster richness) and Pielou's evenness index. These metrics were calculated for the 'all fungi' and for the five most frequently represented phyla (Ascomycota, Basidiomycota, Chytridiomycota,

Glomeromycotina and Zygomycete) in the soil mycobiota and in the wheat root endospheric mycobiota.

The diversity of the wheat and weed root endospheric fungal community was also calculated based on the normalised contingency matrices in the controlled experiment, including sequence cluster richness, Pielou's evenness index, the number of shared sequence clusters using the R VEGAN package (Oksanen et al., 2022) and the percentage of shared sequence clusters. The percentage of sequence clusters shared by wheat and weeds in the pot experiment was calculated as the ratio of the number of sequence clusters shared by weeds and wheat to the number of sequence clusters of the weeds alone.

To assign the enriched or depleted fungi with particular ecological functions, we combined information from the databases FUNGuild (Nguyen et al., 2016), FUNFUN version 0.0.3 (Zanne et al., 2020) and FungalTraits version 1.2 (Pölme et al., 2020) to parse fungal sequence clusters with ecological guilds or traits. First, we used FUNGuild.py script in the Python 3 environment to assign the functions of fungi by uploading our own file of taxa to the FUNGuild database, then we manually added the complementary information about fungal traits and functions from databases FUNFUN version 0.0.3 and FungalTraits version 1.2. An ecological guild is an index with the potential to indicate the functions of fungal species, but it is important to note that the assignment by the above databases to an ecological guild is currently largely at the genus level. In this study, we focused on three guilds: symbiotrophs, plant pathogens and saprotrophs.

## 2.4 | Statistical analyses

### 2.4.1 | Field survey

A Venn diagram was drawn using the R package VENNDIAGRAM (Chen & Boutros, 2011) to detect the shared and single sequence clusters in the soil mycobiota and wheat root endospheric mycobiota in the organic fields. Two coinertia multivariate analyses (Doledec & Chessel, 1994) were then performed to determine if the composition of the weed neighbourhood was related to the soil mycobiota or to the wheat root endospheric mycobiota. For this purpose, only sequence clusters and plant species that were found in at least 3% of the samples were used. The significance of the coinertia was tested using the Monte-Carlo permutation test with the 'randtest' function in the ADE4 package (Dray & Dufour, 2007). In addition, the effects of weed richness on the composition of the soil and wheat root endospheric mycobiota with PERMANOVA were tested using the 'adonis' function of the R package VEGAN (Oksanen et al., 2022). The effects of weed richness on sequence cluster richness of 'all fungi' and of each phylum in the soil mycobiota and wheat root mycobiota in the field sites were tested using a mixed model with negative binomial distributions in the R package LME4 (Bates et al., 2015). The field

site was used as a random factor to control for data dependency (four samples per field site). The normality and homoscedasticity of the model were checked using a graphical representation of the residuals. The marginal ( $R^2_m$ ) and conditional ( $R^2_c$ ) values of  $R^2$  were calculated for all models. These  $R^2$  corresponded to the variance explained by the fixed effects and the addition of fixed and random effects respectively. A Tukey post-hoc test was used for group comparisons of sequence cluster richness and evenness of soil mycobiota diversity and wheat root endospheric mycobiota in the field.

### 2.4.2 | Controlled experiment

We used principal coordinate analysis (PCoA) to identify the composition of the wheat and weed root endospheric mycobiota communities in combined and separate analyses. The least significant difference was also used via the 'LSD.test' function in the *AGRICOLAE* package to compare each weed species along the first and second principal components of the weed and wheat root endospheric mycobiota. We also identified the sequence clusters that were enriched or depleted in wheat root endospheric mycobiota depending on the neighbourhood species. For this purpose, we conducted log2foldchange analysis using R package DESeq2 (Love et al., 2014) to compare each sequence cluster in the root mycobiota of wheat with weeds as neighbours to each sequence cluster in the root mycobiota of wheat in the control treatment without any weed neighbours. After log2foldchange calculation, the sequence clusters whose abundance of log2foldchange was higher than 0.6 which indicates at least 1.52-fold abundance increase for enriched sequence clusters or lower than -0.6 which indicates 0.66 of abundance for depleted sequence clusters and with a significant  $p$  value were kept to count the amount of changed (both enriched and reduced) sequence clusters in each treatment. This cut off was selected via a sensitivity analysis (Habibzadeh et al., 2016).

The effect of weed-mediated change in root endospheric mycobiota on wheat performance was assessed through above-ground biomass. The effect of weed identity on wheat above-ground dry biomass was tested along with the effect of wheat root endospheric mycobiota diversity (i.e. 'all fungi' sequence cluster richness, sequence cluster richness in the phyla Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycotina and Zygomycete) on wheat above-ground dry biomass. In both cases, generalised linear models were used. Significance was tested using a Type II ANOVA after checking for normal distribution of residuals. Linear models were used to detect the effects of weed identity on wheat and weed root endospheric mycobiota sequence cluster richness, on the number of shared sequence clusters, the percentage of shared sequence clusters, the weight of wheat and weed above-ground plant biomass in the controlled experiment. A Tukey post-hoc test was used for group comparisons of sequence cluster richness of weed and wheat root endospheric mycobiota, the number and percentage of sequence

clusters shared by weeds and wheat. All statistical analyses were performed using R software version 4.0.0.

## 3 | RESULTS

### 3.1 | Effects of weed neighbourhood on wheat root mycobiota in the field study

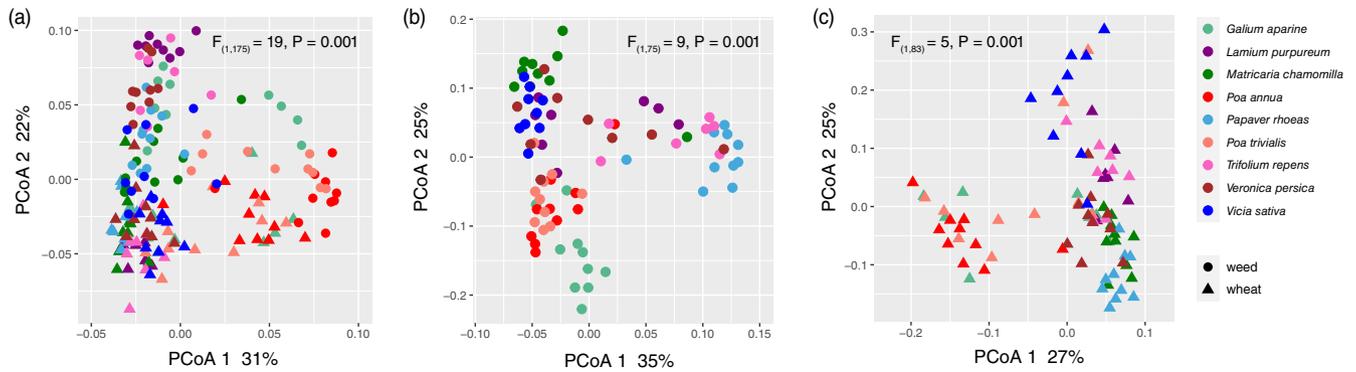
A coinertia analysis showed that, except for Glomeromycotina, soil mycobiota was not influenced by floristic composition in the neighbourhood (Table 1). Floristic richness did not affect the composition (Table 2,  $p = 0.961$ ), the sequence cluster richness or the evenness of 'all fungi' and each phylum of the soil mycobiota (Table S2), indicating a very limited legacy effect of weed species on the soil microbial reservoir. However, we found a significant relationship between floristic composition in the neighbourhood of wheat individuals and the endospheric mycobiota associated with wheat roots, particularly for 'all fungi' (Table 1,  $p = 0.020$ ) and phylum Zygomycete (Table 1,  $p = 0.002$ ). Floristic richness and evenness significantly (Table 2,  $p = 0.021$ ) or marginally significantly (Table 2,  $p = 0.080$ ) affected the composition of wheat root endospheric mycobiota respectively. Floristic richness increased wheat root endospheric mycobiota sequence cluster richness for the whole fungi, in the phyla

**TABLE 1** Coinertia analysis between floristic composition and soil mycobiota, and between floristic composition and root endospheric mycobiota of wheat in the field study. The RV coefficients obtained by coinertia analysis between the same paired data sets highlight the relationship between the floristic species abundance and mycobiota sequence cluster relative abundance of soil or wheat root endosphere. Total inertia of coinertia is related to the explained variance supported by its two first axes.  $p$  values were calculated using a Monte-Carlo test based on 999 permutations. Significant results ( $p < 0.05$ ) are highlighted in bold, and marginal significant results ( $0.05 < p < 0.10$ ) are highlighted in bold and italics.

	RV	Total inertia: Axis 1 and 2 (%)	$p$
<b>Soil mycobiota</b>			
All fungi	0.55	19.9	0.261
Ascomycota	0.49	22.9	0.227
Basidiomycota	0.43	30.4	0.584
Chytridiomycota	0.37	30.6	0.569
Glomeromycotina	0.34	49.6	<b>0.012</b>
Zygomycete	0.36	31.1	0.547
<b>Wheat root endospheric mycobiota</b>			
All fungi	0.56	31.5	<b>0.020</b>
Ascomycota	0.49	30.4	0.104
Basidiomycota	0.46	31.9	<b>0.082</b>
Chytridiomycota	0.34	33.1	0.566
Glomeromycotina	0.29	49.8	0.169
Zygomycete	0.43	48.9	<b>0.002</b>

**TABLE 2** Effect of floristic diversity on soil microbiota and wheat root endospheric microbiota composition in the field study. Floristic diversity is indicated as floristic richness and evenness. Effects were tested via a PERMANOVA analysis. Significant results ( $p < 0.05$ ) are highlighted in bold, and marginal significant results ( $0.05 < p < 0.10$ ) are highlighted in bold and italics.

Parameters	Soil microbiota composition			Wheat root endospheric microbiota composition	
	df	F	p	F	p
Floristic richness	1	0.68	0.961	1.49	<b>0.021</b>
Residuals	58				
Floristic evenness	1	0.089	0.657	1.37	<b>0.080</b>
Residuals	58				



**FIGURE 1** Composition of root endospheric microbiota of weed species and wheat grown using plant-matrix design in the controlled experiment. (a) PCoA of root endospheric microbiota of all weed and wheat plants; (b) PCoA of root endospheric microbiota of all weed plants; (c) PCoA of root endospheric microbiota of all wheat plants with different weed species as neighbours.

Ascomycota, Glomeromycotina and Zygomycete, and floristic richness increased wheat root endospheric microbiota sequence cluster evenness in the phylum Basidiomycota (Table S2).

### 3.2 | Effects of weeds on wheat root endospheric microbiota structure in the controlled experiment

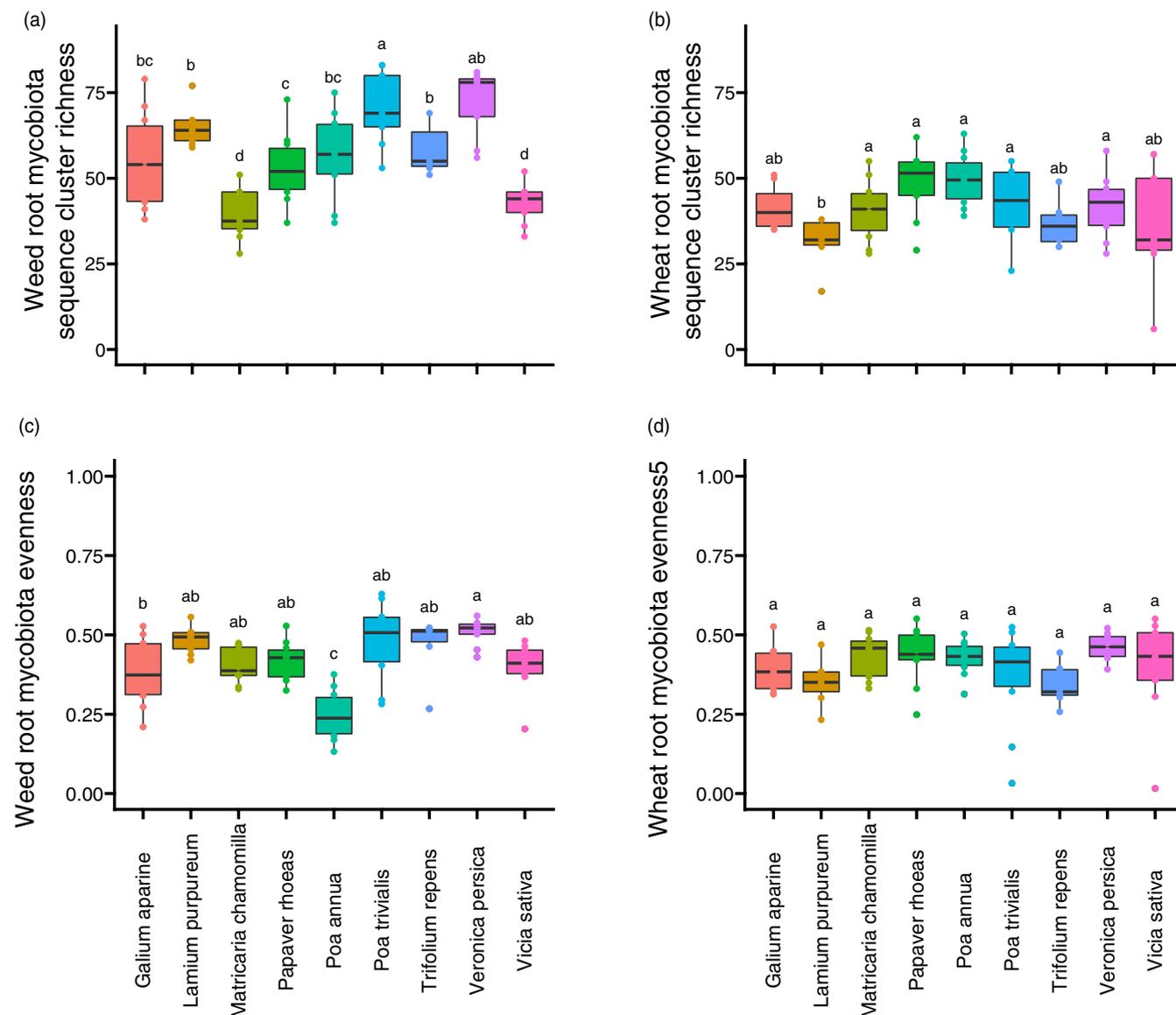
In the controlled experiment, weed species were associated with distinct microbiota composition from that found in wheat plants (Figure 1a,  $p = 0.001$ ). Microbiota composition differed in the roots of each weed species (Figure 1b,  $p = 0.001$ ). Along with the first principal component of weed root microbiota, the biggest differences were found between *P. rhoeas*, *T. repens* and *V. sativa* (Figure S3A), while along with the second principal component of weed root microbiota, the biggest difference was found between *M. chamomilla* and *G. aparine* (Figure S3B). *P. annua* and *P. trivialis* had the most similar root endospheric microbiota composition along both principal components (Figure S3A,B). The effect of weed species was also significant when considering wheat root endospheric microbiota, which clustered depending on the neighbourhood weed species they grew with (Figure 1c,  $p = 0.001$ ). Along with the first principal component of wheat root endospheric microbiota, wheat root endospheric microbiota differed the most between *P. rhoeas*, *M. chamomilla* and *P. annua* treatments (Figure S3C), while along with the second principal component of wheat root endospheric microbiota, *P. rhoeas* and *V. sativa* showed the biggest different effects (Figure S3D). But *P. annua* and *P. trivialis* did not have the same effect on wheat root endospheric microbiota (Figure S3C,D).

### 3.3 | Effects of weeds on wheat root endospheric microbiota diversity in the controlled experiment

The weed species *P. trivialis* displayed the highest root endospheric microbiota sequence cluster richness, the weed species *V. persica* also displayed relatively higher root endospheric microbiota richness, while the two weed species *M. chamomilla* and *V. sativa* had the lowest sequence cluster richness (Figure 2a). Neighbourhood weed identity had a significant effect on the wheat root endospheric microbiota sequence cluster richness (Table S3,  $p < 0.05$ ). The wheat individuals growing with *P. rhoeas*, *P. annua*, *M. chamomilla*, *P. trivialis* or *V. persica* displayed highest root microbiota sequence cluster richness, while the wheat individuals growing with *L. purpureum* displayed lowest root microbiota sequence cluster richness (Figure 2b). The weed species *V. persica* displayed the highest root microbiota evenness, while the weed species *P. annua* had the lowest root microbiota evenness (Figure 2c). No significant differences were found in root endospheric microbiota evenness among individual wheat plants growing with different weed species (Figure 2d).

### 3.4 | Effects of weeds on their ability to transmit root endospheric microbiota to wheat in the controlled experiment

Different weed species shared 10% to 70% sequence clusters (i.e. 5 to 45 sequence clusters) with wheat roots (Figure 3). *G. aparine*, *P. rhoeas*, *P. annua*, *P. trivialis*, *V. persica* and *V. sativa* shared the highest

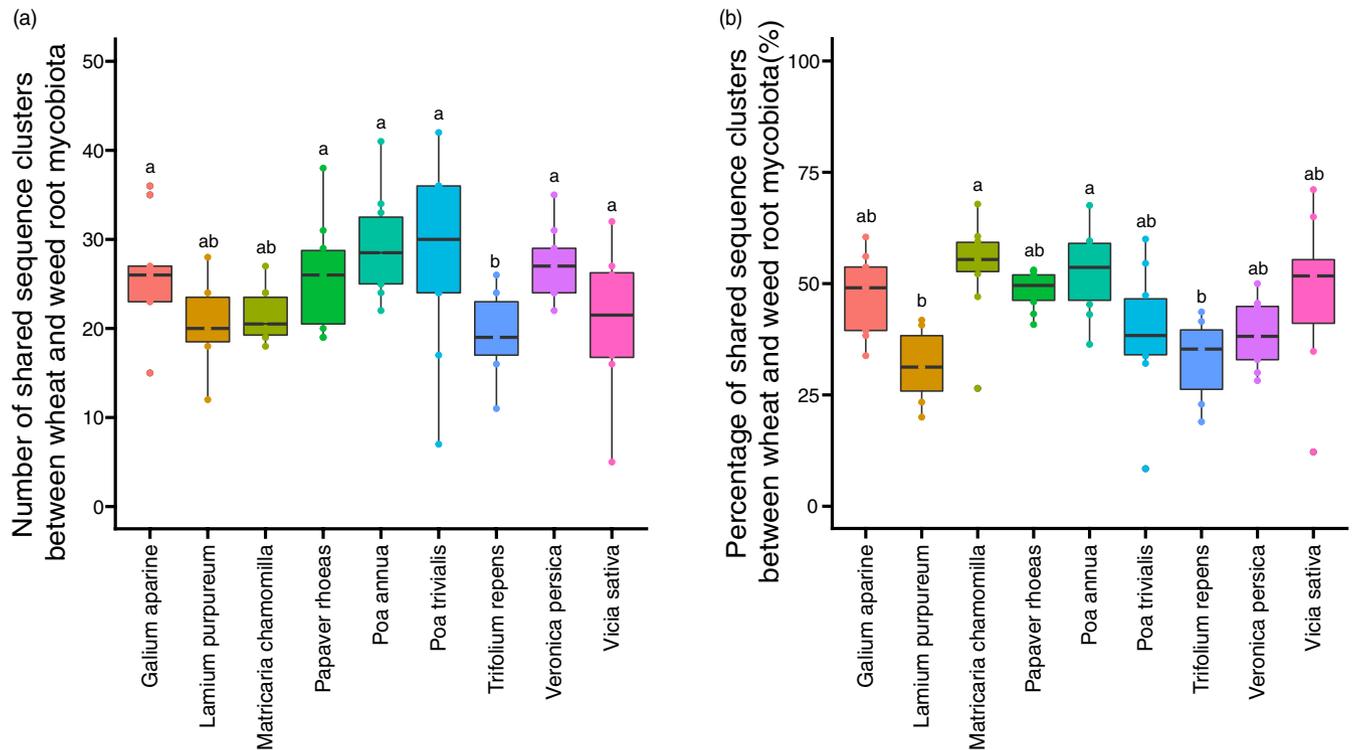


**FIGURE 2** Weed and wheat root endospheric mycobiota sequence cluster richness in the controlled experiment. (a) Root endospheric mycobiota sequence cluster richness of the neighbouring weed species; (b) Root endospheric mycobiota sequence cluster richness of wheat plants. (c) Root endospheric mycobiota evenness of the neighbouring weed species; (d) Root endospheric mycobiota evenness of wheat plants. In (b) and (d), the red dashed line indicates, respectively, the mean root mycobiota sequence cluster richness and evenness of wheat individuals in the control treatment of a single wheat plant growing in the pot. Asterisks indicate the significance level of weeds in promoting wheat root mycobiota diversity compared with red dashed line: \* indicates  $0.01 < p < 0.05$ ; \*\* indicates  $p < 0.01$ . Lowercase letters indicate significant differences in weed identity (Tukey post-hoc test) in all treatments.

number of sequence clusters with wheat (Figure 3a), while *M. chamomilla* and *P. annua* shared the highest percentage of their own root endospheric mycobiota with wheat roots (Figure 3b). The smallest number and the lowest percentage of shared weed root endospheric mycobiota to wheat roots were found for *T. repens* and *L. purpureum* respectively (Figure 3b).

In almost all cases, weed neighbourhoods enriched mycobiota in wheat microbiota compared to wheat alone, only a few sequence clusters were decreased. This enrichment was dependent on the neighbouring weed species (Figure 4a). *L. purpureum* and *P. annua* positively modified the relative abundance of the amount of 35 and 34 sequence clusters, respectively, while *T. repens* had

the least influence on the wheat root endospheric mycobiota (Figure 4a). *P. trivialis* and *V. sativa* reduced the relative abundance of five sequence clusters, and this was the strongest negative effect on the root endospheric mycobiota of individual wheat plants (Figure 4a). Some sequence clusters (e.g. clusters 25 and 30, belonging to phylum Ascomycota) were transmitted successfully to wheat roots by most of the weed species, while other specific sequence clusters (e.g. clusters 16 and 432, belong to genus Geranomyces, Saprotroph) were only transmitted successfully by one weed species (Figure 4b, Table S4). This generalist versus specialist effect was more obvious in weed reduced clusters, cluster 23 (belonging to phylum Ascomycota, family Capnodiales) and cluster 5 (belonging



**FIGURE 3** Root endospheric mycobiota transmission from neighbouring weed plants to focal wheat plants in the controlled experiment. (a) Number of shared root endospheric mycobiota sequence clusters between wheat and neighbouring weed plants; (b) Percentage of shared root endospheric mycobiota sequence clusters between wheat and neighbouring weed plants. Lowercase letters indicate significant differences in weed identity (Tukey post-hoc test) in all treatments.

to phylum Glomeromycotina, genus Gigasporaceae, Symbiotroph) were reduced by most weed species, whereas cluster 85 (belonging to phylum Ascomycota, species Gloeotinia, Pathotroph), cluster 10 (belonging to phylum Ascomycota, class Hypocreales) and cluster 16 (belonging to phylum Chytridiomycota, species Geranomyces, Saprotroph) were only reduced by *Vicia sativa* (Figure 4c, Table S4). Clusters belonging to Glomeromycotina were reduced by weed species *L. purpureum*, *P. annua*, *P. rhoeas*, *P. trivialis* and *T. repens*, the relative abundance of most sequence clusters in the Ascomycota of wheat root mycobiota was increased by the presence of weeds (Figure S4).

### 3.5 | Effects of weeds on wheat performance via their root endospheric mycobiota in the controlled experiment

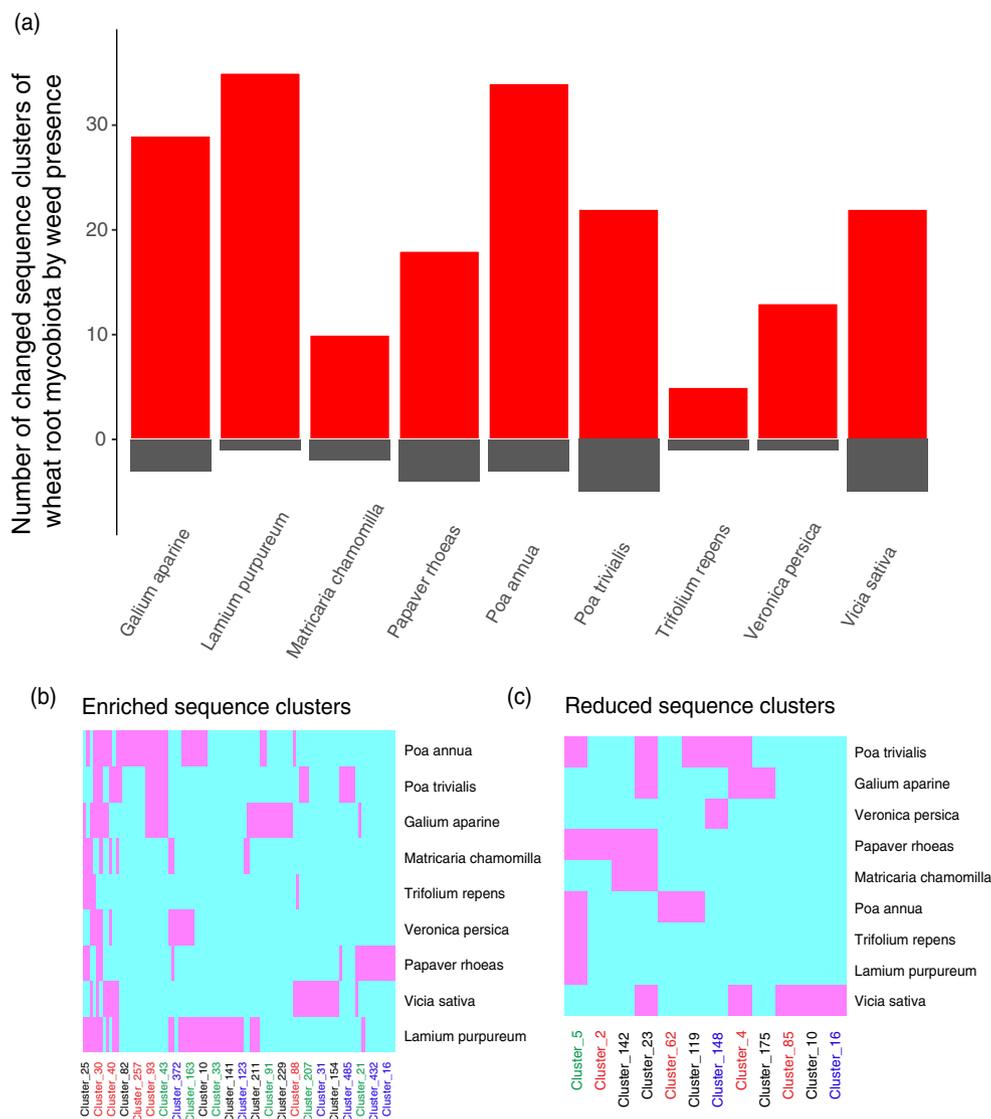
In the controlled experiment, the above-ground dry biomass of neighbouring weeds varied depending on the weed species (Figure 5a). In all treatments with weeds as neighbours, wheat above-ground biomass was not significantly different from that of wheat individuals growing alone without external microbial inoculation (Figure 5b, red dashed line). In six (*G. aparine*, *L. purpureum*, *M. chamomilla*, *T. repens*, *V. persica* and *Vicia sativa*) of the nine treatments with weeds as neighbours, treated wheat individuals had significantly higher above-ground biomass than the individual wheat plants growing

with four wheat individuals as neighbours (Figure 5b, blue dashed line). Neighbourhood weed identity had a significant effect on wheat above-ground biomass (Table S3,  $p < 0.01$ ). *V. persica* and *V. sativa* not only gained growth by themselves but also showed the most improvement in wheat biomass compared to controls (Figure 5), whereas *P. rhoeas* gained in self growth (Figure 5a) but did not promote wheat growth (Figure 5b). The total number of sequence clusters, especially those related to Ascomycota (Table 3,  $p = 0.08$ ) and Basidiomycota (Table 3,  $p = 0.02$ ), associated with wheat root endospheric mycobiota marginally significantly increased wheat above-ground biomass (Table 3,  $p = 0.06$ ).

## 4 | DISCUSSION

### 4.1 | Weed neighbours enriched and shaped composition of wheat microbiota but not by modifying soil microbiota

The composition and richness of neighbouring weeds influenced the composition and richness of endospheric mycobiota associated with wheat roots, whereas little effect was found on bulk soil mycobiota except Glomeromycotina (Table 1,  $p = 0.012$ ) which plays a crucial role in plant–soil feedbacks especially for plant growth and nutrition (Bruns et al., 2018). This suggests that the observed neighbourhood effects of weed plants on the endospheric mycobiota of wheat roots

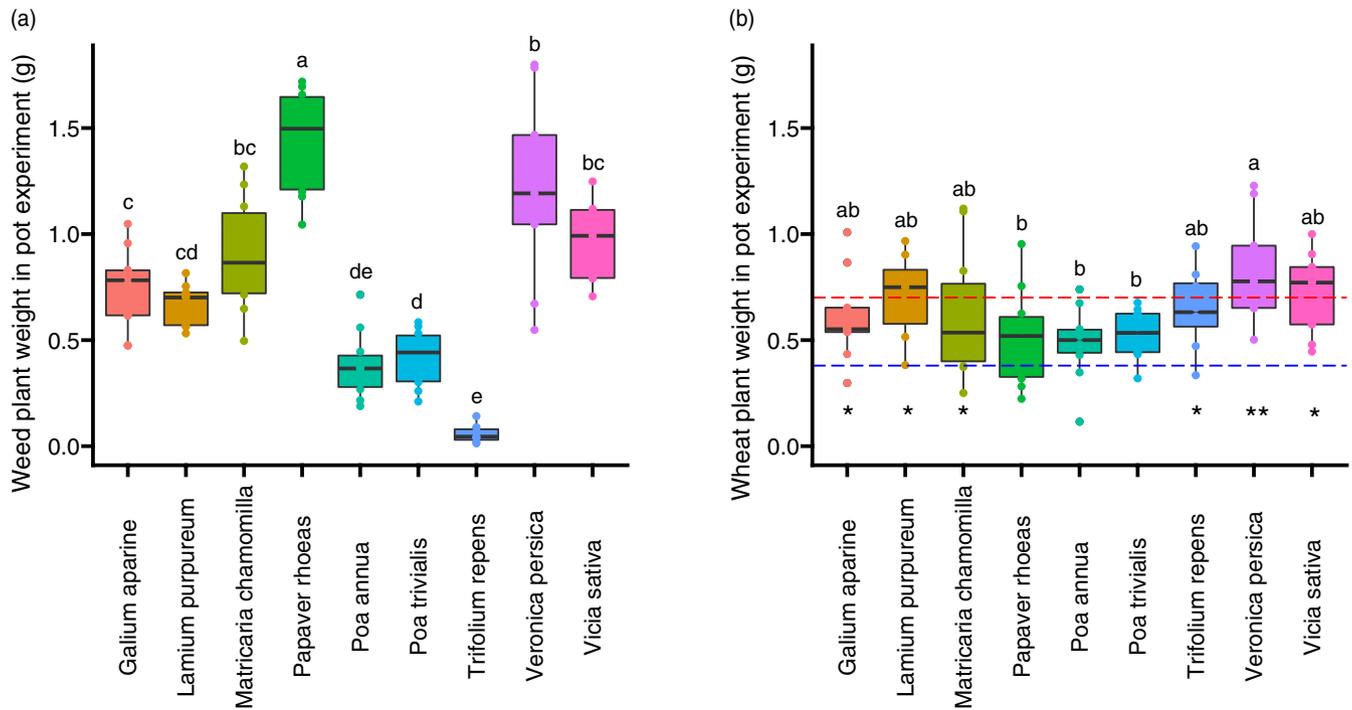


**FIGURE 4** Effect of the neighbouring weed species on the relative abundance of sequence clusters associated with wheat in the controlled experiment. In the three panels, the neighbourhood effects are shown relative to the wheat only control. (a) Number of significantly ( $p < 0.05$ ) modified sequence clusters in wheat root endosphere, the red bars indicate the enriched amount (i.e. relative abundance with  $\log_2\text{FoldChange} > 0.6$ ) of root endospheric mycobiota sequence clusters and the grey bars indicate reduced amount (i.e. relative abundance with  $\log_2\text{FoldChange} < -0.6$ ) of root mycobiota sequence clusters; (b) Identity of enriched sequence clusters in wheat root endospheric mycobiota; (c) Identity of reduced sequence clusters in wheat root mycobiota. In both (b) and (c) the pink grids indicate significantly changed sequence clusters in the wheat root, either increased or decreased relative abundances. The x-axis font colours: green indicates Symbiotroph, red indicates Pathotroph, blue indicates Saprotroph, red indicates Pathotroph and dark colour indicates that no functional trait information was found for this sequence cluster.

were likely due to local microbial dispersal from neighbour plants to crop plants rather than to a change in soil microbial reservoir in which the crop plant recruits. This result contrasts with that obtained in a previous study showing that a neighbour effect led to a legacy effect when the plant communities had been growing in the soil for several years (Bittebiere et al., 2020). In the present study, the limited legacy effect was probably due to (i) the crop rotation and (ii) short life span of the weeds, which were annual plants and only grew in soil for a maximum of 1 year. Local transmission of fungi among plants has already been demonstrated in an experiment performed to test the effect of plant neighbours on *Medicago truncatula* (Mony et al., 2021). Processes of microbial transmission between

plants can be achieved by microbial inoculation via contact between roots or leaves (Enkhtuya et al., 2005; Smith & Read, 2008) or by the development of hyphae (Simard, 2018).

In addition, we demonstrated a positive effect of the diversity of weed neighbours on wheat root endospheric mycobiota diversity in most fungal phyla including Ascomycota, Glomeromycotina and Zygomycete (Table S2). Because plants are associated with a preferential mycobiota (sensu host-preference effect, Vandenkoornhuysen et al., 2002), diverse plant communities provide a higher diversity of niches for micro-organisms, thereby encouraging a bigger range of micro-organisms to coexist locally. Some evidence has shown that richer plant communities increased the diversity of total plant



**FIGURE 5** Plant above-ground biomass of different weed species and wheat in the controlled experiment. (a) Above-ground biomass of weed species; (b) Wheat above-ground biomass depending on the neighbouring weed species. In (b), the red and the blue dashed lines indicate the mean biomass of wheat individuals in the control treatment in which a single wheat plant was grown in each pot and in the control treatment in which the wheat plant in each pot was surrounded by four individual wheat plants respectively. Asterisks indicate the significance level of weeds in promoting wheat growth compared with blue dashed line: \* indicates  $0.01 < p < 0.05$ ; \*\* indicates  $p < 0.01$ . Lowercase letters indicate significant differences in weed identity (Tukey post-hoc test) in all treatments.

**TABLE 3** Effect of different predictors on wheat performance (above-ground dry biomass) in the controlled experiment. The predictors included sequence cluster richness of whole wheat root mycobiota, Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycotina and Zygomycete. Significant results ( $p < 0.05$ ) are highlighted in bold, and marginal significant results ( $0.05 < p < 0.10$ ) are highlighted in bold and italics.

Parameters	df	Chi-square	<i>p</i>
Wheat root mycobiota richness	1	3.59	<b>0.06</b>
Ascomycota sequence cluster richness	1	3.13	<b>0.08</b>
Basidiomycota sequence cluster richness	1	5.51	<b>0.02</b>
Chytridiomycota sequence cluster richness	1	1.44	0.23
Glomeromycotina sequence cluster richness	1	1.29	0.26
Zygomycete sequence cluster richness	1	0.40	0.53
Residuals	73		
Model summary		$R^2 = 0.11$	AIC = 27

microbiota associated with the shoot (Navrátilová et al., 2018). The increased diversity of fungi provided by a diverse neighbourhood is a possible reservoir for transmission to crop plants growing nearby. Here, we demonstrated that, despite an existing soil microbiota that harboured much higher diversity than the microbiota associated

with plants, weed neighbourhoods, even with less abundant cover, significantly influence the root-associated mycobiota of crop plants growing close by (i.e. at a distance of less than 1 m).

#### 4.2 | Weed neighbours affected wheat root endospheric mycobiota and wheat performance

We assessed the ability of neighbourhood plants to influence the wheat root endospheric mycobiota in controlled conditions. Using nine different weed species cultivated in organic field soil as inoculum for wheat plants growing in sterile conditions, we observed differences in the ability of weed species to recruit their own root endospheric mycobiota, and to manipulate the wheat root microbiota. In weeds, these processes, which were linked to the difference in the influence of a target neighbouring plant, can be explained in three steps. First, weed species differ in their ability and use different patterns to recruit root mycobiota, as already shown for AMF by (Vatovec et al., 2005), who classified 14 weed species as strong, weak and nonhost plants for AMF. Plant phylogeny plays a role in structuring their root microbiomes, and a previous study showed that plants that are phylogenetically more distant gain bigger variations in the composition of their associated microbiome (Bouffaud et al., 2014). In the present study, the composition of root endospheric mycobiota of phylogenetically similar weed species such as *P. annua* and *P. trivialis*,

*T. repens* and *V. persica*, was also similar. Second, plant root exudate profiles may also influence the recruitment of root endospheric mycobiota (Pascale et al., 2020; Voges et al., 2019) and their surrounding micro-organisms thereby creating a unique microbial reservoir for their neighbouring plants. Third, plant root traits could explain the transmission of root mycobiota to plant neighbours, as it has been shown that neighbourhood plants' functional proximity in terms of below-ground resource use and uptake strategy was a key predictor of a neighbouring effect on focal plants (Mony et al., 2021).

The ability of weed species to transmit their root endospheric mycobiota to nearby wheat roots also depends on the species. We demonstrated that some sequence clusters were transmitted to wheat roots by most of the weed species tested, including clusters belonging to the Ascomycota phylum. Conversely, some sequence clusters were specifically transmitted by particular weed species to wheat roots. For example, one species of *Geranomyces* belonging to the Chytridiomycota phylum described as parasites of arbuscular mycorrhizae (Simmons, 2011; Wakefield et al., 2010), were only transmitted by *P. rhoeas*. By manipulating this *Geranomyces* species, *P. rhoeas* might improve its own competitive advantage as some studies stated that *P. rhoeas* is associated with relatively low abundance of mycorrhizal fungi, or even has no association with AMF (Gange et al., 1990; Wijesinghe & John, 2001). Future studies on the functions of this neighbour driven microbiota manipulation are required. We also need to compare wheat- and weed-associated microbiota in field conditions and controlled pot experiments in response to similar weed neighbourhoods to strengthen our findings in future studies. In addition, weed species which can grow in most of the sites and soil from only one field were selected to conduct laboratory experiment, the root mycobiota of relatively rare weed species might be interesting to be studied, especially for their microbial recruit abilities in their native or foreign soils.

As expected, competitive interactions between wheat individuals and neighbouring weeds were demonstrated. But this interspecific competition is relatively less intense than wheat intraspecific competition, possibly as a result of pathogen accumulation in monoculture. Interestingly, some weed species promoted wheat growth compared to the 'wheat grown alone' control (e.g. *Veronica persica*). In our experimental design, the wheat growth promotion was necessarily mediated by neighbouring weed species and this phenomenon was shown to be correlated with modifications in wheat mycobiota. Among the weed species studied here, some were particularly beneficial for wheat growth (e.g. *Veronica persica*, *Vicia sativa* or *Matricaria* sp.) relative to their competitive influence. Modifications to wheat mycobiota caused by weed neighbours could increase crop yield if their effects on wheat biomass are confirmed in field conditions.

### 4.3 | Weeds as auxiliaries for crops in sustainable agricultural system

Intensive agriculture has led to a major reduction in soil diversity (Tsiafouli et al., 2015), and disrupted the plant-microbial symbiosis

(Edlinger et al., 2022; Porter & Sachs, 2020). In particular, the long history of plant breeding has reduced the ability of domesticated crop plants to efficiently recruit their own microbiome from surrounding microbial reservoirs. Knowing that wheat breeding may have resulted in wheat plants that are no longer able to efficiently filter or recruit their microbiota endosphere (Mauger et al., 2021), wild neighbour auxiliary plants might be able to mitigate the disturbance of the wheat microbiota in modern crops through their influence on wheat microbiota.

The role of weed plants in agrosystem functioning is already known (Gaba et al., 2020; Marshall et al., 2003), for instance, affecting the composition and interactions of the insect fauna to protect beneficial insects, thereby increasing pollination, providing microclimates for crop development and regulating the development of competitive weeds. Beyond these ecological functions, in the present study, we demonstrated that weed species can help enrich plant microbiota and transmit specific sequence clusters when they grow in the close neighbourhood of crop plants. Such neighbourhood effects, which are likely caused by root-root connections, can also transmit systemic acquired resistance against pathogens to neighbouring plants (Cheol Song et al., 2016), thus helping plants survive and adapt to different environments. Weed neighbourhood effects require in-depth analysis in both controlled and field conditions including screening larger sets of neighbouring weed species in order to identify candidate auxiliary weed plant species that could be promoted in crops through dedicated field management.

Agricultural management is not 'all rocket science' ('Agriculture Isn't All Rocket Science', 2021). Contrary to the current direction, developing ecological approaches to agriculture can be used to favour future sustainable food production, all elements in agricultural systems including both plant diversity, their associated microbiota and the soil microbial reservoir shall be taken into consideration for a more holistic agriculture to obtain higher and more stable crop yields in a more sustainable way. In this context, weed plants could be used as auxiliary plants that provide ecosystem services to targeted crop plants in agricultural systems.

### AUTHOR CONTRIBUTIONS

Cendrine Mony and Philippe Vandenkoornhuys conceived the study and methodology. Claire Ricono, Jie Hu and Paola Fournier collected the data and performed the sequence analyses for weed and wheat root mycobiota, Samuel Mondy processed and performed the sequence analyses for soil samples. Jie Hu and Claire Ricono performed statistical analysis. Jie Hu and Claire Ricono wrote the manuscript with the help of Cendrine Mony and Philippe Vandenkoornhuys. All the authors contributed to the manuscript and gave approval for publication.

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicting interest.

### PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/1365-2745.14073>.

### DATA AVAILABILITY STATEMENT

Data and scripts relevant to this manuscript are available at <https://github.com/HuJamie/Weed-Neighbourhood-effect-on-wheats> and in Zenodo: <https://doi.org/10.5281/zenodo.7541448> (Hu et al., 2023). Sequence data are deposited in the Sequence Read Archive (SRA) under accession number PRJNA811118.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Figure S1.** Experimental design. (A) pre-culture of weed species in soil sampled in an organic field; (B) Plant-matrix design, a sterile pre-vernalsed wheat plant was transplanted to the centre of the pot, and four pre-cultivated individual weed plants (of the same species) were transplanted around it at the same distance from the focal wheat plant. Control treatment 1 contained one individual wheat plant growing alone in one pot. Control treatment 2 contained one individual wheat plant surrounded by four neighbouring wheat plants.

**Figure S2.** Rarefaction curves of soil, wheat and weed root endospheric mycobiota. Curves show the total assigned sequence clusters detected relative to the number of sequences in the soil sampled from field (A), in wheat roots sampled from field (B) and in the roots of wheat and weeds in the controlled experiment (C).

**Figure S3.** Effect of different weed species on the first and second principal components of weed and wheat root endospheric mycobiota. (A) Effect of weed species on the first principal component of the weed mycobiota; (B) Effect of weed species on the second principal component of the weed mycobiota; (C) Effect of weed species on the first principal component of the wheat mycobiota; (D) Effect of weed species on the second principal component of the wheat mycobiota.

**Figure S4.** Changes in the relative abundance of sequence clusters in wheat root mycobiota depending on the weed species in the controlled experiment. The dashed black lines in all panels correspond to the threshold value of no change. The sequence clusters on the left side of the vertical dashed line were reduced and the sequence clusters on the right side of the vertical dashed line were enriched by the influence of the neighbouring weeds considered. The colour of different dots shows the taxonomic identification of the sequence clusters.

**Figure S5.** Soil mycobiota and wheat root endospheric mycobiota sequence clusters in the field study. (A) left panel: proportion of sequence cluster abundance in each phylum (Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycotina

and Zygomycete) of soil mycobiota, (A) right panel: sequence cluster richness of each phylum (Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycotina and Zygomycete) in the soil mycobiota; (B) left panel: proportion of sequence cluster abundance in each phylum (Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycotina and Zygomycete) of wheat endospheric root mycobiota, (B) right panel: sequence cluster richness of each phylum (Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycotina and Zygomycete) in the wheat endospheric root mycobiota.

**Figure S6.** Diversity of soil and wheat root endospheric mycobiota in the field study. Mycobiota diversity is indicated as sequence cluster richness (A), and Pielou's evenness index of the sequence cluster (B). Lowercase letters indicate significant differences between soil and wheat root mycobiota (t-test).

**Figure S7.** Mycobiota composition and dissimilarity of soil and wheat root samples in the field study. (A) PCoA of soil and wheat root mycobiota in the field study; (B) A Venn diagram of soil and wheat root mycobiota in the field study.

**Figure S8.** Weed and wheat root endospheric mycobiota sequence clusters in the controlled experiment. Left panel: proportion of sequence cluster abundance in each phylum (Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycotina and Zygomycete) in all weed and wheat root mycobiota; Right panel: sequence cluster richness in each phylum (Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycotina and Zygomycete) of all weed and wheat root mycobiota.

**Table S1.** Occurrence, and mean coverage percentage of weed species in the neighbourhoods in the field study. Species used for the controlled experiment are highlighted in bold (n=60).

**Table S2.** Effects of floristic richness on soil mycobiota and wheat root endospheric mycobiota diversity in the field study. Mycobiota diversity is indicated as sequence cluster richness and Pielou's evenness index. Random effects in the different fields were tested via linear mixed models.  $R^2_m$  is marginal R squared, denotes variance explained by just fixed effects in the model;  $R^2_c$  is conditional R

squared, denotes variance explained by the entire random model; and AIC denotes Akaike's information criterion. Significant results are indicated by asterisks in brackets after Chisq value: \* indicates  $0.01 < P < 0.05$ ; \*\* indicates  $0.001 < P < 0.01$ , \*\*\* indicates  $P < 0.001$ . n.s.: not significant. Significant results ( $P < 0.05$ ) are highlighted in bold. Upward arrows denote positive effects of explanatory variables in all the models.

**Table S3.** Effect of weed plant identity on wheat and weed root endospheric mycobiota sequence cluster richness, number of shared sequence clusters, percentage of shared sequence clusters, wheat and weed plant aboveground weight in the controlled experiment. Linear models were used to detect the effects of weed identity on each parameter.  $R^2$  denotes variance explained by the model; and AIC denotes Akaike's information criterion. Significant results are indicated by asterisks in brackets after Chisq value: \* indicates  $0.01 < P < 0.05$ ; \*\* indicates  $0.001 < P < 0.01$ , \*\*\* indicates  $P < 0.001$ . n.s.: not significant. Significant results ( $P < 0.05$ ) are highlighted in bold.

**Table S4.** Taxonomic and functional information of weed species enriched and reduced sequence clusters. The font colours of Trophic Mode: green indicates Symbiotroph, red indicates Pathotroph, blue indicates Saprotroph, red indicates Pathotroph, and dark colour indicates that no functional trait information was found for this sequence cluster. In column "Change": E indicates enriched sequence clusters, R indicates reduced sequence clusters, and B indicates both enriched and reduced sequence clusters by certain weed species.

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