From plant genetics to environment of selection: exploring the drivers of fungal communities in the roots and rhizosphere of Brassica napus

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Research Article

Keywords: canola microbiome, Olpidium brassicae, soil fungi, Brassica napus genotype

Posted Date: February 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1328653/v1

Abstract

Purpose

Our aim was to characterize the fungal root and rhizosphere microbiomes of genetically diverse *Brassica napus* lines in a temporally-intensive, multi-site field study to assess the relative contributions of plant genetics, growth stage and environmental conditions to microbiome composition and to identify fungi that were associated with yield performance.

Methods

Sixteen *B. napus* lines were grown across three sites in the Canadian Prairies. Sixteen lines were sampled weekly for ten weeks at one site in 2016, as well as a subset of eight lines at the same site in 2017; the sixteen lines were sampled three times at three sites in 2017. The root and rhizosphere fungal microbiomes were assessed using amplicon sequencing of the fungal ITS region with the Illumina MiSeq.

Results

Overall, *B. napus* line was associated with only 2% of the variation in community structure. Constrained to within a single site-year, this association increased to between 4 and 16% and to between 25 and 37% in a single week within individual sites. The fungal core microbiome consisted of 38 ASVs; *Olpidium*, *Fusicola*, *Fusarium, Gibberella*, *Mortierella*, and *Cutaneotrichosporon* were the most abundant taxa with varied abundance during different *B. napus* growth stages. Thirteen ASVs across three growth stages were highly associated with *B. napus* yield.

Conclusion

Our results point to the potential to exploit the *B. napus* microbiome for improving plant performance by targeting the core taxa identified here as well as those that lead to greater fitness under more specific conditions.

Introduction

Plant roots are surrounded by a diversity of microorganisms in the soil. Some soil microbes are plant pathogens and cause detrimental diseases in their hosts, whereas others are beneficial and not only support plant fitness but also provide ecosystem services, such as *Pseudomonas*, *Bacillus* bacteria and arbuscular mycorrhizal fungi (Padje et al. 2016; Wei et al. 2019). Appreciation of the microbiome in plant essential functions, such as nutrient acquisition and modulation of the immune system has encouraged efforts to harness the potential benefits of microbial diversity through plant breeding and beneficial management practices. Optimizing the selection of microbial communities holds promises for improved plant productivity and more sustainable crop production (Frison et al. 2011; Busby et al. 2017) but to date, the microbiome has not been directly considered in crop breeding programs.

Soil is the primary origin of the microorganisms that colonize roots (Peterson 1959; Pérez-Artés et al. 2005; Bainard et al. 2016). Microbial diversity in the root is widely accepted to have a composition largely derived from a subset of the surrounding rhizosphere soil, indicating that plants have the ability to attract or filter the microbes inhabiting the rhizosphere (Edwards et al. 2015; Van Der Heijden and Schlaeppi 2015; Naylor et al. 2017; Yamamoto et al. 2018). The root microbiome may also be a distinct assemblage rather than a subset of the rhizosphere, whereby some of its members can be transferred between generations through the seeds or aerial organs (Gottel et al. 2011; Lundberg et al. 2012). Plant species and genotype also influence the microbiome in different root compartments (Schweitzer et al. 2008; Bressan et al. 2009; Zancarini et al. 2012; Bazghaleh et al. 2015; Bulgarelli et al. 2015; Wagner et al. 2016; Mina et al. 2020). Plants select microbes mainly through exudation of a variety of line-specific metabolites followed by signaling events that attract or repel specific species (Broeckling et al. 2008; Hu et al. 2018; Sasse et al. 2018). The variation in microbiome composition has been correlated to genetic distance among host species and genotypes (Yeoh et al. 2017; Naylor et al. 2017; Taye et al. 2020). The biochemical profile of plant roots as well as the secretion pattern and composition of root metabolites are highly dependent on physiological stage or plant age (Lucas García et al. 2001; Chaparro et al. 2013; Zhalnina et al. 2018). Soil environmental characteristics including temperature, moisture, and nutrient fluxes also influence the root and rhizosphere microbial composition (Lau and Lennon 2012; Xu et al. 2018; Bardgett and Caruso 2020; Deltedesco et al. 2020) and may exceed the effect of plant physiological stage on the temporal microbiome assemblages (Manici et al. 2017).

Brassica napus is one of the most valuable oilseed crops worldwide. In addition to human consumption, canola and rapeseed are cultivated varieties of *B. napus* and their oil has many non-edible uses in industry such as biofuel and lubricants (Shahidi 1990; Dizge et al. 2009). Spring canola is likewise a widely grown crop in the Canadian prairies contributing about $26 billion CAD to the economy annually (Canola council of Canada 2019). Genotypic differences in the root morphology and architecture have been observed in *B. napus* lines (Würschum et al. 2012; Kiran et al. 2019), which suggests potential for genetic improvement of optimized rooting systems (Arifuzzaman et al. 2019).

The plant fungal microbiome comprises a diversity of species that play key roles in ecological processes, and influence plant performance (Turner et al. 2013; Bazghaleh et al. 2015). Despite its agricultural and ecological importance, the factors driving assembly and diversity of the fungal microbiome associated with the roots and rhizosphere of *B. napus* L., and its potential roles in maintaining plant fitness have remained poorly explored. Recent studies showed that the microbiome of the roots and rhizosphere of canola were consistently different from those of other crop plants (Lay et al. 2018a; Floc'h et al. 2020). However, the role of plant genetic controls, growth stage and environmental variation in shaping the composition of this microbiome have not been compared.

Here, we evaluated the structure and composition of the root and rhizosphere fungal microbiome of sixteen genetically diverse *B. napus* canola lines. A temporally-intensive survey was performed weekly for ten weeks in 2016 to determine the degree of change in the fungal microbiome during the growing season. In 2017, we repeated this work on a subset of eight lines for a multi-year comparison of highly resolved seasonal changes. We expanded the study in

a second year to include three time points with the same 16 lines grown in multiple locations with different soil and climatic factors representative of important canola producing regions in the Canadian prairies. We hypothesized that *B. napus* genetic factors determine the diversity and composition of fungal communities in the root and rhizosphere of *B. napus*. We also hypothesized the composition of the belowground microbiomes are shaped by growth stage and environmental variations related to site-to-site differences, and some specific taxa are related to *B. napus* yield.

Materials And Methods

Experimental design and data collection

Sixteen diverse lines of *B. napus* that represented diverse phenotypes and genotypes were grown at Saskatchewan, Canada in 2016 and 2017 (Table S1). Full details on the data can be found in a previously published data paper (Bazghaleh et al. 2020). Briefly, in 2016, sixteen lines were grown at the Agriculture and Agri-Food Canada (AAFC) Llewellyn Research Farm. In 2017, the same lines were again grown at Llewellyn, as well as at Scott and Melfort (Table S2). Each line was grown in randomized blocks and replicated three times. In 2016, root and rhizosphere samples were collected weekly from the Llewellyn location for 10 consecutive weeks (herein referred to as weeks 1 through 10), starting 3 weeks after sowing. This temporally-intensive sampling was repeated on a subset of eight lines in 2017 to compare between-year differences in environmental conditions (i.e., weather). In 2017, all sixteen lines were sampled at all three locations in weeks 3, 6, and 9 (equivalent to 6, 9 and 12 weeks after sowing) to compare between-location differences (i.e., soil and weather conditions). The three sampled weeks are three typical development stages including leaf development, flowering, and filling stages. Three *B. napus* plants were collected to a 10-cm depth and combined to a single composite sample from each plot. A total of 2,160 root and rhizosphere samples were collected, and DNA extracted from all root and rhizosphere soil samples. Soil adhering to roots after shaking by hand was considered rhizosphere soil. Roots with tightly adhering soil were shaken at 180 rpm for 15 minutes in 0.05M NaCl buffer. After shaking, a subsample of root tissue was rinsed and stored at -80°C for DNA extraction. Buffer-soil mixtures were centrifuged at 5000 rpm for 15 minutes and a subsample of rhizosphere soil was stored at -80°C for DNA extraction.

DNA extraction and library preparation

DNA was extracted from 250 mg of rhizosphere soil using Qiagen PowerSoil extraction kit, and from 50 mg root tissue using Qiagen PowerPlant extraction kit (Hilden, Germany) following manufacturer instructions. DNA quantity was determined following the standard Qubit protocol (Thermo Fisher Scientific, Waltham Massachusetts). DNA from soil and roots was standardized to 5 ng/µL and 1.5 ng/µL, respectively.

DNA was amplified using the ITS1F_KYO1 (CTHGGTCATTTAGAGGAASTAA) / ITS2_KYO2 (TTYRCTRCGTTCTTCATC) primer set (Toju et al. 2018), barcoded using Nextera XT indexes, pooled (384 samples), and then sequenced using the Illumina MiSeq platform using Reagent Kit v2 (500-cycles).

Bioinformatics

In total, over 113 M raw sequence reads were produced and processed using QIIME2 v. 2019.7 (Bolyen et al. 2019). The adaptors and primers of the sequencing reads were trimmed off using Cutadapt version 3.1 (Martin 2011), then the reads were denoised using DADA2 (Callahan et al. 2016) with truncation at 180 bp for forward and 120 bp for reverse reads (Suppl. Method S1). 16,030 unique amplicon sequence variants (ASVs) with an average length of 242 bp were generated with an average of 44 unique ASVs per root and 113 unique ASVs per rhizosphere sample. Sequences were classified using the UNITE database v. 8.0 at 99% sequence identity and identified by best available matching sequences in the database (UNITE Community 2019). The data were analyzed using R v. 3.5.3 (R Core Team 2021) in RStudio (RStudio Team 2019). The feature table and its associated taxonomy and metadata were imported into a phyloseq object using phyloseq v. 1.26.1 (McMurdie and Holmes 2013). Duplicate samples used for checking sequencing quality were removed in the downstream analyses.

Statistical analyses

Before beta-diversity analysis of the fungal community, samples with low reads and rare ASVs were removed from the phyloseq object, and the ASV abundance data were transformed. Specifically, samples with < 2000 reads and ASVs with prevalence lower than 5% across all samples were removed, leading to a removal of 3% of samples and retention of 293 ASVs. Zero abundances were replaced using a Bayes-Laplace approach using the zCompositions v. 1.2.0 (Palarea-Albaladejo and Martín-Fernández 2015) and then transformed to centered log-ratios (CLR) using the CoDaSeq v. 0.99.3 (Gloor and Reid 2016; Gloor et al. 2017). Principal component analysis (PCA) was used to explore the composition of the fungal communities based on a Euclidean distance matrix. Permutational analysis of variance (PERMANOVA) and Shannon index (even depth of 2500 reads) were used to look at compositional differences and diversity of fungal microbiome using vegan v. 2.5-6 (Oksanen et al. 2020). A subset of the ASVs observed in all lines and site-years were identified as core ASVs for constructing phylogenetic tree. The taxonomy of the core ASVs were reconfirmed by Mycobank (https://www.mycobank.org) and National Center for Biotechnology Information (NCBI) databases (https://www.ncbi.nlm.nih.gov/taxonomy). To determine the correlation of the microbial distances and the plant genetic distances among the sixteen *B. napus* lines, the samples (reads > 2000) were grouped according to niches, i.e., root and rhizosphere. To reduce the impact of the rare ASVs on the microbial distances, the ASVs with prevalence higher than 10% across the samples in each group (i.e., root and rhizosphere) were selected, then the mean abundance of each ASV in each *B. napus* line was calculated. Mean abundance data were CLR-transformed and then used for generating the microbial distance (Euclidean) of each pairwise *B. napus* lines. Taye et al. (Taye et al. 2020) calculated the genetic similarity matrix of the 16 *B. napus* lines based on the single nucleotide polymorphisms (SNPs) determined using the Brassica 60K Illumina Infinium SNP array. The correlation of the plant genetic distances and the microbial distances among *B. napus* lines was determined using Pearson's correlation coefficient. Considering the compositional nature of microbiome data, 'selbal' algorithm in the R package *selbal* v.0.1.0 (Rivera-Pinto et al.) was applied to predict which taxa have a close association with *B. napus* yield. Specifically, microbiome data from three growth stages (i.e., weeks3, 6, and 9) were selected, then we filtered the taxa under the assumption that the taxa highly associated with yield of one *B. napus* line should at least present in ≥ 50% of the biological replicates for that line under a given growth stage. In each of the site-years, the average reads of each of the filtered-taxa in the three replicates of each *B. napus* line in weeks 3, 6, and 9 were calculated separately. The three individually replicated plot yields (kg ha^{-1}) for each *B. napus* line were averaged in each site-year, and detailed

information can be found in Mamet et al. (2021). The taxa mostly associated with *B. napus* yield were identified using *selbal.cv()* function in the *selbal* package.

Results

Composition and diversity of the root and rhizosphere fungal microbiomes

Differences in the composition of the fungal root and rhizosphere microbiomes of *B. napus* were substantial (Figs. 1, S1a). In the root, *Olpidium* were the most dominant taxa at Llewellyn (accounting for 87% and 64% average relative abundance in 2016 and 2017, respectively) and Melfort (91%), but the dominance of *Olpidium* was reduced at Scott (29%). In the rhizosphere, the relative abundance of *Olpidium* was much lower than that in the root and *Fusarium*, *Fusicolla*, and *Mortierella* were the dominant genera across site-years. A single ASV representing *O. brassicae* accounted for 98% of the abundance of phylum Olpidiomycota, resulting in lower diversity of root fungi at Llewellyn (2016 and 2017) and Melfort 2017 (Figs. 1 and S2a).

Environmental factors (i.e., site-year) and growth stage significantly affected the composition of fungal microbiome (Figs. 1, 2, and S1b, table S3-S4). Site-year associated with 15% and 16% of the variance of fungal microbiomes in the root and rhizosphere, respectively, whereas growth stage associated with 5% and 8%. Alpha diversity was lowest at mid-season (week 6) due to dominance of a few taxa (Fig. S2b). The temporal patterns of the fungal taxa relative abundance varied in the root and rhizosphere across site-years (Fig. 2). For example, in Llewellyn 2016, the relative abundance of *Olpidium* in the rhizosphere continuously increased from week 1 to 7 and correspondingly, while the relative abundance of *Mortierella, Gibberella*, and unclassified taxa declined. At the same time, the root fungal community was almost entirely dominated by *Olpidium*. In Llewellyn 2017, *Fusarium, Fusicola*, and *Mortierella* were more abundant in the rhizosphere with peak abundance at mid-season, whereas their relative abundances were smaller in the root and fluctuated weekly with *Olpidium* across all the growth stages. In Melfort 2017, the dynamics of the root fungal community was very similar to Llewellyn 2016 as it was dominated by *Olpidium*. In the rhizosphere, *Olpidium* and *Mortierella* were abundant in week 3 but were later reduced, and *Fusarium* and *Cutaneotrichosporon* increased in weeks 6 and 9, respectively. In Scott 2017, *Mortierella* was most abundant in the rhizosphere in week 3 but gradually diminished in weeks 6 and 9, while correspondingly the abundance of *Cutaneotrichosporon* increased. In the root community, the abundance of *Olpidium* increased from week 3 to 9, which corresponded with the reduction of the abundances of *Mortierella, Fusicolla*, and *Fusarium*.

In comparison with niches (i.e., root and rhizosphere), environmental factors and growth stage, the impact of *B. napus* line on the fungal microbiome was much smaller, but still significant (p = 0.001). It associated with 2% of the variance of the fungal microbiomes in the root and rhizosphere (Table S3-S4). However, when environmental conditions were constrained (i.e., in a specific site-year), the effect of *B. napus* line on the fungal microbiome became more obvious. For example, *B. napus* line associated with 4 – 16% of the variance of the fungal microbiome in the root and 5 -13% in the rhizosphere based on each of the site-years (Tables S5-S6), while it was 25 – 41% in the root and 26 – 37% in the rhizosphere at a given sampled week (Table 1). By comparison, the rhizosphere communities were more dispersed among all lines, while the root fungal communities among lines grouped into two clusters (Fig. S1c-d). Many of the lines that had similar root communities had comparatively dissimilar rhizosphere communities (e.g. NAM 13 and NAM 43).

Table 1
Variation of fungal community explained by *Brassica napus* lines in the root and rhizosphere fungal communities at different growth stages in each site-year determined through PERMANOVA. Significant differences are bolded.

Site-year	Growth stage (week)	Variation % in root (*p*-value)	Variation % in rhizosphere (*p*-value)
Llewellyn 2016	1	33.0% (0.088)	32.2% (0.088)
	2	31.6% (0.323)	29.9% (0.138)
	3	34.4% (0.840)	31.3% (0.166)
	4	31.0% (0.567)	30.3% (0.282)
	5	31.9% (0.49)	31.2% (0.620)
	6	31.5% (0.169)	31.4% (0.076)
	7	29.6% (0.53)	30.5% (0.093)
	8	31.8% (0.076)	31.7% (0.069)
	9	31.6% (0.310)	32.7% (0.212)
	10	34.3% (0.167)	33.9% (0.159)
Llewellyn 2017	1	29.3% (0.068)	30.1% (0.096)
	2	25.4% (0.754)	26.3% (0.237)
	3	30.4% (0.125)	30.6% (0.275)
	4	26.0% (0.809)	27.4% (0.827)
	5	26.3% (0.561)	30.9% (0.140)
	6	31.2% (0.106)	**34.5% (0.020)**
	7	28.5% (0.210)	29.2% (0.244)
	8	27.3% (0.182)	27% (0.562)
	9	**32.0% (0.004)**	**31.6% (0.018)**
	10	28.0% (0.095)	**30.1% (0.046)**
Melfort 2017	3	41.3% (0.157)	34.9% (0.287)
	6	**40.1% (0.014)**	34.6% (0.053)
	9	**40.6% (0.033)**	**36.7% (0.001)**
Scott 2017	3	32.5% (0.914)	34.3% (0.272)
	6	32.5% (0.584)	34.0% (0.628)
	9	34.9% (0.657)	33.5% (0.277)

Belowground core fungal microbiome in B. napus

Although the root-associated fungal microbiome is highly dynamic in response to *B. napus* development stage and environmental conditions, some common taxa (a core microbiome) were often associated with the root and/or rhizosphere across all growth stages (i.e., each sampled week), which might be critical to fungal microbiome structure and plant growth. So, we identified the core fungal microbiome of the *B. napus* lines across site-years. Thirty-eight fungal ASVs were shared in all lines and site-years (Fig. 3), but only a few of these ASVs were detected at all sampled weeks. Specifically, seven ASVs occurred in the rhizosphere during all weeks but only a single ASV, *O. brassicae*, was consistently present in the root (Fig. 3). The relative abundance of the taxa occurring at least one sampled week in all lines and site-years varied among the lines (Fig. S3). In addition, the number of the common fungal taxa within an individual line across all site-years ranged between 33 and 69 in the different *B. napus* lines (Fig. S4).

Correlation between B. napus genetic distance and fungal microbiome dissimilarity

To determine whether the fungal community composition is aligned with the SNP-based genetic distances of the *B. napus* lines, their Pearson's correlation coefficient was determined. The linear correlation between the two distances was weak in the root (R = -0.075; p = 0.420) and rhizosphere (R = -0.039; p = 0.670) across all site-years (Figs. 4a and b), as well as in each site-year except for the root (R = -0.330; $p < 0.001$) in Scott 2017 (Fig. S5a-h). Considering the dominant effect *Olpidium* on fungal microbial community dissimilarity in the root, we re-analyzed the correlation between the genetic distances and the root fungal community distances of the *B. napus* lines after the *Olpidium* removal. The correlation between the two distances was still weak in the root (R = -0.022; p = 0.830) across all site-years, but the correlation was greater at Llewellyn 2017 (R = 0.300; p = 0.0016) (Fig. S6a-d).

Fungal taxa mostly associated B. napus yield

Three, five, and four taxa mostly associated with *B. napus* yield were identified in the root in weeks 3, 6, and 9, respectively, whereas two taxa in each of the three growth stages were identified in the rhizosphere. In the root, the yield-associated taxa ASV13935 (class: Leotimycetes) was common in weeks 3 and 6, and ASV10975 (class: Sordariomycetes) was common in weeks 6 and 9. In the rhizosphere, ASV10975 was also common in weeks 6 and 9. Two taxa (i.e., ASV10975 and ASV15009) in week 9 were common in the root and rhizosphere (Table 2). The prevalence of the 13 mostly yield-associated taxa were 38.9% Sordariomycetes, 22.2% Tremellomycetes, 11.1% Dothideomycetes, 11.1% Eurotiomycetes, and 11.1% Leotiomycetes. The regression models of the balances of two groups of taxa (i.e., numerator and denominator) respectively in the root and rhizosphere in weeks 3, 6, and 9 and *B. napus* yield explained 63–72% of yield variation (Fig. S7a and b).

Table 2
Taxa highly associated with *Brassica napus* yield and identified in the root and rhizosphere at three growth stages. Amplicon sequence variants (ASVs) in N correlated with *B. napus* yield, whereas ASVs in DEN group was negatively correlated with *B. napus* yield. The ASVs in common across growth stages and

Niche	Growth stage	ASV	Group	Phylum	Class	Order	Family	Genus	Sp
Root	Week 03	**ASV13935**	[b]NUM	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Tetracladium	Tet
		ASV15000	[c]DEN	Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Filobasidium	Un
		ASV7026	DEN	Ascomycota	Eurotiomycetes	Chaetothyriales	Trichomeriaceae	Knufia	Kn
	Week 06	**ASV10975**	NUM	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Nectria	Ne
		ASV13935	NUM	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Tetracladium	Tet
		ASV5451	NUM	Ascomycota	Sordariomycetes	Hypocreales	Unclassified	Unclassified	Un
		ASV10074	DEN	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Exophiala	Ex
		ASV13038	DEN	Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	Vis
	Week 09	**ASV10975**	NUM	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Nectria	Ne
		ASV7952	NUM	Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	Mycosphaerella	My
		ASV11181	DEN	Basidiomycota	Tremellomycetes	Holtermanniales	[d]Holtermanniales_f.l.s	Holtermanniella	Ho
		ASV15009	DEN	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Gibberella	Gib
[a]Rhizo	Week 03	ASV11500	NUM	Ascomycota	Dothideomycetes	Pleosporales	Leptosphaeriaceae	Leptosphaeria	Lep
		ASV11048	DEN	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Cystofilobasidiaceae	Cystofilobasidium	Cys
	Week 06	**ASV10975**	NUM	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Nectria	Ne
		ASV3413	DEN	Unclassified	Unclassified	Unclassified	Unclassified	Unclassified	Un
	Week 09	**ASV10975**	NUM	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Nectria	Ne
		ASV15009	DEN	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Gibberella	Gib

[a]Rhizo: Rhizosphere; [b]NUM: numerator; [c]DEN: denominator; [d]Holtermanniales_f.l.s: Holtermanniales_fam_Incertae_sedis

Discussion

In this study, we (a) simultaneously investigated the effects of host plant genotype and seasonal changes at multiple locations on the diversity and composition of fungal microbiome in the root and rhizosphere of *B. napus*; (b) explored the core fungal microbiome of the *B. napus* lines; (c) determined the correlation between *B. napus* genetic distances and fungal community distances in the root and rhizosphere; and (d) extracted the fungal taxa highly associated with *B. napus* yield across site-years at three plant developmental stages.

Dynamics of the fungal microbiome

Here, we showed that the composition of root and rhizosphere fungal microbiomes were shaped by *B. napus* line, field site, year to year differences, and growth stage ($p < 0.001$). This provides strong support for our hypotheses that both plant genetics and environment play significant and interacting roles in shaping the *B. napus* fungal root and rhizosphere microbiomes.

A recent study showed that the composition of bacterial communities in the rhizosphere also varies among the same sixteen *B. napus* lines (Taye et al. 2020), which together with our results suggest the potential for breeder-directed selection of genotype-specific root-associated microbial communities. Similar to our results, other studies reported small but significant effects of host plant lines on the root microbiome across different environments. For instance, one study suggested that the effect of plant genotype on microbiome in various plant tissue habitats is only two and three percent, while the habitat plays a bigger role and shape the composition of plant microbiome by 30 and 24 percent for bacterial and fungal communities, respectively (Cregger et al. 2018). However, our temporally-intensive interrogation showed that *B. napus* line had a larger effect when considered within the context of a single sampled week. A previous report has shown that the plant microbiome is dynamic and only by tracing it from origin to senescence, can we understand the factors that determine the

initial assembly of microbial community (Kristin and Miranda 2013). Variable genotype-by-environment interactions suggest that breeding for plant traits that consistently establish a beneficial microbiome may need to be adapted for dominant environmental conditions (Wagner et al. 2016; Morella et al. 2019).

We found the effect of the plant on the rhizosphere microbiome depended on location and site-year. For example, we detected an increase in the abundance of *O. brassicae* from week 1 to 8 in the rhizosphere in Llewellyn 2016, while at the same site in 2017 fluctuations in the abundance of major genera were observed throughout the growing season. In the root, *Olpidium* dominance was observed in two site-years (i.e., Llewellyn 2016 and Melfort 2017). Although the root community was dominated by *O. brassicae* at Llewellyn in both 2016 and 2017, increases in the abundance of *Fusicola* and *Fusarium* in 2017 support the hypothesis that seasonal changes in environmental conditions can significantly affect the root microbiome composition.

The biggest difference in the fungal community was detected between Scott and all other locations, highlighted by the greatest alpha diversity at Scott as well as greater similarity between root and rhizosphere communities. Our results agree with other studies showing that in agricultural ecosystems, the effect of soil type on the composition of rhizosphere microbial communities is usually stronger than the effect of plant genotype (Philippot et al. 2013). Plants and their microbiomes are affected by environmental variables, including temperature, moisture level, and soil pH which directly or indirectly shape the composition and diversity of the microbiomes. While these environmentally induced effects can cause direct microbial responses, they can indirectly cause plant responses leading to changes in its microbiome composition (Carvalhais et al. 2013). At Scott, soil pH was 5.5, significantly lower than at Melfort (pH 6.5) and Llewellyn (pH 7) (Table S2). Soil pH is a key determinant for fungal community composition (Rousk et al. 2010; Chen et al. 2014) therefore, a lower soil pH may have impeded the ability of *O. brassicae* to dominate root and rhizosphere communities or it created a more conducive environment for the growth of fungi including diverse species. Rousk et al. (2009) reported a fivefold increase in fungal growth with lower soil pH, but lower pH (< 6) can reduce the production of zoospores and infection of the root by *Olpidium* species (Iwamoto et al. 2017). Another contributing factor to this large difference in the microbiome composition could be the later planting date at Scott as was re-planted due to hail.

When plants move into their reproductive phase, they allocate less carbon and other resources to roots (Peiffer et al. 2013). We detected the lowest alpha diversity in week 6 (flowering stage), highlighting the increasing dominance of *O. brassicae* from the early growth stage to flowering stage, and becoming less dominant toward the end of growth stage. It is notable that distinguishing between the co-occurring effects of growth stage and environmental variables such as precipitation and temperature on the root microbiome is difficult and likely requires multiple years of field testing.

Fungal core microbiome

We intensively assessed the fungal microbiome in diverse *B. napus* lines across different growth stages in the four different field conditions. We detected a relatively large number of fungal ASVs in our dataset, however only a small fraction of them were ubiquitously shared among the *B. napus* lines and site-years. The core fungal microbiome of *B. napus* had two major components: 1) a group of 38 taxa that were detected in all lines and in all site-years and, 2) a subgroup of 8 of these ASVs that were found in the rhizosphere (7 ASVs) or root (1 ASV) in all sampled weeks.

Within the core microbiome of *B. napus*, the subgroup of 8 ASVs that were detected across all the sampled weeks represent functionally diverse groups of fungi. *Olpidium brassicae* is an obligate root-infecting pathogen that, in other *Brassicae* species, also serves as a vector for a wide range of plant disease-causing viruses (Hartwright et al. 2010). However, *B. napus* plants are normally asymptomatic or growth mildly reduced under *O. brassicae* infection. Because the strains of *O. brassicae* specific to *B. napus* plant are not the vectors of some viruses which caused serious diseases (Lay et al. 2018b). *Olpidium brassicae* are present in the root and rhizosphere of *B. napus* plant, and it is often the most dominant species in the root of *B. napus* plant, especially in monoculture systems (Hilton et al. 2013). Four of the seven fungal ASVs identified in the rhizosphere at all sampled weeks were reported as plant pathogens. Although *Fusarium hostae* were commonly associated with *B. napus* plant, it cannot cause serious *B. napus* disease, like fusarium wilt caused by *Fusarium oxysporum* (Geiser et al. 2001; Younesi et al. 2021). *Fusarium hostae* was reported to causes *Fusarium* root and crown rots in wheat (Gebremariam et al. 2016). *Gibberella* is the teleomorph of *Fusarium* detected among core ASVs of *B. napus*, and *G. baccata* can cause cankers and blights on a wide range of plants (Desjardins 2003). *Alternaria alternata* can cause black plot and/or stem canker in many plants, such as pear, strawberry, tomato and tobacco (Tsuge et al. 2013), and it is also the pathogen of alternaria leaf spot of canola (Al-Lami et al. 2019). *Cylindrocaprpon* sp. was reported to be the pathogen for blackfoot disease of grapevines (Petit and Gubler 2005). Although these pathogens infected *B. napus* root during the entire growth stage, the plant did not show obvious symptoms. One reason might be that *B. napus* plant is not the ideal host, restricting the infection by these pathogens. The other possibility is the antagonism between some endophyte fungal species and these pathogens (Heydari and Pessarakli 2010). The other core ASVs which appeared in all lines across the site-years, but not at each sampled week may be keystone taxa that regulate the fungal microbiome structure in a temporal way to respond the change of *B. napus* plant growth (Banerjee et al. 2018).

Correlation of B. napus phylogeny and fungal community dissimilarity

Plant and root-associated microbiomes have established the relationship of mutual selection and adaptation during long evolutionary history. Plant phylogenetic distance may be correlated with the assembly dissimilarity of root-associated microbiomes (Bouffaud et al. 2014; Naylor et al. 2017; Fitzpatrick et al. 2018; Wang and Sugiyama 2020). However, the sensitivity to plant phylogenetic distance varied between root-associated bacterial and fungal microbiomes. Specifically, the root-associated bacterial microbiome usually is more responsive than the fungal microbiome to plant phylogenetic distance or plant species (Bonito et al. 2014; Wang and Sugiyama 2020; Li et al. 2021), and root microbiome is more responsive than the rhizosphere (Naylor et al. 2017; Fitzpatrick et al. 2018). Here, rhizosphere fungal community dissimilarity had no correlation with *B. napus* genetic distance based on SNPs, and the root fungal community dissimilarity correlated with *B. napus* genetic distance in some site-years. In contrast, the bacterial community dissimilarity was significantly and positively correlated with *B. napus* genetic distance (Taye et al. 2020). Consistent with previous studies, our work indicates that the influence of plant phylogenetic distance on the root-associated fungal community is not as strong as it is for bacterial community, especially for intraspecies genetic variation.

Relationship of the specific fungal taxa and B. napus yield

Fungal taxa highly associated with *B. napus* yield were largely different among three growth stages (i.e., weeks 3, 6, and 9), indicating the temporal change of the relative abundance of the recruited taxa by the plant during its growth and development. The association of bacterial taxa or community composition with *B. napus* traits (i.e., yield and root) from the same field experiment was also impacted by growth stage (Mamet et al. 2021; Taye et al. 2022). Indeed, their function at a specific growth stage might affect plant physiology and development, which ultimately influences *B. napus* yield (Kumar and Bais 2012). Meanwhile, the several shared taxa between growth stages and between the root and rhizosphere might be more influential to *B. napus* yield than the others due to their longer and broader effect in the root and/or rhizosphere of *B. napus*. The taxa ASV13935 (species: *Tetracladium* sp) shared in the roots in weeks 3 and 6 was positively correlated to *B. napus* yield, which is consistent with the finding in a landscape-scale study (Hilton et al. 2021). The taxa ASV10975 (species: *Nectria ramulariae*) identified in both the root and rhizosphere in weeks 6 and 9 was also positively correlated with *B. napus* yield. Conversely, ASV15009 (species: *Gibberella baccata*) which co-occurred with ASV10975 in week 9 in both the root and rhizosphere, was negatively associated with *B. napus* yield. However, the two taxa were assigned to the same family (i.e., *Nectriaceae*) which was previously considered to contain numerous plant and human pathogens (Lombard et al. 2015). *Nectria ramulariae* (anamorph: *Cylindrocarpon obtusiusculum*) and *Gibberella baccata* (anamorph: *Fusarium lateritium*) were also reported as plant pathogens (Clark et al. 1990; Hirooka et al. 2012). Modes of pathogenicity for the two organisms are different, *Gibberella baccata* was classified as a destructive plant pathogen (Desjardins 2003), and *Nectria ramulariae* was reported as plant pathogen only in a very few studies (Hirooka et al. 2012; Wenneker et al. 2016), and the species within the genus *Nectria* (anamorph: *Cylindrocarpon*) were usually considered as only weak plant pathogens (Jankowiak et al. 2016). The enriched *Nectria ramulariae* might compete with *Gibberella baccata* for resources and niche which limited the abundance of *Gibberella baccata* and reduced the damage of *Gibberella baccata* to *B. napus* plant (Abdullah et al. 2017; Moreno and López-Moya 2020). These identified fungal taxa commonly existed across the site-years based on the filter criteria, but the function of most of them is still unknown. Whether they affect *B. napus* plant directly or in an indirect way, such as regulating the abundance of other microbial taxa need to be tested in future work.

Conclusions

Our work identified a core fungal microbiome, common to genetically diverse *B. napus* lines grown across varied conditions. We also observed an association of *B. napus* line with root and rhizosphere microbiome community composition that was strongest under discrete conditions, i.e., a single time point within a site. The thirteen taxa that were highly associated *B. napus* yield provide a promising avenue of exploration to enhance crop productivity by taking manipulating ecology-based crop growth enhancement solutions. Together, these results point to the potential to exploit the *B. napus* microbiome for improved plant performance by targeting core taxa as well as those that lead to greater fitness under more specific conditions such as limited fertility or moisture stress.

Declarations

Acknowledgements

We sincerely appreciate the technical assistance of Alix Schebel and Kimberly Hamonic for sample processing and library preparation. We thank Charlotte Norris, the team of field technical staff at AAFC farms in Saskatoon, Melfort, and Scott who managed the field site-years, and our summer students Lauren Reynolds, Cordell VanGenderen, Yolanda Iannucci, and Kira Blomquist for their assistance.

Funding

This work is supported by a grant from the Plant Phenotyping and Imaging Research Centre (P2IRC) to B.L.H, S.D.S and S.V.. P2IRC is a digital agriculture research center funded by the Canada First Research Excellence Fund (CFREF) from the Natural Sciences and Engineering Research Council (NSERC), managed by the Global Institute for Food Security (GIFS), and located at the University of Saskatchewan (U of S).

Competing Interests

The authors declare no competing interests.

Author Contributions

Y.L. and N.B. led the writing and analysis. B.L.H. co-wrote the manuscript and co-managed the project implementation with S.D.M. S.V. selected the germplasm, and designed and implemented the field site-years. N.B. prepared the DNA sequencing libraries for 2017 samples. All authors contributed to writing and revision of the manuscript.

Data Availability

The raw sequences were deposited to the sequence read archive (SRA) repository of the National Center for Biotechnology Information (BioProject PRJNA575004, Accessions: SAMN13414364 - SAMN13415317; SAMN13416986 - SAMN13417833; SAMN13416203 - SAMN13416971).

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Figures

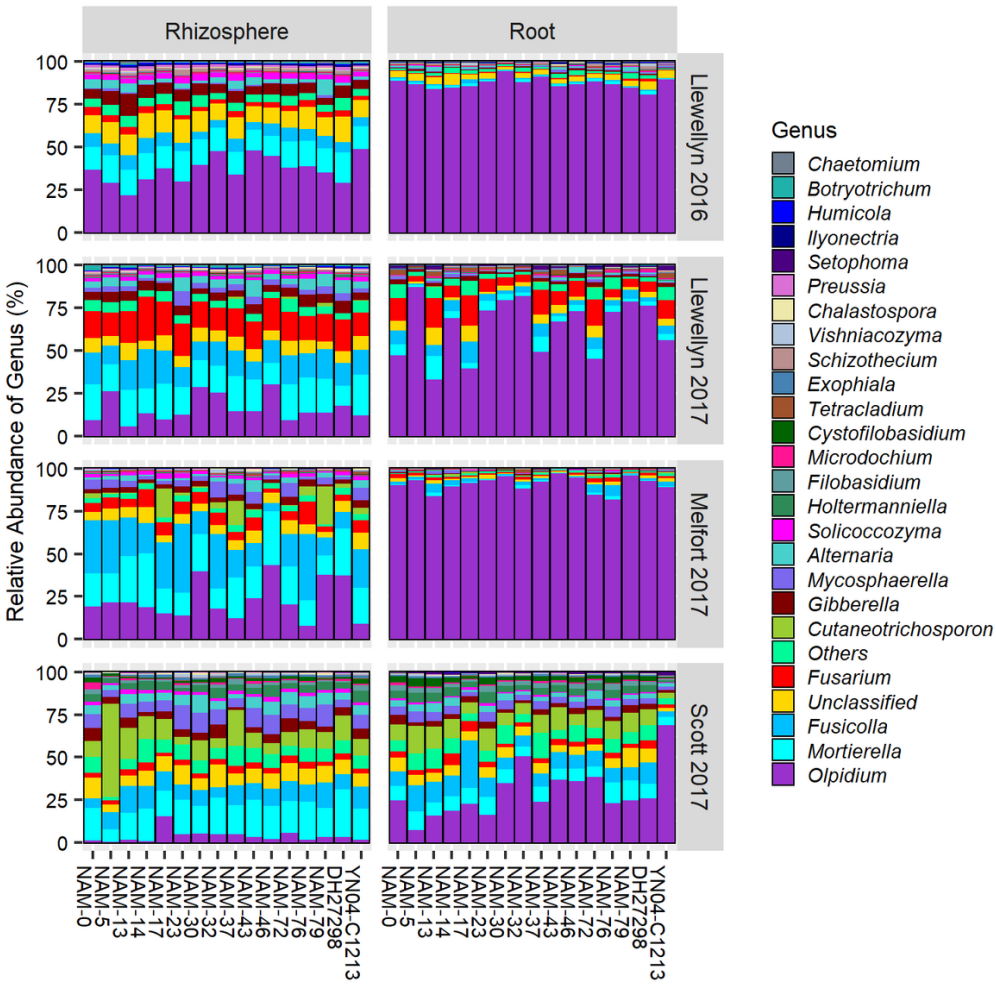

Figure 1

Relative abundance of fungal taxa in the root and rhizosphere of *Brassica napus* lines at the genus level in each site-year

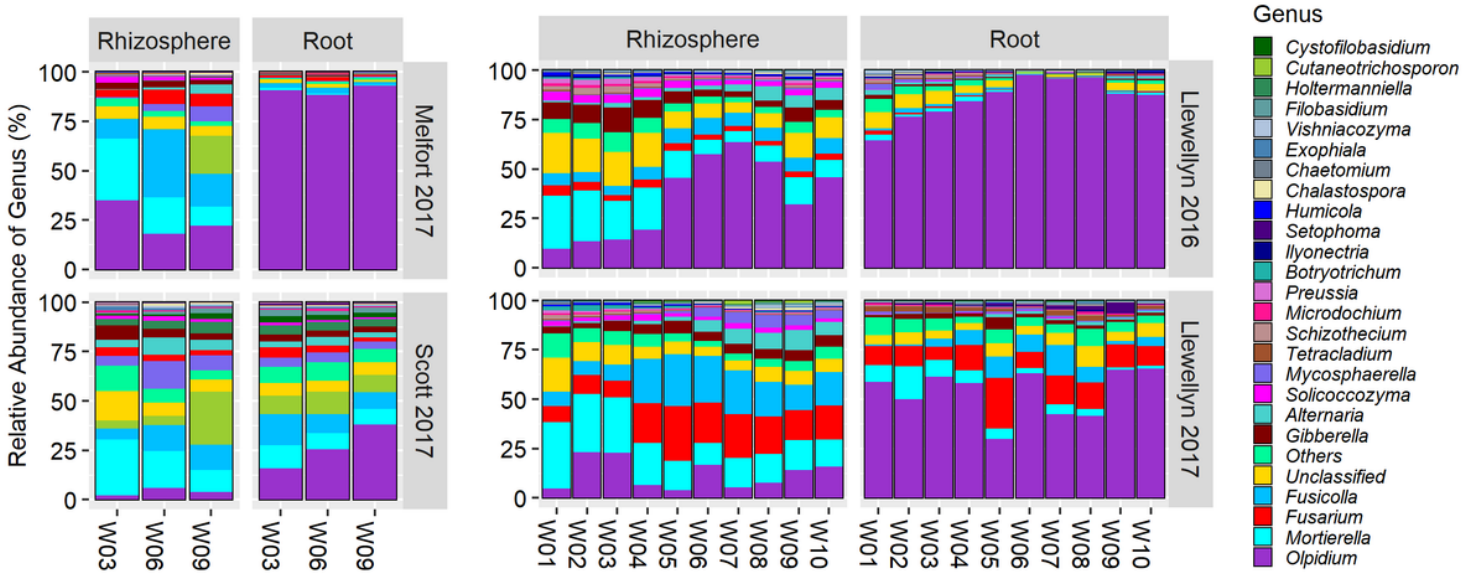

Figure 2

Temporal changes in the relative abundance of fungal taxa in the root and rhizosphere of *Brassica napus* at the genus level in each site-year; W01-10: Week 1-10

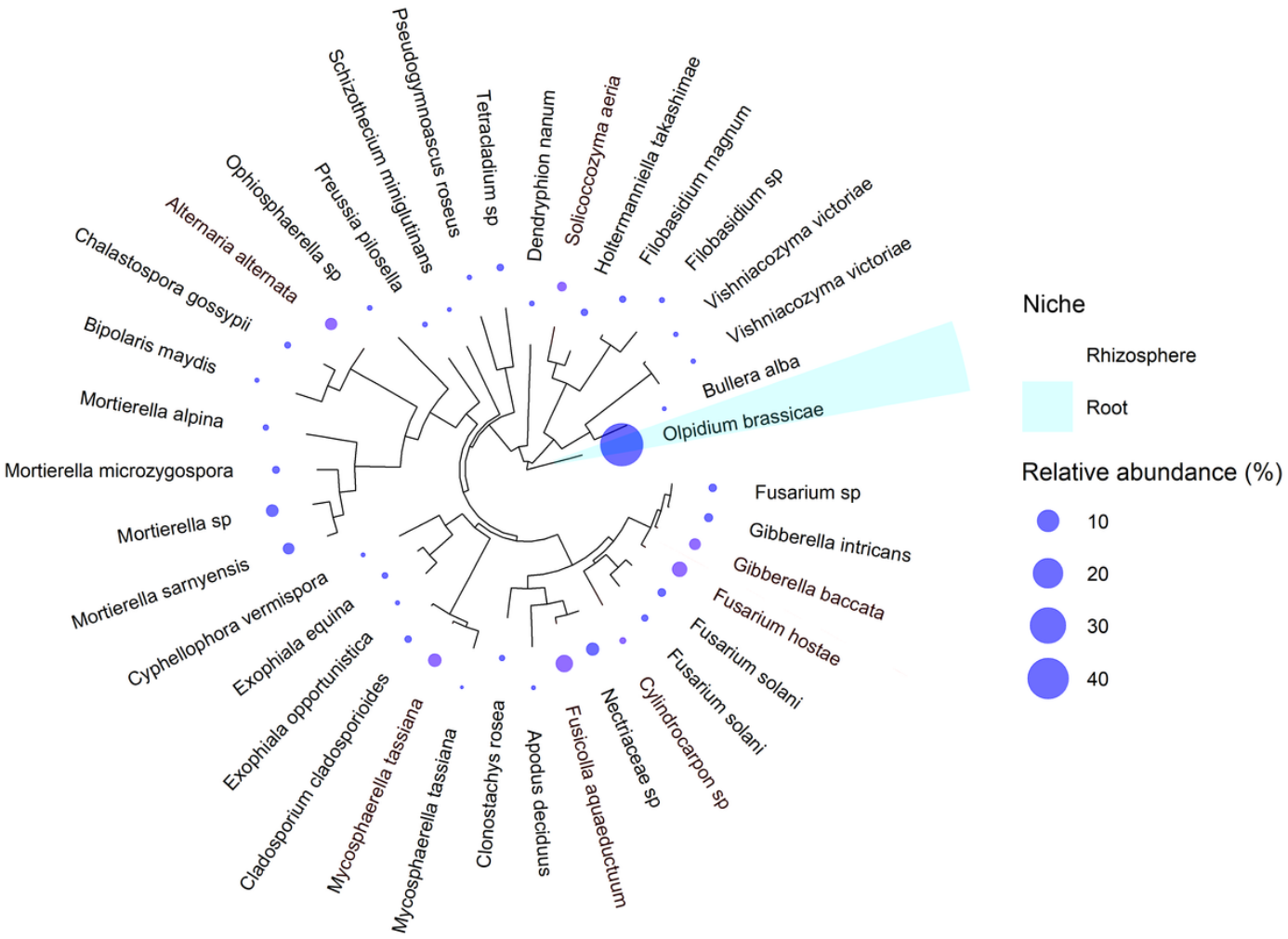

Figure 3

Phylogeny and relative abundance of core fungal ASVs (detected in all lines across site-years) in the root and rhizosphere microbiomes of *Brassica napus*. Fungal ASVs in pink boxes were found in the rhizosphere in all sampled weeks, whereas *Olipidium brassicae* (light blue box) was ubiquitous across all sampled weeks

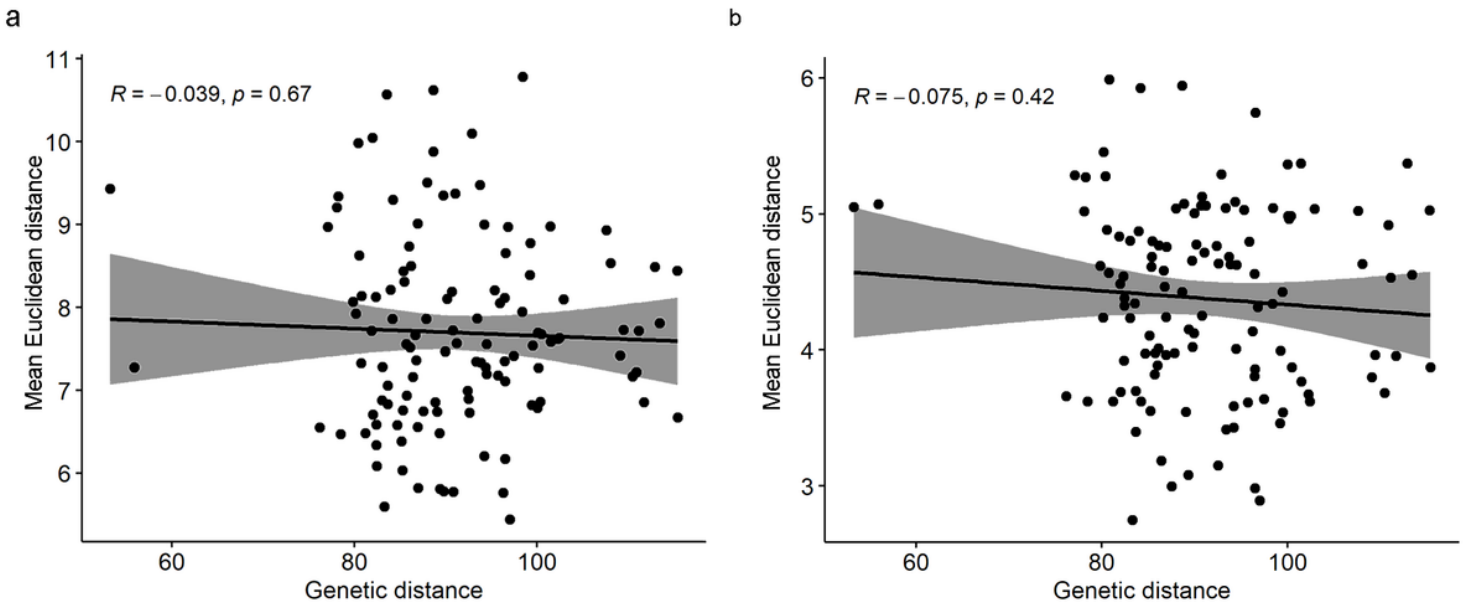

Figure 4

Correlation between fungal community dissimilarity and plant genetic distance among *Brassica napus* lines. (a) rhizosphere samples across all site-years; (b) root samples across site-years

Supplementary Files

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- SupplementaryInformation.docx