Plant Soil
https://doi.org/10.1007/s11104-026-08723-0

RESEARCH ARTICLE

Microbiome analysis identifies a microbial marker for root rot resistance across four contrasting soils

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Received: 25 September 2025 / Accepted: 1 June 2026
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Abstract

Aims Soil-borne pathogens can severely compromise legume cultivation and, consequently, plant-based protein production by triggering root rot, particularly in pea (*Pisum sativum* L.). Although some pea genotypes exhibit enhanced performance compared to others under root rot stress, breeding efforts have so far achieved only limited resistance. Microbiome-mediated breeding might show more success in alleviating root rot. We hypothesized that some root-associated microbes serve as resistance markers across diverse soils. We therefore determined whether certain fungi or mycobiome attributes consistently associated with a genotype's resistance across a diverse range of soils.

Methods Root-associated fungal communities of eight pea genotypes differing in root rot resistance were characterized by ITS amplicon sequencing in four soils with contrasting infestation levels under controlled conditions. We employed alpha and beta diversity, as well as differential abundance analyses, to detect genotype- and soil-specific patterns and associations with resistance traits.

Results Both genotype and soil identity influenced alpha diversity. Evenness was reduced in infested soils, as OTU richness increased. Fungal community composition covaried with infestation level, genotype and previously defined resistance levels of the genotypes. A single OTU (OTU3), showing 100% sequence similarity to a previously identified heritable, resistance-associated hub taxon (*Dactylonectria*), was consistently enriched in resistant genotypes across soils and was positively correlated with seed emergence.

Responsible Editor: Ben Jackson.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-026-08723-0.

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Published online: 24 June 2026

Conclusions Our results reveal OTU3 as a common microbial resistance marker, highlighting the potential of microbiome-mediated root rot resistance breeding in legumes. The uncovered phenomenon may represent a promising avenue to promote sustainable legume cultivation.

Keywords *Pisum sativum* · Resistance breeding · Microbial marker · Root rot complex · Root microbiome · Legumes

Abbreviations

OTU	Operational Taxonomic Unit
RRI	Root Rot Index
SDW_{Rel}	Relative Shoot Dry Weight
ANOVA	Analysis Of Variance
CLR	Centered Log-Ratio
PCA	Principal Components Analysis
RDA	Redundancy Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance

Introduction

In recent years, the consumption of pulse legumes, especially protein-rich peas (*Pisum sativum L.*), has gained in importance, since many consumers look for alternative protein sources to animal-based products, either for dietary restrictions or environmental concerns (Sanchez-Sabate and Sabaté 2019). This has led to an increase of 29% in the area dedicated to green and dry pea production from 2000 (7.5 million ha) to 2022 (9.7 million ha) worldwide (FAOSTAT 2024). Furthermore, peas are commonly used in crop rotations due to their symbiosis with nitrogen-fixing bacteria, which support the growth of pea plants and restore the nitrogen reserves of the soil (Herridge et al. 2008; Lal 2017).

A major challenge for pea production worldwide is yield depression or soil fatigue, a phenomenon in which the yield falls below expectations. It is now known that this can be caused by the build-up of soil pathogens over the seasons (Fuchs et al. 2014). Ultimately, these pathogens can cause pea root rot, an interaction between a complex of microorganisms that, in a synergistic manner, damages the root system and eventually can lead to plant death (Bainard et al. 2017; Wille et al. 2019).

Pesticides and mineral fertilisers are the traditional solution to increase yields and thereby meet the world population's food demands and to solve disease-related problems (John and Babu 2021). However, new directives encourage avoiding them due to their reported damage to the biosphere and impact on the natural resources (Programme et al. 2022). Plant breeding programs are a sustainable alternative to boost the resistance and productivity of crops in response to biotic and abiotic stress. A variety of resistant pea accessions are already available, but they provide only partial resistance to one or a few pathogens (Bodah et al. 2016; Sharma et al. 2022). Multiple studies have already reported the potential use of quantitative trait loci (QTL) for the selection of pea genotypes with resistance against *Aphanomyces euteiches*-caused root rot (Lavaud et al. 2016; Pilet-Nayel et al. 2002; Wu et al. 2021), although only partial resistance has been achieved. This situation highlights the need for alternative strategies, such as leveraging the root microbiome. In recent years, research on harnessing the root microbiome to promote disease resistance has gained significant attention (Toyota and Shirai 2018). The potential applications are numerous (Du et al. 2025), for example, as a modulator of plant-pathogen interactions (Tiwari et al. 2022), or a prediction tool for crop performance (Chang et al. 2017). The root microbiome has so far not been exploited by breeding programs (Trivedi et al. 2020).

The holobiont concept has substantially reshaped our understanding of plant–microbiota associations, considering them as a discrete ecological unit (Vandenkoornhuyse et al. 2015). When considered as part of the holobiont, the microbiota can be seen as extended plant traits that can be studied in the context of plant resistance, with individual taxa occurring at characteristic abundances. The application of the holobiont concept has already been applied in biomedical research as a tool to predict colorectal cancer in multiple cohorts through so-called microbial markers (Yu et al. 2017). In plant sciences, the term 'microbial marker' is mainly associated with the genetic markers that allow the identification of a specific microbial taxon and their use to diagnose a plant disease (Sapre et al. 2021). Wille et al. (2021) introduced the term microbial markers in plant breeding and suggested the potential of certain microbial organisms to assess the resistance of pea genotypes.

To better understand the role of the root microbiome against pea root rot, we established a screening

assay under controlled conditions (Wille et al. 2020). Further, we defined suitable resistance traits: 1) Root Rot Index (RRI), indicating the severity of the root rot; 2) seed emergence, and 3) the shoot dry weight of a plant in infested soil relative to the same genotype grown in sterilised soil (SDW_{Rel}) (Wille et al. 2020). A further study evaluated preselected microbial markers for resistance using qPCR, based on the quantification of potentially relevant taxa identified in the literature and reported that the pathobiome highly depends on the host genotype (Wille et al. 2021). It remains unknown whether there are other, possibly more effective key microbial markers for disease resistance. Gfeller et al. (2025) made a first attempt to shed light on this through the microbiota analysis of 252 pea genotypes to identify resistance-associated OTUs and Ariza-Suarez et al. (2025) tested the association of those genotypes with disease phenotypes finding a QTL associated with disease severity. For several key OTUs, the genetic loci controlling their abundance were identified (Schneider et al. 2025). While promising resistance markers were identified in the one soil studied, it remains unknown whether these markers for resistance also apply to other soils or whether the resistance markers are soil-specific, which would significantly limit their usefulness.

To investigate how different genotypes modulate their root mycobiota when challenged with root rot pathogens in different soils, we used high-throughput sequencing to characterise the root fungal community of eight pea genotypes grown in four different soils. This allowed us to screen for microbial markers associated with resistant pea genotypes across various soils. Our main hypothesis is that some microbial taxa (markers) function as an extended plant phenotype or genotype that can predict plant resistance across diverse soils. This study examines the genotype-microbiome interactions as a factor to be considered in future breeding strategies. We identified a microbial marker associated with resistant genotypes across all infested soils tested and that shows correlations with seed emergence.

Material and methods

Plant material and experimental setup

The samples analysed in this study originated from eight pea genotypes grown in four contrasting soils, building on an experiment previously described in Wille et al. (2021). The eight genotypes, comprising gene bank accessions and cultivars, were selected based on their contrasting resistance to root rot. Three of them (Respect, G89 and S22) were known to be susceptible, while the remaining five (S64, G78, EFB.33, S91 and S134) exhibited partial resistance in previous studies (Baćanović-šišić et al. 2018; Wille et al. 2020). Five of the eight genotypes examined in the present study were also evaluated in an infested field over a period of three years, where they showed the expected level of resistance (Vonzun et al. 2024), indicating that our screening system can predict field relevant root rot resistance. Seven surface-sterilized seeds from each genotype were sown in 600 mL pots containing sieved soil from one of four soils originating from different agricultural fields supplemented with sterile quartz sand in a 2:1 (v:v) ratio. The soils were known to differ in their abundance of pea root rot pathogens, and were classified accordingly: Feldbach (uninfested), Kirchlindach (infested), Puch (infested), and Neu-Eichenberg (infested). The soil from Feldbach was considered to be uninfested based on information provided by the farmer and the quantification of the pathogens reported in (Wille et al. 2021). Some causal agents of root rot were also present in Feldbach soil; however, at very low concentrations compared with those in the other soils. This could explain why roots grown in the Feldbach soil showed weak symptoms. Soil characteristics and coordinates can be found in the Supplementary Table 1. The pea plants were grown for 29 days in a growth chamber at a mean temperature of 20 °C and 85% relative humidity with a 16h/8h light/dark cycle, receiving 200 µmol m^{-2} s^{-1} of photosynthetically active light. The pots were randomized in four blocks, each containing all genotype x soil combinations, and watered from below by flooding the trays to a height of 4 cm for 30 min every three days, as determined by prior optimization trials. After germination, the peas were thinned out to 5 plants per pot. The full factorial experimental design consisted of 2 factors: soil (4 levels) and genotype (8 levels) and was replicated 4 times, with each replicate being sown in four consecutive days and harvested in the same order. Of the 64 pots, 8 showed no plants. All of these corresponded to the genotype Respect.

Consequently, only one replicate of Respect was available in Feldbach, three in Puch, two in Kirchlindach, and two in Neu-Eichenberg.

Seedling emergence and RRI were recorded as described in Wille et al. 2020. Briefly, the RRI scores progresses from 1 (no symptoms) to 6 (disintegration of the root system), taking into account the appearance of local or generalised lesions, like browning, rotting, or the loss of root integrity. To determine pea performance in the absence of root rot stress and soil-borne microbes, in parallel, the complete set of plants was also grown in the four X-ray-sterilised soils (30–100 kGy, Synergy Health Däniken AG, Switzerland). This allowed us to calculate the SDW_{Rel} as the ratio of the shoot dry weight of the non-sterilized samples to that of the sterilized sample control, as an additional resistance-associated phenotype.

DNA extraction and sequencing

All the roots from one pot were washed and pooled in a single sample. Then they were lyophilised and ground to a fine powder. DNA was extracted from 20 mg of root powder using the Mag-Bind® Plant DNA DS 96 Kit (Omega Bio-Tek, Norcross, United States) following the instructions of the manufacturer. The amplicon libraries were prepared and sequenced by Génome Québec (Montreal, Canada) as previously described (Gfeller et al. 2025), where library preparation was done in a two-step PCR process using the primer pair ITS1f (Gardes and Bruns 1993) and ITS2 (White et al. 1990) targeting the ITS1 region, and the obtained amplicons were sequenced on an Illumina MiSeq sequencer.

Bioinformatics

The bioinformatic analysis was performed by the Genetic Diversity Centre (GDC), ETH Zurich. The complementary reads were merged, the priming sites were trimmed and the sequences were quality-filtered and denoised, using USEARCH (v11.0.667; Edgar 2013). The resulting sequences were clustered into zero radius OTUs (zOTUs) with UNOISE3 (Edgar 2016b). An additional clustering of the zOTUs at 97% sequence identity was done with UPARSE (v11.0.667; Edgar 2013). Taxonomic assignment was performed with SINTAX (v11.0.667, Edgar 2016a) against the UNITE_v82_Fungi database (last updated on 04.02.2020). To obtain a putative taxonomy for the OTUs not assigned by the default bioinformatic pipeline, the above taxonomic assignment was complemented with an additional method. For the OTUs of highest interest, we performed a complementary BLAST search against the NCBI ITS database (September 2025 https://blast.ncbi.nlm.nih.gov/Blast.cgi). When multiple best hits with different reference sequences occurred at the lowest taxonomic rank (e.g., species), we assigned the OTU to the next higher level (e.g., genus).

In a previous study, heritable hub OTUs associated with root rot resistance were identified across 252 pea genotypes grown in an infested soil (Kirchlindach; Gfeller et al. 2025). To evaluate whether these OTUs are also present in the roots of peas grown in the soils of this study and whether they remain associated with root rot-resistant genotypes, we mapped the OTU sequences of three key OTUs of the previous study (fOTU1508, fOTU1517, fOTU22) against the fungal sequences obtained in this study. This was performed by a local *blastn* (NCBI, 2.9.0+) search facilitated by the R package *rBLAST* (Hahsler and Nagar 2024).

Data analysis

The statistical analysis and visualisation of the data were performed using R (v4.3.2; R Team 2023) and the *tidyverse* package collection (Wickham et al. 2019). A summary of all statistical analyses is provided in Supplementary Fig. 1. The shared OTUs between soils were visualised using the *UpSetR* package (Gehlenborg 2019). To discriminate the infestation of the soils in the analysis, we created a new variable called "condition" that grouped the soils into infested (Kirchlindach, Puch and Neu-Eichenberg) and uninfested (Feldbach). To examine the effects of previously determined genotype resistance levels on mycobiome attributes and individual taxa, genotypes were classified into a "susceptibility" variable (susceptible: Respect, G89, S22; resistant: S64, G78, EFB.33, S91, S134).

To assess the effect of genotype and soil condition or soil origin and their interactions on alpha diversity, the OTU counts were rarefied on a loop 500 times to the minimal sequencing depth present in the dataset to avoid detection bias (Bodenhausen et al. 2013; Schloss 2024) using functions from the R packages

phyloseq (McMurdie and Holmes 2013) and *vegan* (Oksanen et al. 2022). For each iteration and sample, the Shannon index (as $H\prime = -\sum p_i * \log(p_i)$, where p_i is the relative abundance of each OTU), OTU richness (S) and Pielou's evenness (as $J = H\prime/\log(S)$; Pielou 1966) were calculated and averages of the 500 iterations were used for downstream analysis. The normality of the distribution of the residuals and the homoscedasticity of the error variance were checked for all linear models. Differences in the within-soil variances of the four soils were found and dealt with using a weighted model that accounted for them. For this, a generalized least squares (gls) model using the *nlme* package (Pinheiro et al. 2023) was fitted to test for the effects of soil, genotype and their interactions on the diversity indices. To perform an analysis of the variance (ANOVA), the Wald Chi-square test statistics and *p*-values were calculated using the *Anova()* function of the *car* package (Fox and Weisberg 2019).

Beta diversity analysis was performed on a filtered data set in which taxa containing less than 4 sequences in less than 4 samples were removed. The OTU counts were then centered log-ratio (CLR) transformed using the *aldex.clr()* function from the package *ALDEx2* (Fernandes et al. 2014). The effect of genotype and soil condition or soil origin and their interaction on beta diversity was assessed by performing a Permutational Multivariate Analysis of Variance (PERMANOVA) on Euclidean distances and visualizing the ordination of the samples using a Principal Components Analysis (PCA). After the effect of the soil was assessed, a redundancy analysis (RDA), performed with the package *vegan* (Oksanen et al. 2022) constraining for genotype susceptibility or RRI was performed while accounting for the differences of the soils.

Differential abundance analysis was conducted using functions from the *ALDEx2* package. First, the effect of soil condition on OTU abundance was evaluated using Welch's t-test with the function *aldex.ttest()* to compare infested and uninfested samples. Next, a generalized linear model (GLM) implemented within *ALDEx2* was used to assess the effects of both soil condition and pea genotype on CLR-transformed abundance data. A modified version of the *aldex.glm*() function (adapted by GitHub user "chassenr") was employed to control for soil effects when comparing genotype susceptibility. Log_2 fold changes were calculated using the *aldex.effect*() function, defined as the difference in $\log_2$-transformed OTU abundance between susceptible and resistant genotypes. Finally, the correlation between individual resistance traits (RRI, seed emergence, and SDW_{Rel}) and CLR-transformed abundance was assessed using the *aldex.corr*() function.

Results

Alpha diversity is associated with genotypes, soils and infestation level

To test whether specific microbial taxa or microbiome attributes consistently associate with pea genotype or a genotype's resistance level against root rot across diverse soils, we profiled the ITS1 region of root-associated fungal communities of eight genotypes grown in four soils and analysed alpha and beta diversity, and identified differentially abundant OTUs. Amplicon sequencing provided 13,176 to 47,002 reads per sample with an average of 33,578 reads (Supplementary Fig. 2) and no major differences were observed in the sequencing depth of the different soils or genotypes. The fungal community was dominated by Ascomycota, followed by Basidiomycota (Supplementary Fig. 3).

Evenness was significantly higher in the uninfested soil compared to the infested soil, and a genotype effect was observed (Fig. 1a, Supplementary Fig. 4). However, no significant interaction of the condition with the genotype was observed. It should be noted that roots grown in the uninfested soil also exhibited mild symptoms, although these were much weaker than those observed in the other soils (Supplementary Fig. 5). The differences among the infested soils were also investigated, revealing that the evenness of the soils was slightly statistically different, but that there was a more significant genotype effect (Fig. 1b). The correlation between evenness and RRI was significant and negative when considering all soils (Fig. 1c). This significant correlation was lost when looking at the infested soils only, indicating that the uninfested soil is driving this correlation. OTU richness showed an opposite pattern, with higher values in the infested soils and lower in the

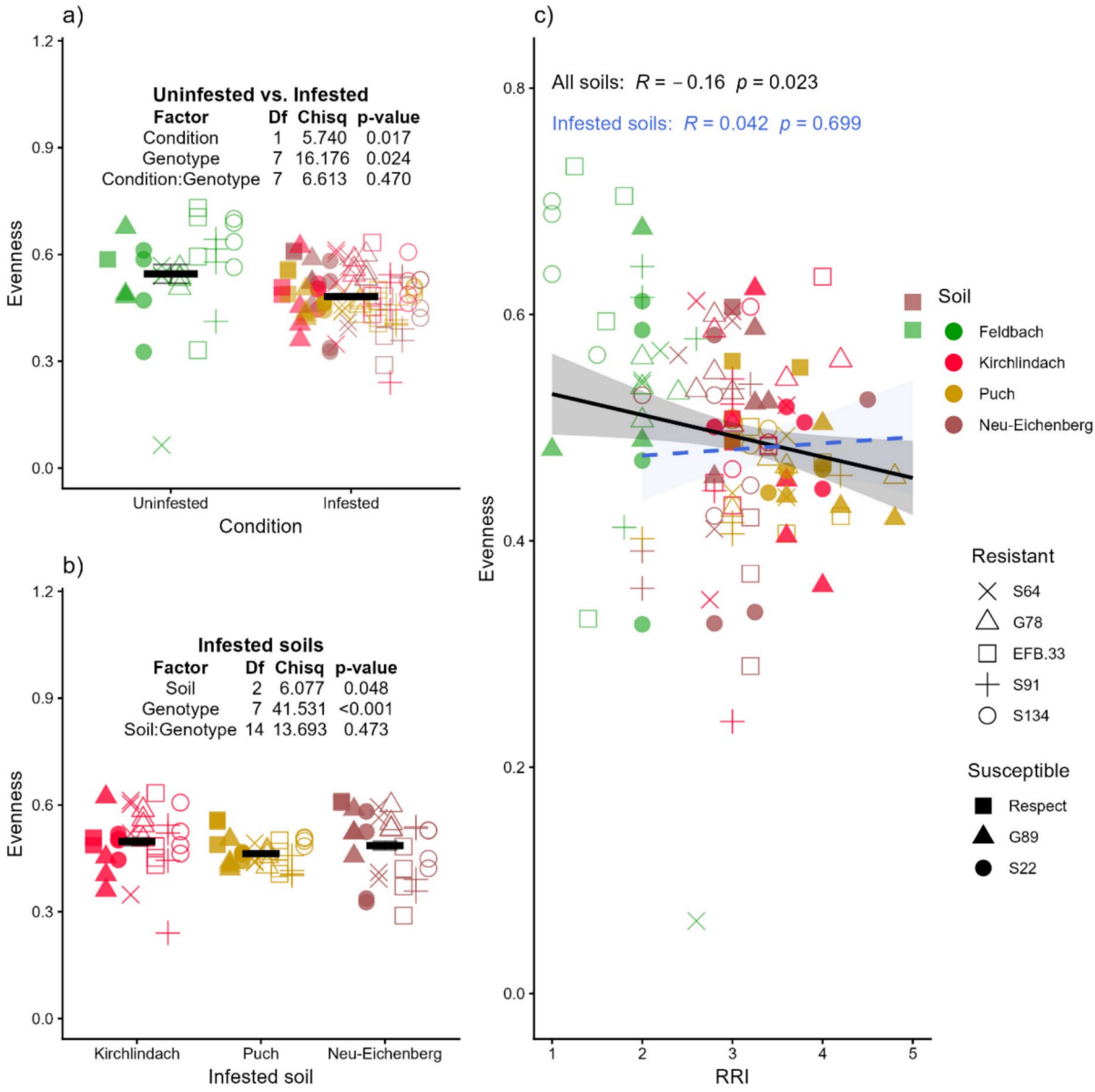


Fig. 1 Pielou's evenness in (**a**) all soils, and (**b**) infested soils only, with their respective Analysis of Variance table. **c** Correlation of the evenness with the Root Rot Index (RRI). R: Pearson's correlation index; *p*: *p*-values; Solid line: significant correlation; Dashed line: not significant correlation; Filled shapes: susceptible genotypes; Empty shapes: resistant genotypes

uninfested (Supplementary Fig. 6). Genotype susceptibility did not have any effect on OTU richness or evenness (Supplementary Table 2). No significant correlations of richness and evenness with seed emergence and SDW_{Rel} were found, except for evenness and emergence when considering all four soils (Supplementary Fig. 7).

Fungal community structure differs between soils and genotypes

We studied the fungal community structure to identify variations in the root mycobiota that could explain the differences in pea resistance to root rot. For this purpose, a PCA on the CLR-transformed

OTU abundances was conducted, and the statistical significance of the experimental factors was checked with PERMANOVA. Considering all soils, PC1 (11.6%) clearly separated the soils into infested and uninfested and PC2 (6.8%) separated the three infested soils (Fig. 2a). These differences in community composition were confirmed by PERMANOVA, which revealed a significant condition effect (infested versus uninfested), genotype effect and a significant condition x genotype interaction.

To investigate if there are differences in the fungal communities within the infested soils, the same approach was followed but only considering the three infested soils. In Fig. 2b, PC1 and PC2 explained 8.96 and 6.14% of the variation and showed that the community of each of the soils was distinct from the others, as confirmed by the PERMANOVA results. The different genotypes tended to cluster within the same soil, mainly according to their susceptibility, as supported by PERMANOVA.

To further investigate differences between susceptible and resistant genotypes, an RDA constrained for susceptibility was performed in the three infested soils, revealing that it significantly explained 2% of the variation in fungal community composition (Fig. 3a). Similarly, when the RDA was constrained for RRI, it explained 4% of the total variation. A PERMANOVA to test plant resistance traits revealed that RRI could explain 9.8% of the variation in fungal community composition if all the soils were considered, while the seed emergence and SDW_{Rel} could only explain around 2% each, regardless of the soils selected (Supplementary Table 3).

Potential microbial markers for root rot-resistant genotypes identified by differential abundance analysis and correlations

To assess the effects of soil condition on individual taxa, pairwise comparisons were performed between infested and uninfested soils across all the genotypes, and the effect sizes were determined. The OTUs with effect sizes greater than 1 or smaller than −1 are shown in Supplementary Table 4. OTU25 (classified as *Fusarium* sp.*)* had the greatest association with the uninfested soil, while OTU549 (classified as *Leohumicola* sp.) had the greatest association with the infested soils. Moreover, 30 OTUs were shared across all soils, and nearly as many (27) were unique to the uninfested soil (Feldbach) (Supplementary Fig. 8). The infested soils Kirchlindach, Puch and Neu-Eichenberg had 2, 3 and 10 unique OTUs, respectively, and shared 7 OTUs: OTU75, OTU76, OTU84, OTU174, OTU294, OTU665 and OTU1191. Taxonomic assignment of these OTUs can be found in the Supplementary Table 5.

The effect of the genotypes on OTU abundance was tested at three different levels (considering all the genotypes, Table 1): First, the impact of the genotypes on CLR-transformed abundances was investigated considering all the soils together, where only OTU3 displayed a significant effect. In second place, to eliminate the influence of the uninfested soil, only the 3 infested soils were considered. Only OTU2, OTU3, OTU10, OTU35 and OTU132 were shown to be controlled by genotype across the infested soils. Finally, to study the soil-genotype interactions reported in the beta diversity analysis, the genotype effect was studied in each of the soils individually. A total of 16 OTUs were reported to have a significant genotype effect in at least one soil, but only OTU3 exhibited this effect in more than one soil. OTU3 was differentially abundant among the three infested soils (Kirchlindach, Puch and Neu-Eichenberg), with remarkably high relative abundances.

To go one step further in understanding the relationship between the OTUs and the genotypes, a comparison of the abundance of the taxa in susceptible and resistant genotypes was performed for all soils, infested soils and individual soils (Table 2). When all soils were considered together, only OTU3 showed significantly higher abundance in the resistant genotype. Restricting the analysis to infested soils, OTU3 and OTU35 were more abundant in the resistant genotypes, whereas OTU93 and OTU549 were enriched in the susceptible ones. At the level of individual soils, significant associations with resistant genotypes were detected only in Kirchlindach, where both OTU3 and OTU35 displayed higher abundance in the resistant genotypes.

As seen previously, the correlations drawn with all the soils considered risk of being influenced by uninfested soil. For this reason, only the infested soils were considered to draw correlations between the OTU abundance and the resistance traits (Supplementary Table 6). From all the taxa sequenced, only OTU3 and OTU35 showed a positive correlation with

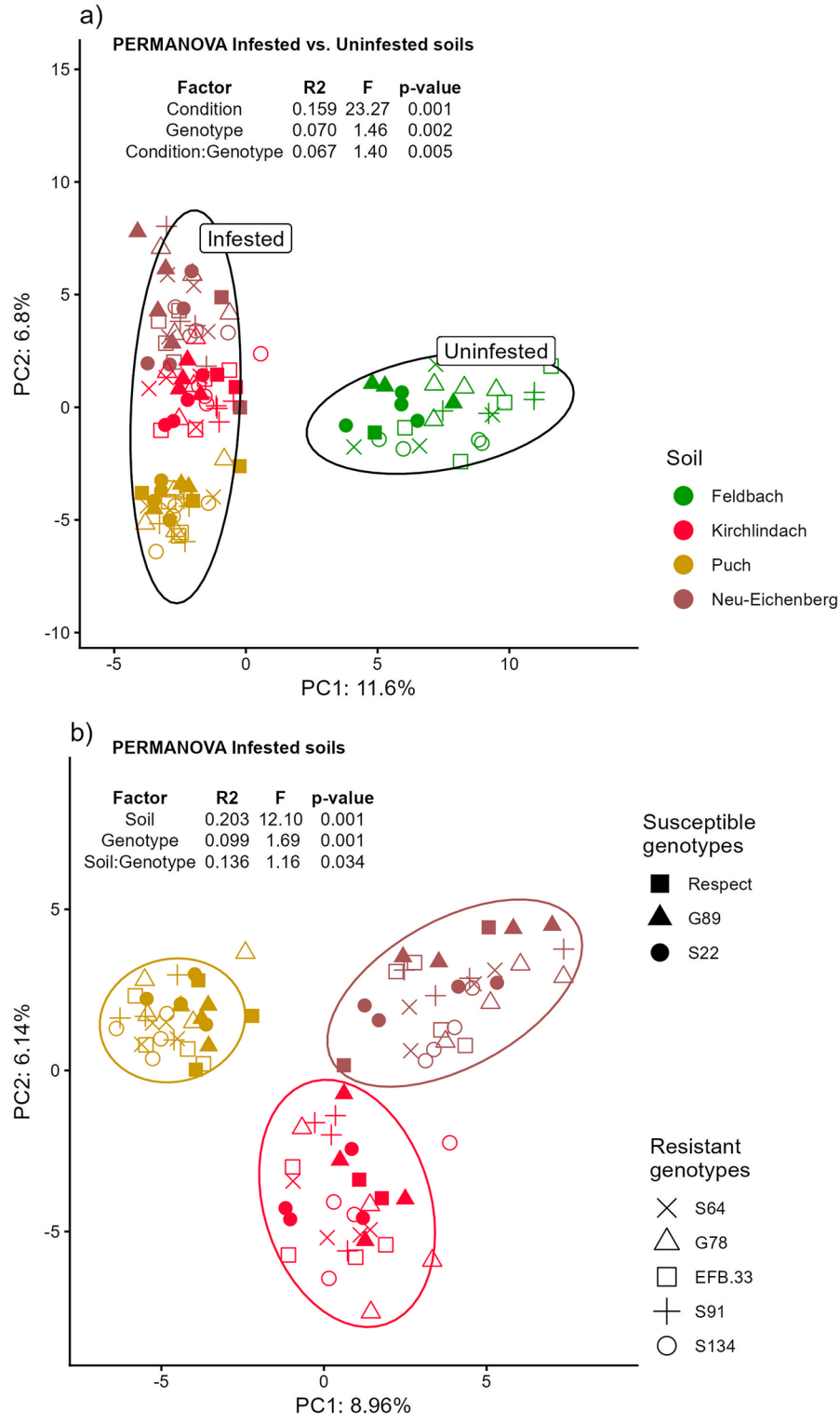


Fig. 2 Principal Components Analysis of the centered log-ratio transformed abundances of fungal Operational Taxonomic Units according to soil and genotype for all the soils (**a**) and only the three infested soils (**b**), accompanied by their respective Permutational Multivariate Analysis of Variance (PERMANOVA) results. Open and closed shapes indicate susceptible and resistant genotypes, respectively

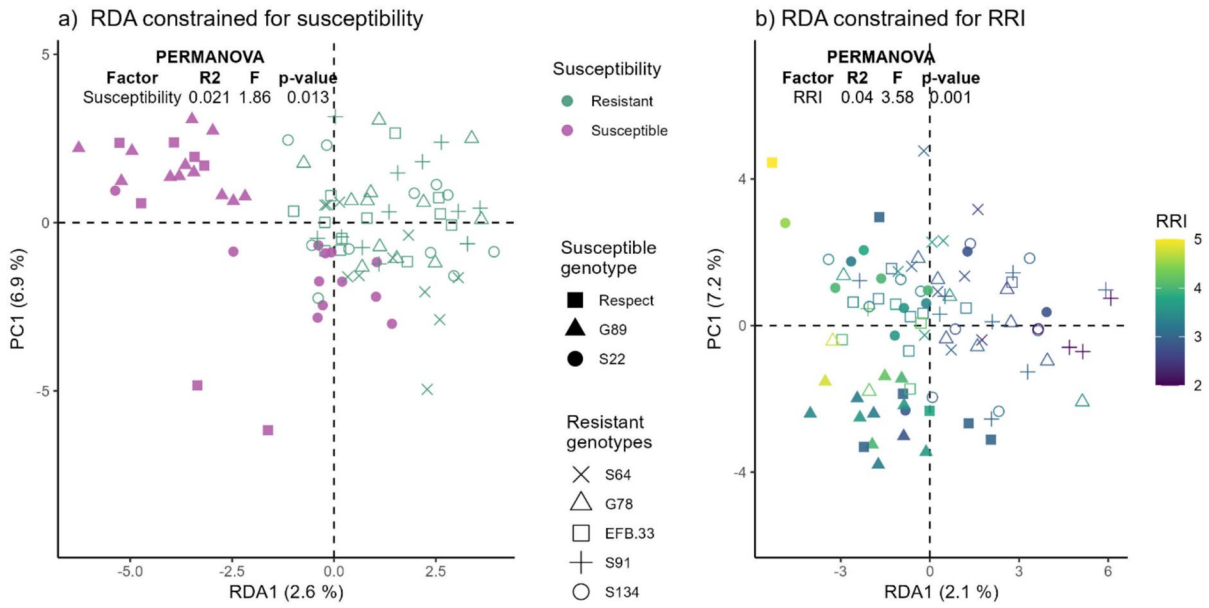


Fig. 3 Redundancy analysis (RDA) and Permutational Multivariate Analysis of Variance (PERMANOVA) tables of the centered log-ratio transformed abundances constrained for susceptibility (**a**) and Root Rot Index (RRI) (**b**) for the samples of the three infested soils (Kirchlindach, Puch and Neu-Eichenberg). Empty symbols: Resistant genotypes; Full symbols: Susceptible genotypes

Table 1 Corrected *p*-values of the genotype effect on centered log-ratio transformed abundance and mean relative abundance across genotypes of Operational Taxonomic Units (OTU) with at least one significant result for individual soils

	Feldbach (Uninfested)		Kirchlindach(Infested)		Puch(Infested)		Neu-Eichenberg(Infested)		All soils	All infested soils
OTU	FDR *p*-value	Abund. (%)	FDR *p*-value	Abund. (%)	FDR *p*-value	Abund. (%)	FDR *p*-value	Abund. (%)	FDR *p*-value	FDR *p*-value
OTU2	0.207	5.01	0.179	23.28	**0.004**	12.11	0.060	11.83	0.221	**0.005**
OTU3	0.193	10.42	**0.011**	12.65	**< 0.001**	29.25	**0.026**	23.38	**0.018**	**0.001**
OTU9	0.265	1.33	0.566	0.05	**0.041**	0.25	0.228	0.14	0.776	0.765
OTU10	0.416	11.19	0.065	26.24	0.177	24.34	0.091	27.91	0.192	**0.003**
OTU25	0.170	31.63	0.330	2.35	**0.042**	0.76	0.182	8.14	0.793	0.423
OTU35	0.064	0.69	**0.034**	1.28	0.078	1.43	0.183	0.27	0.393	**0.034**
OTU36	0.564	0.02	0.291	0.5	**0.033**	0.87	0.887	0.12	0.893	0.647
OTU76	0.617	< 0.01	**0.029**	0.4	0.661	0.13	0.080	0.2	0.864	0.150
OTU82	0.072	0.87	**0.019**	0.37	0.575	< 0.01	0.488	< 0.01	0.306	0.401
OTU132	0.407	0.17	0.355	0.12	0.519	0.04	**0.031**	0.1	0.289	**0.047**
OTU188	NA	NA	NA	NA	0.570	< 0.01	**0.032**	0.09	0.503	0.361
OTU887	**0.031**	0.29	0.457	0.01	0.570	< 0.01	NA	NA	0.459	0.659
OTU1191	0.502	< 0.01	0.447	0.94	**0.049**	0.13	0.066	0.38	0.879	0.393

In bold are the p-values smaller than 0.05. Underlined are the OTUs with a significant genotype effect in at least 3 soils. *NA* OTUs that have not been detected or that have been filtered out due to a low mean relative abundance (< 0.01%), *FDR* false discovery rate correction of the *p*-values for multiple OTU testing

Table 2 Corrected *p*-values for Welch's *t*-test and log_2FC for the comparison of centered log-ratio transformed abundance in resistant (negative values, green colour) or susceptible (positive values, purple colour) genotypes

	Feldbach		Kirchlindach		Puch		Neu-Eichenberg		Infested soils		All soils	
OTU	**FDR *p*-value**	**log_2FC**	**FDR *p*-value**	**log_2FC**	**FDR *p*-value**	**log_2FC**	**FDR *p*-value**	**log_2FC**	**FDR *p*-value**	**log_2FC**	**FDR *p*-value**	**log_2FC**
OTU3	0.999	-2.535	**0.037**	-1.229	0.090	-1.105	0.512	-0.943	**<0.001**	-1.102	**0.008**	-1.015
OTU35	1.000	0.528	**0.026**	-1.952	0.144	-1.350	0.872	-1.639	**<0.001**	-1.653	0.332	-1.183
OTU93	0.978	2.610	0.515	2.748	1.000	0.411	0.769	2.543	**0.048**	1.878	0.057	2.115
OTU549	1.000	0.216	0.533	1.207	0.619	0.615	0.702	1.160	**0.008**	1.008	0.121	1.116

In bold the significant *p*-values. *FDR* false discovery rate correction of the p-values for multiple OTU testing

seed emergence, which aligns with previous observations of the associations of these OTUs. Others, like OTU549 or OTU93, exhibited a strong negative correlation with SDWRel or a positive correlation with RRI, respectively (Supplementary Table 7).

Taken together, OTU3 has repeatedly appeared in the results as a taxon associated with plant resistance to root rot. It has a higher abundance in the resistant genotypes across all soils and it is positively correlated with seed emergence. A closer inspection of its distribution across the soils revealed interesting patterns (Fig. 4), where a tendency to increase its abundance with the genotype resistance can be seen, reaching very high percentages of relative abundances in S134, the most resistant genotype based on the RRI across all soils.

Furthermore, the two most resistance-associated and heritable OTUs identified in a previous study (fOTU1508, fOTU1517, Gfeller et al. 2025) were mapped to OTU3 with 100% and 95.9% sequence similarity, respectively. The OTU3 sequence is assigned by NCBI nucleotide BLAST to the genera *Dactylonectria* sp. or *Ilyonectria* sp. (Supplementary Table 5).

Discussion

In this study, we identified differences in the mycobiota of roots from different pea genotypes grown in four soils with contrasting root rot pathogen levels as established in (Wille et al. 2021).This provides a

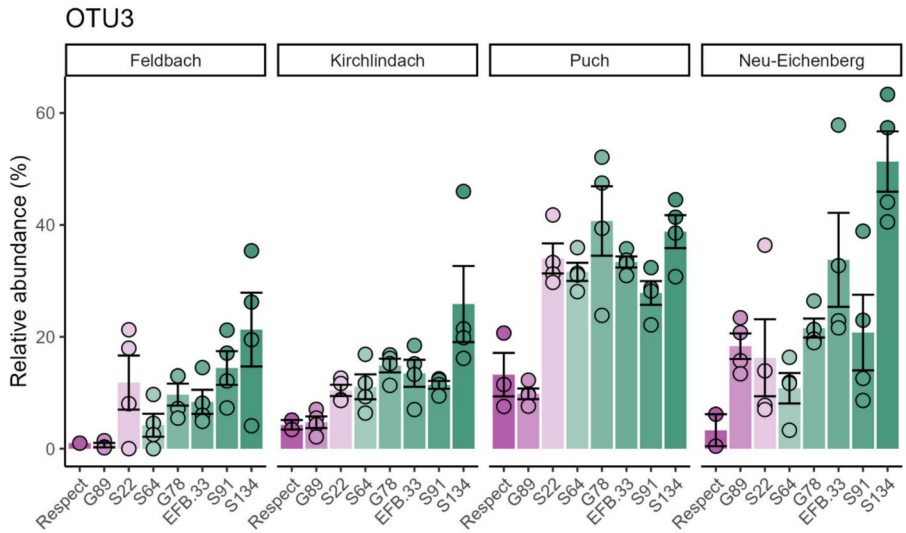


Fig. 4 Mean relative abundance (bars) and standard error bars of OTU3 across soils. The genotypes are ordered from the most susceptible (purple tones) to the most resistant (green tones) based on the mean RRI for each genotype across all soils. Each point represents the relative abundance of OTU3 in the roots from a single pot

frame to assess how genotypes, genotype resistance levels and soil infestation modulate the root-associated fungal taxa abundance and how these taxa associate with root rot resistance traits. We successfully identified a potential microbial marker for resistant genotypes that is highly abundant and enriched in roots from resistant genotypes and correlates positively with seed emergence.

Alpha diversity is affected by soil infestation level and pea genotypes

We first explored the richness of fungal communities to test if there were differences between the infested and uninfested soils and among genotypes. The richness of the root mycobiota was higher when grown in infested soils than in the uninfested one. This trend goes against the assumptions that higher diversity translates into less disease pressure (van Elsas et al. 2012). More recent studies have also shown that the presence of keystone taxa or microbial functions can be more important in maintaining soil and plant health than diversity measures such as richness alone (Banerjee et al. 2018, 2019; Qiu et al. 2024). Other studies have found that in cases of root rot, alpha diversity (richness and Shannon index) is higher in rhizospheres of diseased compared to healthy plants, most likely due to the creation of new habitats from the decomposing root tissues (Liu et al. 2024). In our study, more OTUs might have entered the diseased roots due to a breakdown of the physical and/or biochemical plant defence.

Next, we investigated the evenness of the fungal communities, which showed the opposite pattern as the richness: the uninfested soil was more even than the infested ones. This behaviour of the evenness is consistent with previous observations listed in Chaparro et al. (2012), where higher evenness is considered an indicator of healthy soils or systems, since there are no taxa dominating over the rest of the community. Similar to the richness, evenness alone does not necessarily explain the health of a system. While alpha diversity indices are simple and easy-to-evaluate measures to characterise microbial communities, they can fail to capture the complexity of the networks that ensure the viability of the system and might not reflect the functional diversity (Pan et al. 2021; Xun et al. 2021). While an effect of the pea genotype on alpha diversity was found, it was not related to the susceptibility of the genotypes, indicating that other species-specific traits are of importance to influence alpha diversity. In line with this finding, root rot-related traits did not show a consistent correlation with any of the alpha diversity indices. The only two significant correlations seemed to be driven by the presence of the uninfested soil.

Fungal communities are shaped by soil, genotype and root rot resistance

Analysing the community composition revealed that the uninfested soil is unique and distinct from the infested soils, as shown by the PCA. This observation was further confirmed by the high number of shared OTUs among the infested soils. Focusing on the three infested soils, a clear separation by soil was observed for the root mycobiota. In accordance with our results, previous research has identified the soil origin as a key driver of the root microbiome (Bell-Dereske et al. 2023; Yang et al. 2023), as well as the effect of the soil pathogen infestation level (Liang et al. 2024).

We also investigated the effects of susceptibility and RRI through a constrained analysis, which revealed that both factors influenced the community structure, albeit weakly. A greater effect of the plant resistance traits was observed when all the soils were considered, especially for RRI, whose R^2 values spanned from 9.8% with all the soils to 4% with only the infested ones. This once again highlights the significant impact of soil infestation levels on the analysis. In accordance with our results, Flemer et al. (2022) also reported an effect of RRI on the microbial community, showing that biotic stressors are capable of modifying the endosphere microbiome. Zhang et al. (2023) also demonstrated that the genotype and genotype susceptibility can modulate the rhizosphere composition. These findings suggest that the underlying relationships between plant, soil and microbial community are complex, and cannot be fully understood when studied from a single perspective (e.g. in a single soil). It is necessary to understand pathobiome dynamics and the resistance to pathogens across different environments to draw conclusions that are relevant for agricultural practices.

Identification of microbial markers for resistance through differential abundance analysis

To identify microbial markers for root rot resistance, we explored associations between OTU abundances and soils, pea genotypes and genotype susceptibility. This revealed several OTUs, such as OTU25, that are associated with the uninfested soil. Interestingly, although OTU25 may have a beneficial effect, it was assigned to the *Fusarium* genus, a group that includes several pathogens, but also contains fungi with reported beneficial roles (Iida et al. 2022). The most striking finding was OTU3, which was differentially abundant between genotypes in infested soils and was consistently associated with resistant genotypes. Moreover, OTU3 showed a strong correlation with seed emergence across all soils and the three infested soils. Notably, it showed 100% sequence identity with a highly heritable hub OTU associated with root rot resistance that was previously identified in Gfeller et al. (2025), where it was likewise strongly correlated with seed emergence. The finding that this taxon is associated with root rot resistance in two different studies and three different soils provides evidence that OTU3 might act as a microbial marker for root rot resistance. In a separate study, genotype S134, which exhibited the highest abundance of OTU3 in the roots and the highest resistance to root rot based on the RRI in our analysis, was also identified as the most resistant genotype under agriculturally relevant field conditions across three consecutive years (Vonzun et al. 2024). It should be noted that bacteria and oomycetes were not assessed in this study; however, previous work suggests that, in the soils tested here, fungi are most strongly associated with root rot and resistance to root rot (Gfeller et al. 2025; Wille et al. 2021).

Our study not only revealed potential microbial markers for resistance, like OTU3, but also other taxa that are associated with the susceptible genotypes. OTU93, for example, is positively correlated with RRI and is assigned to the family Orbiliaceae, which includes some nematode-trapping fungi and might have thrived on nematodes that entered the diseased roots (Zhang et al. 2020). Another taxon that was associated with the susceptible genotypes was OTU549, which was negatively associated with SDW_{Rel}, a plant trait indicative of tolerance against soil pathogens, and was assigned to the genus *Leohumicola,* which has been found in high abundance in diseased peas before (Xu et al. 2012).

Taxonomic assignment of OTU3 revealed that it belongs to the genera *Dactylonectria* or *Ilyonectria*. These genera, together with other closely related species collectively referred to as 'Cylindrocarpon-like asexual morphs,' have been implicated in numerous cases of black foot disease in grapevine worldwide, occurring in both symptomatic and asymptomatic plants (Aigoun-Mouhous et al. 2019; Lawrence et al. 2019; Berlanas et al. 2020; Carbone et al. 2023). They have also been reported as the causal agent of black root rot in strawberry (Chen et al. 2021) and in blackberry (Sánchez et al. 2019). Coincidentally, an *Ilyonectria* strain has also been isolated from infected pea plants used as a cover crop (León et al. 2021). In contrast to all the studies mentioned above, this current work is the first to report an association between *Dactylonectria* or *Ilyonectria* and root rot resistance. It remains to be evaluated whether OTU3 is merely a marker or an active agent. Possible mechanisms involved in the biocontrol activity of OTU3 include niche competition, direct antagonism of pathogens, pathogen spore germination inhibition or plant-mediated effects (Köhl et al. 2019; Narayanasamy 2013; Nassary 2025). The isolation of OTU3 from root material will enable the precise identification of the taxa, allowing inoculation experiments to determine the nature of the association with the resistant genotypes and shed light on the underlying mechanism.

Our findings highlight OTU3 as a robust and conserved microbial marker for pea root resistance. Previous studies relied on the preselection of markers from the literature and their targeted quantification, such as Wille et al. (2021). In contrast, we analyzed the entire root-associated fungal community of peas grown in four different soils. This community-wide approach revealed that the literature-based markers could not be validated across different soils, highlighting the importance of taking the entire microbiome into account. We provide evidence that the newly detected microbial marker, OTU3, is consistently detectable and valid across diverse soils and in independent controlled conditions studies (Gfeller et al. 2025) Integrating the detection of this taxon into pea breeding strategies could facilitate the identification of novel resistant or partially resistant lines, analogous to the use of classical genetic markers. This complementary approach has the potential to advance

breeding for resistance to soilborne pathogens and, in the long term, further promote legume cultivation.

Acknowledgements We thank the Genetic Diversity Centre (GDC) at ETH Zurich for their contribution to the processing of the raw sequencing reads.

Author contributions Monika M. Messmer, Pierre Hohmann, and Bruno Studer designed the research. Lukas Wille and Mario Kurmann performed research. Ignacio Romero-Lozano analysed data with input from Natacha Bodenhausen and Valentin Gfeller. Ignacio Romero-Lozano wrote the first draft of the manuscripts with input from Valentin Gfeller. All coauthors contributed to the internal revision process of the manuscript. All authors approved the final version of the paper.

Funding Open access funding provided by Research Institute of Organic Agriculture. This work was funded by the Root2Res project (EU Horizon Europe no. 101060124 and Swiss State Secretariat for Education, Research and Innovation (SERI) no 23.00050), the LIVESEED project (EU Horizon 2020 no. 727230 and SERI no. 17.0009) and MICIU/AEI/10.13039/501100011033 and FSE + under grant reference no RYC2022-037997-I. The opinions expressed and arguments employed herein do not necessarily reflect the official views of the EC and the Swiss government. Neither the European Commission/SERI nor any person acting on behalf of the Commission/SERI is responsible for the use which might be made of the information provided on this publication.

Data availability The raw sequencing datasets are available in the European Nucleotide Archive (ENA, http://www.ebi.ac.uk/ena) at EMBL-EBI under the study accession PRJEB97121. R code and data to reproduce the statistical analysis and visualizations are available under https://github.com/FiBL/Pea_Microbiome_Analysis.git.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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