

Driver taxa and conserved core-hub communities shape rhizosphere bacterial networks across *Brassica napus* developmental stages

Zelalem M. Taye ^{a,b,c}, Bobbi L. Helgason ^d, Steven D. Siciliano^d, and Eric G. Lamb ^b

^aFaculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, Canada; ^bDepartment of Plant Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada; ^cDepartment of Forest and Conservation Science, The University of British Columbia, Vancouver, BC, Canada; ^dDepartment of Soil Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada

Corresponding author: Zelalem Taye (email: zelalem.taye@ubc.ca)

Abstract

The rhizosphere harbors intricate microbial networks that influence plant health and productivity. Understanding the structure and dynamics of these networks is crucial for developing microbiome-based strategies to enhance crop performance. In this study we examined rhizosphere bacterial communities across *Brassica napus* developmental stages, aiming to (1) identify indicator genera and infer functional traits, (2) determine bacterial genera driving community assembly, (3) assess growth-stage-specific driver genera, and (4) identify core-hub microbial communities shaping community structure. Network analysis revealed a highly complex rhizosphere microbiome, with microbial network complexity peaking at the flowering stage, which exhibited the highest network connectivity (up to 179 exclusive edges). This suggests a strong influence of plant developmental stage on microbial community assembly. We identified 40 driver genera forming novel associations across growth stage transitions, including 10 conserved driver genera present throughout development and stage-specific driver genera during vegetative–flowering (5) and flowering–maturity (20) transitions. Despite dynamic shifts, core-hub communities remained stable across growth stages, suggesting an early-established, conserved microbial foundation. Indicator taxa and predicted functions showed stage-specific enrichment of nitrogen and carbon cycling traits, including early-stage dominance of nitrification and nitrogen fixation functions and late-stage shifts toward carbon degradation. These functional shifts likely reflect plant nutrient requirements, root exudation, and responses to fertilization. Together, these findings highlight both stability and plasticity in bacterial taxa of rhizosphere microbiomes and offer valuable targets for further microbial assembly process dissection for microbiome-informed crop improvement strategies.

Key words: *Brassica napus*, canola, microbial assembly, microbial network, plant–microbe interactions, rhizosphere

1. Introduction

The rhizosphere is a dynamic environment where microorganisms interact and form intricate biological networks that play fundamental roles in plant health and productivity (Kim et al. 2011; Hallam and Mccutcheon 2015). Microbial community assembly and ecosystem functions are dependent on the interactions within these networks (Fuhrman 2009; Hallam and Mccutcheon 2015). Highly interconnected (interacting) microbes can also mediate plant–microbiome interactions, transmitting host effects to the microbial community via microbe–microbe associations (Agler et al. 2016). In some cases, these associations can lead to the formation of beneficial microbial communities that promote nutrient uptake, mitigate abiotic stresses, and prevent pathogen attacks in plants (Bulgarelli et al. 2013). Understanding these microbial interactions is essential for optimizing crop per-

formance through microbiome engineering and inoculants (Hallam and Mccutcheon 2015; Ren et al. 2015).

Brassica napus is a globally significant oilseed crop cultivated for edible oil production, biofuel, and phytoremediation. The potential of the plant microbiome to enhance crop production in *B. napus* has been recognized (Farwell et al. 2006; Lay et al. 2018; Taye et al. 2020). Taye et al. (2020), for example, showed that *B. napus* rhizosphere microbial composition and abundance was under fine scale plant genetic and growth stage control. Phenology-dependent microbial associations have been linked to agronomic traits such as seed yield (Mamet et al. 2022; Li et al. 2023), root length (Taye et al. 2022), and seed quality (Asad et al. 2022). While studies have examined rhizosphere microbial composition and abundance, little is known about bacteria–bacteria networks in *B. napus* and how they change across development stages.

Network-based analysis approaches have proven effective in unraveling complex microbial relationships in soil ecosystems (Schwager et al. 2014; Cardona et al. 2016; Dixon et al. 2024). By analyzing microbial co-occurrence patterns, these methods identify key taxa that likely shape community structure including (1) core-hub communities, which form stable, highly connected microbial networks and play crucial role in maintaining community structure (Agler et al. 2016), and (2) driver genera, which introduce new connections and re-shape microbial community assembly across compared states or conditions (Kuntal et al. 2019).

Recent studies of microbial networks have identified key microbial taxa in *B. napus*, including the bacterial genus *Pseudarthrobacter* (Floc'h et al. 2020b) and fungal species such as *Olpidium brassicae* (Floc'h et al. 2020a). In addition, microbial community assembly in *B. napus* rhizosphere, root, and leaf compartments appears to be growth-stage dependent (Bell et al. 2022). However, the extent to which bacterial co-occurrence networks shift across growth stages remains unexplored. In this study, we use co-occurrence network analysis in 16 diverse lines to investigate rhizosphere microbial co-occurrence networks across *B. napus* developmental stages. Our objectives are to (1) identify indicator genera and infer functional traits of bacterial communities, (2) determine bacterial genera driving community assembly, (3) assess growth-stage-specific driver genera, and (4) identify core-hub microbial communities shaping community structure. By characterizing these co-occurrence networks, we aim to provide a foundation for microbiome-informed breeding and microbiome engineering strategies to enhance *B. napus* productivity.

2. Materials and methods

2.1. Sampling design and growth stages considered

The experiment was conducted in 2016 at a single site near Saskatoon, SK, Canada (latitude 52.181366, longitude -106.502941). The soil is a dark brown Chernozem (Soil Classification Working Group 1998), with a clay loam texture. The field was managed with an oilseed, wheat, barley, fallow rotation with fallow in the growing season prior to this experiment. Site description, experimental design, sampling methods, DNA extraction, amplification and sequencing protocols, and the *B. napus* genotypes used in this study are described in detail by Bazghaleh et al. (2020). Briefly, the experimental design was a randomized complete block design with three replicates. Roots with attached rhizosphere and bulk soil were collected weekly for 10 weeks starting 18 days after seeding and ending 81 days after seeding. The corresponding phenotypic stages for the sampling weeks were 2–3 leaf stage (week 1, 18 days after seeding (DAS)), 4–5 leaf stage (week 2, 25 DAS), 6–9 leaf stage (week 3, 32 DAS), flowering (weeks 4–7, 39–70 DAS), maturity (weeks 8 and 9, 67 and 74 DAS), and harvest (week 10, 81 DAS). At each sampling three random canola plants were harvested from each plot and rhizosphere soils (i.e., soil remaining attached to the root following shaking to dislodge bulk soil) were collected, composited from the

three plants, extracted in NaCl buffer, and centrifuged. Pellets were stored at -80°C until further analysis. In this study, the weekly datasets were grouped into three broad phenological stages corresponding to vegetative (weeks 1–3), flowering (weeks 4–7), and maturity (weeks 8–10) growth stages. The reason for this grouping was to compare microbial assembly patterns across growth stages.

Sixteen diverse *B. napus* genotypes varying in seed color, glucosinolate, erucic acid and fiber contents and origin were used to represent genetically diverse germplasm. Breeding lines from the Agriculture and Agri-Food Canada (AAFC) canola breeding program and lines from a larger diversity collection comprised of accessions representing the variation found across spring *B. napus* housed within various germplasm collections were included (Bazghaleh et al. 2020). In the present study, these genotypes were not used as treatment factors; instead, samples from all genotypes were aggregated within each growth stage to ensure robust network construction generally applicable to the canola rhizosphere microbiome.

2.2. Sequence data processing

The dataset used in the present study was generated in experiment previously reported in Bazghaleh et al. (2020) and Taye et al. (2020). Sequencing and data processing procedures are described in detail in those studies. DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, catalogue number 12955-4) and 16S rRNA genes were amplified using the primer set 342 F-806R. Amplified PCR products were sequenced on an Illumina MiSeq. A total of 144, 192, and 144 samples were collected for the vegetative, flowering, and maturity stages, respectively, and sequencing yielded 142, 191, and 143 high-quality samples for downstream analysis. To account for difference in sequencing depth, sequence counts were adjusted based on an internal standard (*Aliivibrio fischeri*) count. Taxa ASVs were agglomerated at the genus level and both samples and taxa with zero sums were removed. Abundance values were converted to relative abundance values. The whole dataset was used for the indicator taxa and FAPROTAX database based putative microbial trait predictions across growth stages. For the co-occurrence network-based analysis genera which were present in all three growth stages (vegetative, flowering, and maturity) were subsetted. Unclassified genera were also removed before conducting data analysis. This filtering step ensured that all downstream analyses, particularly the network comparisons, were based on confidently annotated and ecologically interpretable taxa.

2.3. Indicator taxa and microbial traits across growth stages

Indicator genera were determined by multilevel pattern analysis. We used the “multipatt” and “indval.g” functions in the R package IndicSpecies to analyse the strength and statistical significance of the relationship between rhizosphere bacterial genera occurrence and abundance with vegetative, flowering, and maturity growth stages of *B. napus* (De Cáceres and Legendre 2009). Ideal indicator species are characterized by high exclusivity and fidelity. Indicator species with

a significant p -value (<0.05 , computed using 9999 permutations) were determined (De Cáceres et al. 2012). The prokaryotic functional traits were predicted using the FAPROTAX database (Louca et al. 2016) based on the 16S rRNA marker gene through the trans_func class in Microeco R package (Liu et al. 2021). Differential abundance of the traits was calculated using ANOVA method in the trans_diff class (Liu et al. 2021).

2.4. Co-occurrence network construction

We used co-occurrence network analysis to characterize complex microbial co-occurrences at three growth stages (vegetative, flowering, and maturity). The compositionality corrected by renormalization and permutation (CCREPE) algorithm in CCREPE R package (Schwager et al. 2014) was used for network inference among the relative abundances of bacterial genera. Correlation-based methods suffer from limitations notable from challenges of working with relative abundance data (Hirano and Takemoto 2019). The CCREPE approach mitigates the limitation of using compositional data for co-occurrence inference by providing compositionally corrected p -values and z -scores for all pairwise correlations and an N -dimensional checkerboard score which provides similarity measure more appropriate for compositional data analysis (Schwager et al. 2014). Statistically significant ($p < 0.05$) Spearman correlations between pairs of genera were calculated by re-sampling and a null distribution of correlation values obtained from 1000 iterations. An edge (association) was assigned between pairs of genera for every significant positive and negative correlation ($p < 0.05$), the Benjamini–Hochberg false discovery rate was used to reduce errors resulting from multiple comparisons. The edge lists created with the significantly correlated bacterial genera were subsequently used as an input for further network analysis using the netShift tool (Kuntal et al. 2019), with the specific objectives of identifying driver taxa responsible for network rewiring and core-hub communities.

2.5. Overall change in co-occurrence pattern and core-hub community detection

We used the common sub-networks between the compared growth stage association network pairs to make comparisons of graph properties. A common sub-network is a smaller network derived from the subset of nodes common to both comparison pairs in the original network (Kuntal et al. 2019). To quantify overall changes in bacterial co-occurrence patterns, the graph properties of the common sub-networks including total nodes, total and exclusive edges, average path length, graph density, and cluster coefficient were used. To quantify the overall extent of change in individual bacterial genera association across growth stages the Jaccard edge index was used. The Jaccard edge index of compared networks is the ratio between the number of intersecting edges in the two networks over their union. Since the compared networks are common sub-networks, biases of the Jaccard edge index due to unequal node content are minimized.

To identify core-hub communities, first we used a community detection algorithm called Fastgreedy (Yang et al. 2016)

in the NetShift tool to separately assign community membership to each bacterial genera (nodes) of the compared networks. Then assigned communities with highest distribution of nodes with highest coreness centrality values (the number of maximally connected neighbouring genera which has at least that many number of connections) were regarded as core-hub communities. To further evaluate how the membership of core-hub community members evolved across growth stages, the similarity between all identified communities in the compared networks was calculated in terms of shared nodes (genera). We used the quantified similarity between communities to identify conserved core-hub communities across growth stages. Community detection algorithms are widely used in biological network analysis to detect closely interacting nodes (Newman 2006; Lai et al. 2010; Kuntal et al. 2013) as such communities could be performing a function with some degree of independence with respect to other communities in the network and could facilitate stable occurrence of a group of taxa possessing attributes desirable for interdependent growth or to meet other ecological requirements.

2.6. Changes in association of a single taxon and driver genera detection

We used the netShift tool method (Kuntal et al. 2019) to identify driver genera that have a significant role in shaping *B. napus* rhizosphere microbial community assembly. The driver genera were identified using the Neighbour Shift (NESH) score and betweenness values (Kuntal et al. 2019). This method allows quantification of directional changes in the individual bacterial genera co-occurrences by exploring significant overall changes in community patterns. For example, when comparing the bacterial network at flowering with the network at vegetative stage, bacterial genera with an altered set of associations (high NESH score) and increased importance (increase in betweenness score) holds key significance in microbial co-occurrence and is predicted to be a driver taxon for flowering stage. The identified driver genera were further filtered to identify those that were drivers across growth stages and those exclusive to only one growth stage.

3. Results

3.1. Growth stage-associated indicator bacterial genera in the rhizosphere of *B. napus*

Multipattern analysis identified distinct growth stage-specific indicator taxa in the rhizosphere of *B. napus* across different growth stages. During the vegetative stage, *Candidatus solibacter* was the sole indicator taxon ($\alpha < 0.05$). At the flowering stage, six taxa were significantly associated with this phase, including *Erwinia*, *Acidovorax*, *Ralstonia*, *Paenibacillus*, *Rahnella*, and *Clostridium* ($\alpha < 0.01$). In the maturity stage, eight indicator taxa were identified: *Nannocystis*, *Dokdonella*, *Chitinophaga*, *Niabella*, *Lacibacter*, *Ochrobactrum*, *Actinocorallia*, and *Amycolatopsis*, most of which exhibited strong indicator values ($\alpha < 0.01$) (Table 1). These findings suggest that microbial community composition in the rhizosphere shifts

Table 1. Indicator value (IndVal) and associated significance value (*p*-value) of indicator species analysis of bacterial genera at three growth stages of *Brassica napus* (vegetative, flowering, and maturity).

	A	B	IndVal	<i>p</i> -value
Vegetative (number of indicator species = 1)				
<i>Candidatus solibacter</i>	0.8330	0.0493	0.2030	0.0439
Flowering (number of indicator species = 6)				
<i>Erwinia</i>	0.8242	0.4583	0.6150	0.0001
<i>Acidovorax</i>	0.8163	0.3646	0.5460	0.0001
<i>Ralstonia</i>	0.8664	0.3333	0.5370	0.0002
<i>Paenibacillus</i>	0.8853	0.3073	0.5220	0.0001
<i>Rahnella</i>	0.7537	0.2188	0.4060	0.0021
<i>Clostridium</i>	0.9667	0.1562	0.3890	0.0001
Maturity (number of indicator species = 8)				
<i>Nannocystis</i>	0.7033	0.1748	0.3510	0.0003
<i>Dokdonella</i>	0.7478	0.1608	0.3470	0.0001
<i>Chitinophaga</i>	0.8526	0.1049	0.2990	0.0001
<i>Niabella</i>	0.8014	0.1049	0.2900	0.0011
<i>Lacibacter</i>	0.6401	0.1189	0.2760	0.0011
<i>Ochrobactrum</i>	0.7535	0.0979	0.2720	0.0017
<i>Actinocorallia</i>	0.9323	0.0699	0.2550	0.0002
<i>Amycolatopsis</i>	0.7974	0.0769	0.2480	0.0107

Note: The value for “A” indicates the how specific the genus is to the growth stage (i.e., $A = 1$ means the genus is only present in that growth stage). The value for “B” indicates the probability of finding the genus in only the samples of the growth stage (i.e., $B = 1$ means the genus is only present in the specific growth stage through all the samples). The stat value (indVal) indicates how associated the genera is to the growth stage.

dynamically throughout canola development, highlighting potential microbial interactions and functional roles at each growth stage.

3.2. Carbon- and nitrogen-related potential functional profiles across *Brassica napus* growth stages

Microbial potential functional profiles related to carbon and nitrogen cycling in *B. napus* rhizosphere varied significantly across plant growth stages (Table 2 and Table S1). Among carbon metabolism traits, hydrocarbon degradation-related functional profile including general hydrocarbon degradation, increased progressively from the vegetative stage to flowering and reached their highest relative abundance at maturity (Table 2). This pattern suggests an intensification of microbial hydrocarbon processing capacity as the plant developed. In contrast, methanotrophy remained at low relative abundance throughout all growth stages, with no significant differences detected (Table S1).

Nitrogen-related potential functional profiles exhibited distinct temporal patterns. Nitrogen fixation-related profiles were significantly enriched during the vegetative stage, then declined at flowering and remained low at maturity (Table 2). Conversely, functional profiles involved in nitrate reduction, nitrate respiration, and nitrogen respiration peaked at flowering, with significantly higher relative abundance compared to both vegetative and maturity stages. Nitrification, nitrate/nitrite denitrification and total denitrification-related functional profiles were significantly more abundant during

the vegetative stage and declined through flowering and maturity (Table 2).

These findings indicate shifts in rhizosphere microbial potential functional profiles, with early enrichment in nitrogen-fixing and nitrifying-associated traits, followed by a transition towards reductive nitrogen-related profiles mid-season and a dominance of hydrocarbon degradation-related profiles by maturity.

3.3. *Brassica napus* rhizosphere bacterial network complexity through plant development

Our network-based analysis approach bridges metagenomic profiling with culture-based microbial assembly experiments by identifying driver and core-hub taxa that can be targeted for detailed investigation and plant breeding.

The common sub-networks between the vegetative and flowering, flowering and maturity, and vegetative and maturity growth stages had 30, 50, and 26 bacterial genera (nodes), respectively.

The relatively small Jaccard edge indices between the compared microbial networks for the vegetative–flowering, vegetative–maturity, and flowering–maturity networks (0.16, 0.273, and 0.213 respectively) indicate substantial microbial community rewiring (i.e., presence of large numbers of new edges (connections) at one growth stage which are completely absent in another) across *B. napus* growth stages. The smaller the Jaccard edge index, the greater the microbial rewiring between *B. napus* growth stages, thus the greatest network changes occurred with the vegetative to flowering transition

Table 2. Carbon and nitrogen metabolism traits across growth stages in *Brassica napus* rhizosphere.

Microbial trait	Growth Stage	N	Mean relative abundance (%)	SD	SE
Carbon metabolism					
Hydrocarbon degradation	Vegetative ^c	142	0.119	0.563	0.047
	Flowering ^b	191	0.618	0.974	0.07
	Maturity ^a	143	1.231	1.381	0.115
Aromatic hydrocarbon degradation	Vegetative ^c	142	0.109	0.523	0.044
	Flowering ^b	191	0.598	0.976	0.071
	Maturity ^a	143	1.196	1.313	0.11
Nitrogen metabolism					
Denitrification	Vegetative ^a	142	1.772	2.348	0.197
	Flowering ^b	191	0.772	1.2	0.087
	Maturity ^b	143	0.741	1.326	0.111
Nitrogen fixation	Vegetative ^a	142	7.827	6.345	0.532
	Flowering ^b	191	3.869	3.762	0.272
	Maturity ^b	143	4.297	2.786	0.233
Nitrate reduction	Vegetative ^b	142	12.114	13.643	1.145
	Flowering ^a	191	22.107	15.548	1.125
	Maturity ^b	143	13.682	10.46	0.875

Note: The table presents microbial traits, the three plant growth stages, number of samples (N), mean relative abundance, and associated standard deviation (SD) and standard error (SE). Significant differences among growth stages ($\alpha < 0.05$) are indicated by superscript letters.

Table 3. Global graph properties of the common sub-networks of positively correlated co-occurring bacterial networks among the vegetative, flowering, and maturity growth stages.

Network metrics (most common network)	Network comparisons	Growth stages		
		Vegetative	Flowering	Maturity
Total nodes	Vegetative–flowering	30	27	–
	Flowering–maturity	–	50	49
	Vegetative–maturity	26	–	24
Total edges	Vegetative–flowering	32	124	–
	Flowering–maturity	–	243	121
	Vegetative–maturity	29	–	55
Exclusive edges	Vegetative–flowering	10	102	–
	Flowering–maturity	–	179	57
	Vegetative–maturity	11	–	37
Average path length	Vegetative–flowering	3.41	1.78	–
	Flowering–maturity	–	2.03	3.04
	Vegetative–maturity	3.38	–	2.54
Graph density	Vegetative–flowering	0.07	0.35	–
	Flowering–maturity	–	0.208	0.10
	Vegetative–maturity	0.09	–	0.207
Cluster coefficient	Vegetative–flowering	0.19	0.59	–
	Flowering–maturity	–	0.52	0.42
	Vegetative–maturity	0.2	–	0.46

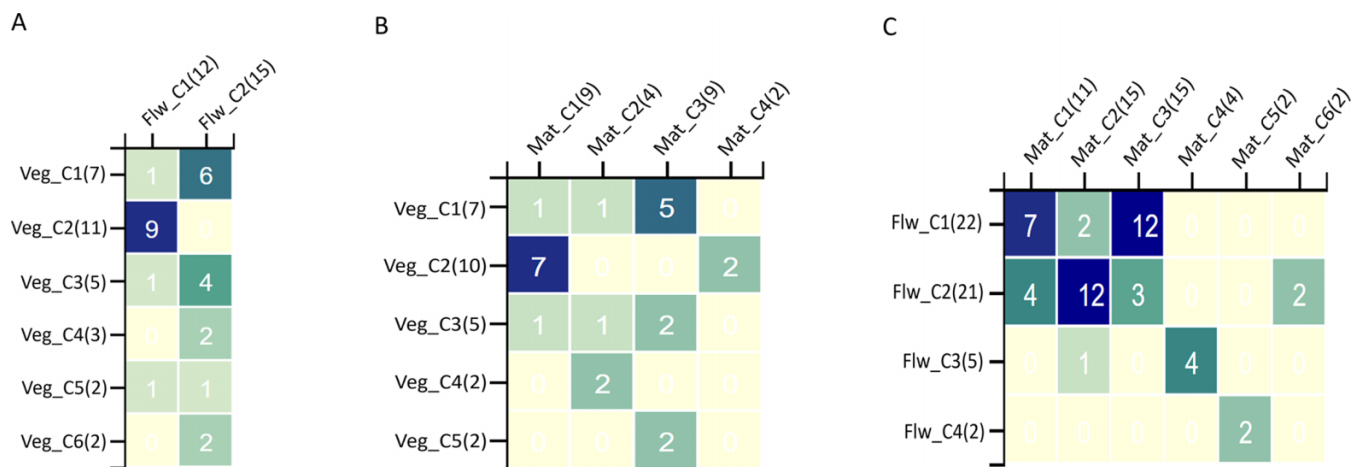
Note: Values indicate comparisons among the common sub-networks between pairs of stages. The “–” is shown when a growth stage is not being compared.

and the least rewiring during vegetative to maturity transition.

The global network properties showed a major rewiring of the bacterial network at flowering, with the highest number of total and exclusive edges, graph density,

and clustering coefficient, and the shortest path length (Table 3). This suggests greater cross talk, tighter associations, and more modular structure among bacterial genera at flowering compared with vegetative and maturity stages.

Fig. 1. Rhizosphere bacteria network community shuffling plots. The heatmaps show the vegetative (“Veg_”) and flowering (“Flw_”) network communities along the vertical axis and the flowering (“Flw_”) and maturity (“Mat_”) network communities along the horizontal axis. The identified communities in each growth stage are represented by a community id: C1, C2, etc. with a count of the total number of nodes in the community in brackets. Numerical values inside the heatmap show the number of intersecting nodes between the compared communities. Similar (conserved) communities are shown with blue gradient (deep blue indicating most similar community) while dissimilar ones are shown as green gradient (deep green indicate most dissimilar pairs).



3.4. Core-hub communities in *B. napus* rhizosphere microbial community and their conservation across growth stages

There were 22, 18, and 64 conserved microbial edges among the vegetative–flowering, vegetative–maturity, and flowering–maturity comparisons, respectively. Consecutive growth stages shared higher numbers of edges and the flowering–maturity comparison had the highest number of cross-growth stage conserved microbial edges.

3.4.1. Vegetative to flowering comparisons

The Fastgreedy (Yang et al. 2016) community detection algorithm identified six and two distinct sub-communities in the vegetative and flowering stage networks, respectively (Figs. 1A and S1). The community shuffling plot shows that “Veg_C1”, “Veg_C2”, and “Veg_C3” were distinct core-hub communities at the vegetative stage. The other three communities at vegetative stage did not have taxa with high coreness centrality value. A “core-hub community” has the majority of its nodes with high coreness centrality values. The flowering stage had both its identified communities as core-hub (Fig. 1A).

The first core-hub community at the vegetative stage (Veg_C1) is composed of seven genera, of which *Balnei-monas*, *Pseudomonas*, *Reyranella*, *Skermanella*, and *Steroidobacter* show the highest coreness centrality values. Veg_C2 is composed of 11 genera, of which *Agromyces*, *Couchioplanes*, *Dactylosporangium*, *Labrys*, *Phycoccus*, and *Pseudonocardia* show the highest coreness centrality values, whereas Veg_C3 is composed of five genera of which *Agrobacterium*, *Sphingobacterium*, and *Stenotrophomonas* show the highest coreness centrality values. The two communities at flowering had 12 (“Flw_C1)

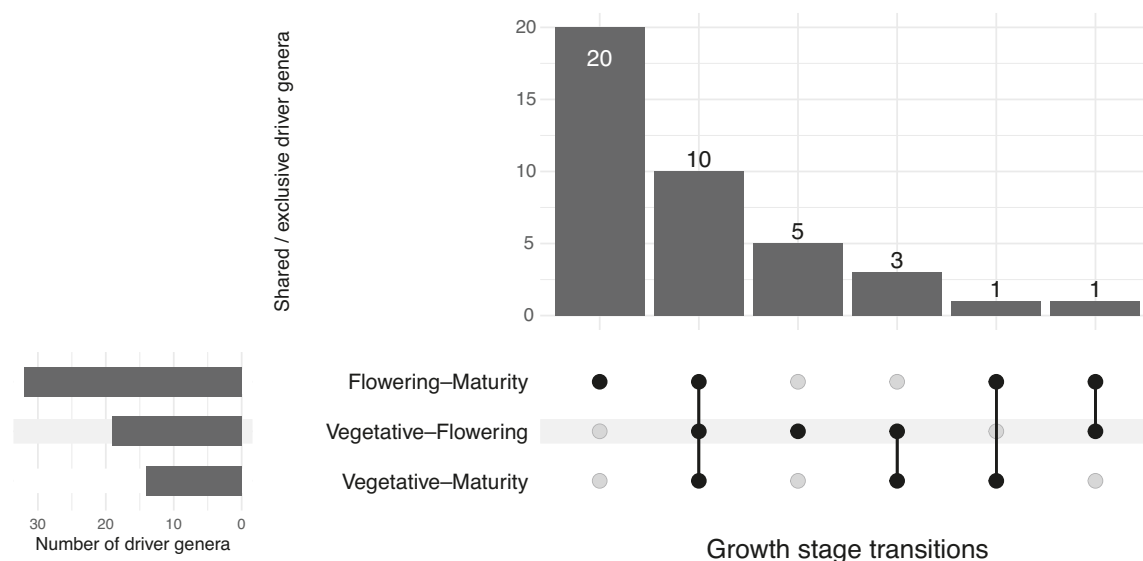
and 15 (“Flw_C2”) bacterial genera (Fig. 1A). The microbial communities identified at the vegetative stage were generally conserved at flowering. For example, 6 of the 7 members of C1 and 9 of the 11 members of C2 at the vegetative stage remained together at flowering. The limited community shuffling through time indicates that *B. napus* microbial community organization is set early in plant development (Fig. 1A).

3.4.2. Vegetative to maturity comparisons

There were five and four distinct sub-communities in the vegetative and maturity stage networks, respectively (Fig. 1B). The community shuffling plot shows that “Veg_C1” and “Veg_C2” were core-hub communities at the vegetative stage that had majority of its members with highest coreness centrality values (Figs. 1B and S2). Similarly, the maturity stage showed two core-hub communities (“Mat_C1” and “Mat_C3”) (Figs. 1B and S2).

The core-hub community at the vegetative stage in community Veg_C1 comprised 7 genera and in Veg_C2 10. The genera with high coreness centrality value in Veg_C1 were *Balnei-monas*, *Pseudomonas*, *Reyranella*, *Skermanella*, and *Steroidobacter*. In Veg_C2, *Agromyces*, *Couchioplanes*, *Dactylosporangium*, *Labrys*, *Phycoccus*, and *Pseudonocardia* were with high coreness centrality value (Fig. S2). These bacterial genera were also identified as members of core-hub community in the vegetative to flowering comparison (Figs. 1A and S1). Similar to the vegetative–flowering transition, most community members identified at the vegetative stage were also conserved at maturity. For example, 5 of the 7 members of C1, and 7 of the 10 members of C2 at the vegetative stage remained together at maturity (Fig. 1B).

Fig. 2. Distribution of shared and growth stage-specific driver genera across vegetative–flowering, vegetative–maturity, and flowering–maturity transitions in the *Brassica napus* rhizosphere. The horizontal bars at the left bottom indicate the number of driver genera identified in each growth stage transitions. The top vertical bars indicate the number of driver genera either unique to a specific growth stage transition or shared among transitions. Black circles and connecting lines below the bars show which growth stage transition(s) each bar represents.



3.4.3. Flowering to maturity comparisons

There were four and six distinct sub-communities in the flowering and maturity stage networks, respectively (Fig. 1C). The community shuffling plot shows that “Flw_C1” and “Flw_C2” were distinct core-hub communities at the flowering stage (Figs. 1C and S3). The maturity stage showed one core-hub communities (“Mat_C2”) (Fig. S3). Twelve and seven of the 22 members in C1, and 12 of the 21 in C2 at flowering remained together at maturity (Fig. 1C). All growth stage comparisons showed the presence of bacterial sub-communities conserved across growth stages. Some of the sub-communities with majority of their members with high coreness centrality score were identified as core-hub communities (Figs. 1A–1C).

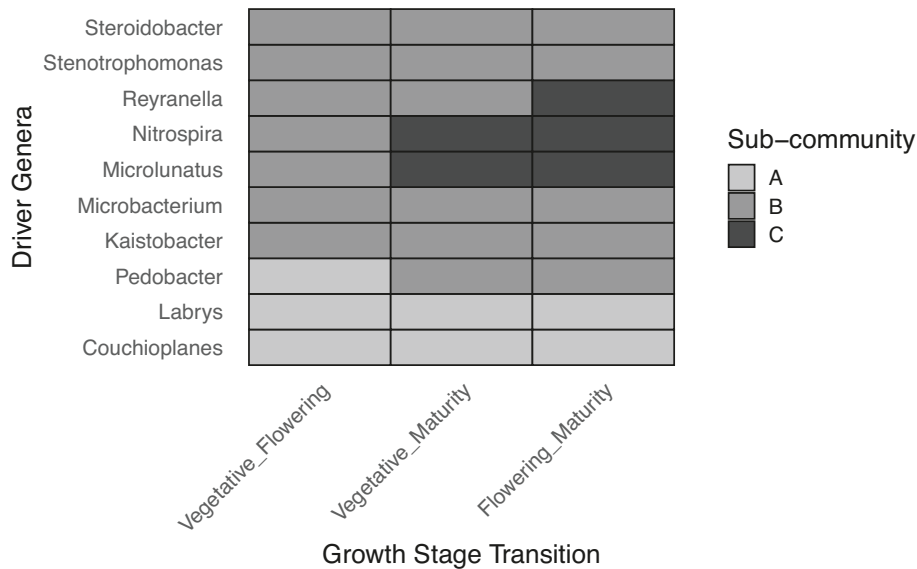
3.5. Potential driver bacterial genera in *B. napus* rhizosphere at different growth stages

We defined driver taxa as a bacterial genus at a specific growth stage with an altered set of associations (high NESH score) and an increase in betweenness compared with another growth stage and holds a key significance in microbial community interactions. Nineteen, 14, and 32 driver genera were identified in the vegetative–flowering, vegetative–maturity, and flowering–maturity comparisons, respectively (Table S2, S3, and S4). The top ten driver genera with the highest numbers of exclusive microbial connections during the vegetative–flowering transition grouped into two sub-communities: *Stenotrophomonas* (15 exclusive connections), *Reyranella* (14), *Balneimonas* (13), *Skermanella* (12), and *Sphingobacterium* (10) belonged to one sub-community, while *Couchioplanes* (11), *Mycobacterium* (11), *Phy-*

coccus (11), *Pseudonocardia* (11), and *Agromyces* (10) belong in another (Table S2). The top ten driver genera with the highest numbers of exclusive microbial connections during the vegetative–maturity transition grouped into three sub-communities: *Labrys* (8 exclusive connections), *Couchioplanes* (5), *Agrobacterium* (4), and *Geodermatophilus* (4) belonged to one sub-community, while *Balneimonas* (6), *Skermanella* (6), *Stenotrophomonas* (5), *Pedobacter* (4), *Reyranella* (4), and *Steroidobacter* (4) belonged in another (Table S3). The exclusive connections *Stenotrophomonas* has with *Balneimonas* and *Skermanella* at maturity stages was a negative correlation (Fig. S4). The top driver genera with the highest numbers of exclusive microbial connections during the flowering–maturity transition were *Agrobacterium* (6) and *Labrys* (6), which belonged to the same sub-community, followed by *Pedobacter* (5) and *Stenotrophomonas* (5), which also clustered within a second shared sub-community (Table S4). Notably, the exclusive connections between *Agrobacterium* and *Labrys*, *Pedobacter* and *Labrys* at the maturity stage were negative correlations (Fig. S4). Driver genera established a higher number of exclusive edges in the vegetative–flowering stage transition and decreased at the flowering–maturity transition (Table 3).

All growth stages shared 10 driver genera. The vegetative–flowering and vegetative–maturity transitions shared three driver genera; vegetative–flowering and flowering–maturity transitions and vegetative–maturity and flowering–maturity transitions each shared one driver genus. The highest number of exclusive driver genera (20) was present in the flowering–maturity transition followed by vegetative–flowering (5) (Fig. 2, Table S5).

Fig. 3. Driver genera shared across all growth stages. Heatmap shows the community membership of identified driver genera. Similar colors across columns indicate that those genera belong to the same sub-community within the growth stage indicated and similar color across rows indicate that those genera maintained similar community membership across the growth stages.



3.5.1. Driver genera influencing microbial assembly across growth stages

The 10 driver genera identified to play role in microbial assembly in the vegetative–flowering, vegetative–maturity, and flowering–maturity growth stage transitions (Fig. 2 and Table S5) were grouped into two main sub-communities (Fig. 3). Figure 3 shows how community membership changed with reference to their community membership at vegetative–flowering–maturity transitions. The shared driver genera mainly maintained their membership across growth stages. For example, the sub-community with members *Kaistobacter*, *Microbacterium*, *Microlunatus*, *Nitrospira*, *Reyranella*, *Stenotrophomonas*, and *Steroidobacter* was stable through the growth stages except for *Microlunatus* and *Nitrospira* forming a distinct community at vegetative–flowering transition in which *Reyranella* also joined this community at flowering–maturity transitions. Similarly, the second sub-community with members *Couchioplanes*, *Labrys*, and *Pedobacter* maintained their membership throughout the growth stages except *Pedobacter* (Fig. 3). The three driver genera shared between vegetative–flowering and vegetative–maturity transitions (*Methylibium*, *Phycococcus* and *Rubrobacter*) (Table S5) did not remain in the same sub-communities.

3.5.2. Driver genera influencing microbial assembly exclusively at one growth stage

The five driver genera identified to play role in microbial assembly exclusively at vegetative–flowering transition (Table S5) were grouped into two sub-communities (Fig. 4). The first sub-community contained *Agromyces* and *Geodermatophilus* while the second sub-community contained *Balneimonas*, *Devosia*, and *Sphingobacterium* (Fig. 4). The 20 driver genera that were exclusive to flowering–maturity (Fig. 4) transi-

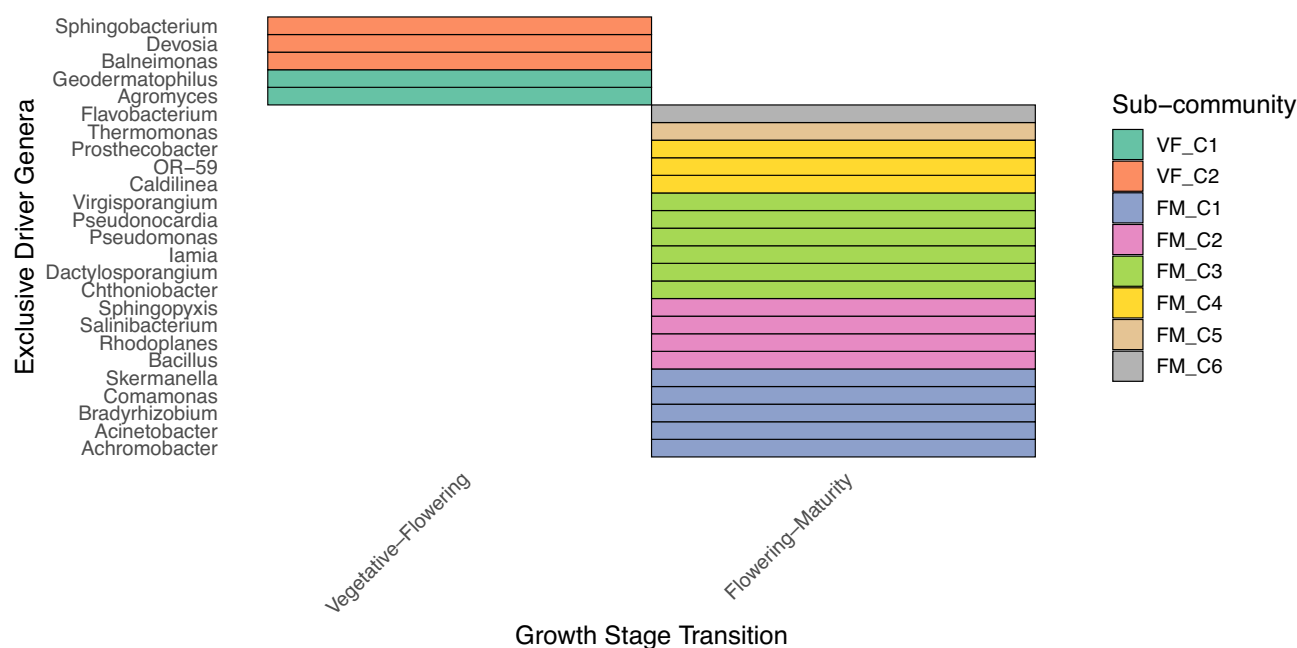
tion were mainly grouped into four sub-communities with two taxa being in two other sub-communities (Fig. 4). The first sub-community contained *Achromobacter*, *Acinetobacter*, *Bradyrhizobium*, *Comamonas*, and *Skermanella*. The second community contained *Bacillus*, *Rhodoplanes*, *Salinibacterium*, and *Sphingopyxis*. The third community contained *Chthoniobacter*, *Dactylosporangium*, *Iamia*, *Pseudomonas*, *Pseudonocardia*, and *Virgisporangium*. The fourth community contained *Caldilinea*, OR-59, and *Prostheco bacter*. The vegetative–maturity transition had no exclusive driver genera.

4. Discussion

Here we detail how plant developmental stage structures *B. napus* rhizosphere microbial network assembly and potential functional capacity. Understanding the microbial community assembly processes under field conditions is crucial to identify the microbial community composition and organizational structures associated with high crop productivity. The single-site, single-year study design, combined with diverse canola genotypes and intensive weekly sampling, allowed us to construct robust microbial networks and compare rhizosphere dynamics across growth stages. However, confirming the universality of these patterns in canola will require multiyear, multisite studies.

This study identified growth stage specific indicator taxa, potential functional shifts in nitrogen and carbon cycling, and the presence of both core-hub communities and driver genera that shape the microbial assembly in *B. napus* rhizosphere. Previous observations of plant growth stage-specific control on the abundance of individual bacterial genera (Taye et al. 2020, 2022) clearly extend to bacterial community assembly processes. Beneficial and detrimental microbial effects on the plant are not only dependent on the high abun-

Fig. 4. Driver genera identified exclusively at vegetative–flowering and flowering–maturity. Heatmap colors show community membership of identified driver genera (VF and FM refers to the growth stages transitions vegetative–flowering and flowering–maturity and the C1, C2 etc. indicate the community membership at the respective growth stages). Similar labels within column indicate that those genera belong to the same sub-community within the growth stage indicated.



dance or activity of individual taxa but also in the complex microbial interactions (Bulgarelli et al. 2013; Hallam and Mccutcheon 2015). In this regard, our understanding of these microbial interactions and their relevance to microbial assembly, plant health and development is very limited. The identification of core-hub communities and driver genera provides community-level insights into *B. napus* rhizosphere microbial assembly and informs strategies for microbiome optimization. Building on this, our network-based analyses identify candidate taxa for targeted microbiological experiments, enabling direct testing of microbial associations, elucidation of assembly mechanisms, and the construction of synthetic communities to evaluate their effects on plant growth and health, thereby bridging genomics-based insights with culture-based validation.

4.1. Indicator bacterial taxa across *B. napus* growth stage

The sole indicator species at the vegetative growth stage, *Candidatus Solibacter* (Acidobacteria), is known for its oligotrophic nature, involvement in soil carbon cycling and is best suited for low nutrient (carbon source) conditions (Ward et al. 2009).

In contrast, the flowering stage exhibited the greatest number of indicator taxa, including *Erwinia*, *Acidovorax*, *Ralstonia*, *Paenibacillus*, *Rahnella*, and *Clostridium*. Many of these genera are metabolically diverse and known to respond to elevated carbon and nutrient availability, consistent with increased rhizodeposition during flowering (Chaparro et al. 2014). For example, *Paenibacillus* is frequently reported as associated with plant growth-promoting capabilities and nitrogen trans-

formation potential (Grady et al. 2016). *Ralstonia* is also known to colonize root environments and interact with plant signaling pathways (Hardoim et al. 2015). The emergence of *Clostridium*, an anaerobe capable of nitrogen fixation and organic matter fermentation (Wiegel et al. 2006), further suggests micro-niche diversification within the rhizosphere during reproductive development.

At maturity, eight indicator taxa were identified, including *Nannocystis*, *Dokdonella*, *Chitinophaga*, *Niabella*, *Lacibacter*, *Ochrobactrum*, *Actinocorallia*, and *Amycolatopsis*. Some of these taxa are associated with complex organic matter degradation (Rosenberg 2014; Shobade et al. 2024) and the enrichment of these genera might reflect the shift from active nutrient acquisition to senescence-associated processes, including root tissue turnover and accumulation of complex detritus in the rhizosphere.

4.2. Dynamics of nitrogen- and carbon-related potential functional profiles across *B. napus* growth stages

The rhizosphere microbial community exhibited distinct putative functional profile shifts in carbon- and nitrogen-associated traits across canola growth stages, reflecting dynamic plant–microbe interactions modulated by plant developmental needs and fertilization history.

Putative functional profiles related to nitrate reduction peaked during the flowering stage (~22.1% mean relative abundance), significantly higher than at the vegetative (~12.1%) and maturity (~13.7%) stages. This pattern coincides with peak nitrogen demand during flowering, when reproductive tissues form and nitrogen is rapidly translocated

(Hirel et al. 2007). Given that urea dissolves quickly and is converted to ammonia, and most fertilizer was applied in the fall and at seeding, it is likely that the bulk of fertilizer-derived nitrogen was available during the vegetative stage rather than later stages. The observed enrichment of taxa associated with putative nitrate-reducing functional profiles at flowering may therefore reflect other processes, such as delayed mineralization, microbial turnover, or plant-driven changes in rhizosphere nitrogen dynamics, rather than direct fertilizer availability. This discrepancy highlights the complexity of linking functional inferences to fertilization regimes and warrants further work with higher-resolution nitrogen measurements across growth stages.

Putative functional profiles associated with nitrogen fixation and denitrification were significantly enriched at the vegetative stage (~7.8% and ~1.8%, respectively) and declining thereafter. These early enrichments suggest that microbial communities may buffer nitrogen availability when fertilizer-derived nitrate is not yet fully accessible to plants (Philippot et al. 2013). Their reduction by flowering could likely be due to nitrate-mediated suppression of nitrogenase activity (Kraiser et al. 2011) and the competitive advantage of nitrate-reducing taxa. Future studies that integrate microbial functional profiling with direct soil nitrogen speciation and flux measurements would help disentangle how fertilizer timing, transformation, and plant demand jointly shape rhizosphere nitrogen cycling.

Putative microbial traits associated with hydrocarbon degradation and aromatic hydrocarbon degradation increased progressively across the growth stages. Mean relative abundances of hydrocarbon degradation rose from ~0.12% at vegetative to ~0.62% at flowering, and further to ~1.23% at maturity. A similar pattern was seen for aromatic hydrocarbon degradation (~0.11% to ~0.60% to ~1.20%). This trend may reflect the accumulation and diversification of root exudates and decaying organic matter over time, especially as plants transition toward senescence (Chaparro et al. 2014; Zhahnina et al. 2018).

Collectively, these observations indicate a developmentally-synchronized shift in putative microbial functional profiles, with nitrogen-cycling-associated traits dominating early to mid growth stages in response to plant nutrient uptake dynamics and fertilization, while carbon degradation-associated traits expand later as the plant's contribution to belowground organic matter intensifies. These patterns reflect changes in the relative abundance of microbial taxa associated with these functional potentials, highlighting the dual role of rhizosphere microbiota in nutrient provisioning and decomposition in response to plant developmental cues and soil management practices.

4.3. Increased *B. napus* microbial community organization across growth stages

This is the first study to examine how *B. napus* rhizosphere microbial networks shift during plant development. The small Jaccard edge indices and growth stage related exclusive edges and driver taxa observed demonstrate microbial network rewiring, with substantial rewiring at flower-

ing. Similar increases in microbial network complexity with development have been observed in other species including wild oat (Shi et al. 2016). These developmental changes are likely driven by increasing plant influences on the rhizosphere environment. Plants alter their rhizosphere conditions including pH, moisture, oxygen, and organic carbon over time (Hinsinger et al. 2009; Chaparro et al. 2014). This variation in resource availability is often an important driver of bacterial network structure (Foster et al. 2012). In the case of *B. napus*, levels of rhizodeposition are reported to generally decrease with age (Nguyen 2003). We therefore propose two mechanisms to explain the observed higher network complexity at flowering. First, at the vegetative stage higher rhizodeposition (Nguyen 2003) may enable the plant to directly attract more bacterial genera; as the plant grows and amount of rhizodeposition decreases the plant may shift strategy to only modulating the activity of a selected "few" bacterial taxa which in turn interact with other bacteria increasing the network complexity and organization. A more detailed understanding of *B. napus* rhizodeposition patterns is needed to clearly link it with microbial assembly processes. Given that the identity of the exudates produced vary depending on the plant, environmental, and management conditions (Mimmo et al. 2011; Town et al. 2023), further study needs to consider these aspects. Second, assuming that the level of rhizodeposition may generally decrease across growth stages (Nguyen 2003), bacterial taxa may increase the strength of their interactions with other taxa to establish a mutually beneficial community that better utilizes available resources. Other possible contributing factors could be the plant's morphophysiological changes at flowering. For example, from the same experiment an increasing trend in overall root length during vegetative stage followed by stability at flowering was observed (Taye et al. 2022). Whether the root morphological and associated physiological changes at flowering are linked with the increased microbial organization at flowering needs to be investigated. Root associated microbes are known to release phytohormones and volatile compounds which can regulate plant growth and morphogenesis (Ortiz-Castro et al. 2009), hence, whether canola plants are also altering their root architecture at the same time needs to be evaluated.

4.4. Core-hub communities shaping *B. napus* rhizosphere microbial assembly

The *B. napus* rhizosphere microbial network is characterized by a set of conserved microbial interactions and communities across growth stages. Consecutive growth stages had a greater number of shared edges with flowering and maturity stages having the highest number of shared edges. These conserved microbial associations suggest that bacterial genera that are recruited and establish associations with each other at early stages maintain these interactions through plant growth and play a role in shaping the rhizosphere microbial assembly. In addition to the growth stage conservation of core-hub taxa observed in this study, a recent study also showed conservation of hub bacterial taxa across years and locations (Floc'h et al. 2020b), suggesting presence of *B. napus* driven processes that lead to conservation of core taxa.

The persistence of core-hub communities across *B. napus* growth stages suggests that these taxa may share stable, plant-driven niches and engage in interactions that support co-occurrence. Previous work in *B. napus* has shown fine-scale plant genetic influence on rhizosphere bacteria, which may contribute to the structuring of these networks (Taye et al. 2020). While mechanisms such as metabolic complementarity or stress mitigation have been proposed in other systems (Morris et al. 2011; Hallam and Mccutcheon 2015), our results primarily indicate that conserved core-hub taxa form the backbone of the rhizosphere network across development stages. Together, these findings suggest that both plant-driven selection and stable microbial associations likely contribute to the observed conservation of core-hub communities.

Insights from the conserved core-hub communities identified in the *B. napus* rhizosphere provide a framework for designing targeted bacterial–bacterial and plant–microbe interaction studies. The repeated occurrence of specific taxa as highly interconnected nodes across growth stages could suggest that these genera might play consistent structural roles in rhizosphere network assembly (Agler et al. 2016; Layeghifard et al. 2017). However, whether these core-hub communities represent functionally coordinated groups or arise from shared plant-driven niches cannot be resolved from network inference alone. Experimental validation using cultured isolates and synthetic communities will be required to disentangle the mechanisms underlying their nonrandom aggregation and to assess their implication for plant health and ecosystem services. Such integration of network-based inference with microbiological experimentation is a critical next step toward evidence-based microbiome optimization.

4.5. Potential driver bacterial genera shaping *B. napus* rhizosphere microbial assembly

This study demonstrated that both conserved and growth stage-specific driver genera play roles in the rewiring of bacterial interactions in the *B. napus* rhizosphere (Figs. 3 and 4, Table S5). Across growth stage transitions, we identified 10 shared driver genera, along with 5 and 20 driver genera exclusive to the vegetative–flowering and flowering–maturity transitions, respectively. The presence of shared driver genera across growth stages suggests that a subset of bacterial genera consistently occupy crucial positions within the canola rhizosphere microbial network, whereas the presence of growth-stage exclusive drivers indicate dynamic reorganization of microbial interactions during plant development. Whether the exclusive driver taxa identified at each growth stage follow changes in plant development or are triggers for plant physiological changes cannot be resolved from network inference alone and requires experimental validation.

The shared driver genera tended to remain in same community across growth stages, suggesting stable network organization. For example, *Pedobacter*, *Kaistobacter*, *Microbacterium*, *Microtholunatus*, *Nitrospira*, *Reyranella*, *Stenotrophomonas*, and *Steroidobacter* largely maintained community membership throughout development, as did *Couchioplanes* and *Labrys* (Fig. 3). This stability suggests that interactions among these

driver genera are conserved over time, while their connections with other members of the rhizosphere community shifts across growth stages. These patterns highlight a structured yet dynamic microbial assembly process and identify driver genera as priorities for future studies aimed at validating ecological interactions and identifying factors underlying network rewiring. In *Arabidopsis thaliana* and *Boechera stricta*, the rhizosphere microbial community has been shown to modulate the timing of flowering (Wagner et al. 2014; Lu et al. 2018), and transplantation of the rhizosphere microbiome from *A. thaliana* to *Brassica rapa* caused a shift in flowering phenology (Panke-Buisse et al. 2014). Given that *B. napus* and *A. thaliana* are both members of the Brassicaceae, similar mechanisms linked to flowering are possible in *B. napus*. Notably, several driver genera identified here, including *Flavobacterium*, *Mycobacterium*, *Pedobacter*, *Pseudomonas*, and *Steroidobacter* have also been recently shown to be associated with canola yield in recent field studies (Li et al. 2023), supporting their relevance to crop performance.

5. Conclusion

In conclusion, we have shown here that growth stage alters *B. napus* rhizosphere microbial community assembly, spanning from indicator taxa shifts to putative functional transitions and network restructuring. Early growth stages were characterized by enrichment of microbial taxa associated with nitrogen fixation and denitrification potential. By flowering, nitrate reduction-associated profiles peaked, coinciding with high plant nitrogen demand, while maturity was marked by greater enrichment of microbial taxa linked to carbon-degrading potential. These transitions occurred within conserved yet dynamic microbial networks, where core-hub communities persisted across development and driver genera modulated stage-specific community rewiring, with overall microbial network complexity highest at flowering. The importance of the *B. napus* microbiome for productivity, including seed yield is reported (Mamet et al. 2022; Li et al. 2023). This suggests the possibility to incorporate ecological considerations for microbiome optimization besides the individual differentially abundant and core bacterial genera considerations (Taye et al. 2020). Combining both the individual bacteria and community perspectives can be complementary and can lead to a better understanding of *B. napus* rhizosphere microbial assembly and ultimately the optimization of positive plant–microbial interrelationships. Notably, these microbial changes occurred under a fertilization regime that included fall and spring application. Given that most fertilizer-derived nitrogen would have been available in early growth, the enrichment of nitrate-reducing functions at flowering highlights uncertainties in the timing and transformation of nitrogen in the rhizosphere. Future studies coupling microbial functional profiling with direct measurements of soil nitrogen pools and fluxes, under tailored fertilization strategies, will be critical for optimizing *B. napus* performance through microbiome-informed management. This study also identifies a cohort of core-hub communities and driver genera that represent valuable targets for future dissection of microbial assembly processes and their potential

application in canola management. Finally, while the present study focused on growth stage-driven microbial assembly patterns across diverse canola genotypes, future work explicitly examining genotype-specific network structures will be important to resolve how plant genetic variation shapes microbial interactions and to further refine microbiome-based strategies for canola improvement.

Acknowledgements

We thank Steve Shirlcliffe, Alix Schebel, Shanay Williams-Johnson, Zayda Morales, Jennifer Bell, Cordell Vandenderen, Yolanda Iannucci, Sarah Valentine, Triann Littlepine, Jennifer Dechka, Kira Blomquist, and many others for field and laboratory assistance.

Article information

History dates

Received: 31 August 2025

Accepted: 12 January 2026

Accepted manuscript online: 23 January 2026

Version of record online: 27 February 2026

Copyright

© 2026 The Authors. This work is licensed under a [Creative Commons Attribution 4.0 International License](#) (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Data availability

The datasets generated for this study can be accessed from the NCBI Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA575004>) under accession number PRJNA575004.

Author information

Author ORCIDs

Zelalem M. Taye <https://orcid.org/0000-0002-3923-0968>

Bobbi L. Helgason <https://orcid.org/0000-0003-1664-8250>

Eric G. Lamb <https://orcid.org/0000-0001-5201-4541>

Author contributions

Conceptualization: ZMT, BLH, SDS, EGL

Data curation: ZMT

Formal analysis: ZMT

Funding acquisition: BLH, SDS, EGL

Investigation: ZMT

Methodology: ZMT, BLH, SDS, EGL

Project administration: BLH, SDS, EGL

Supervision: EGL

Validation: ZMT

Visualization: ZMT

Writing – original draft: ZMT

Writing – review & editing: ZMT, BLH, SDS, EGL

Competing interests

The authors declare that they have no competing interests.

Funding information

This study is supported by a grant from the Plant Phenotyping and Imaging Research Centre (P2IRC) and a Saskatchewan Wheat Development Commission Scholarship to ZT. P2IRC is a digital agriculture research center funded by the Canada First Research Excellence Fund (CFRCF) from Natural Science Engineering Research Council (NSERC), managed by the Global Institute for Food Security (GIFS), and located at the University of Saskatchewan (U of S).

Supplementary material

Supplementary data are available with the article at <https://doi.org/10.1139/cjss-2025-0084>.

References

- Agler, M.T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.-T., Weigel, D., and Kemen, E.M. 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol.* **14**: e1002352. doi:[10.1371/journal.pbio.1002352](https://doi.org/10.1371/journal.pbio.1002352). PMID: 26788878.
- Asad, N.I., Wang, X.-B., Dozois, J., Azarbad, H., Constant, P., and Yergeau, E. 2022. Early season soil microbiome best predicts wheat grain quality. *FEMS Microbiol. Ecol.* **99**: 1–13. doi:[10.1093/femsec/fiac144](https://doi.org/10.1093/femsec/fiac144).
- Bazghaleh, N., Bell, J.K., Mamet, S.D., Moreira, Z.M., Taye, Z.M., Williams, S., et al. 2020. An intensive multilocation temporal dataset of fungal and bacterial communities in the root and rhizosphere of *Brassica napus*. *Data Brief*, **31**, 106143. doi:[10.1016/j.dib.2020.106143](https://doi.org/10.1016/j.dib.2020.106143).
- Bell, J.K., Mamet, S.D., Helgason, B., and Siciliano, S.D. 2022. *Brassica napus* bacterial assembly processes vary with plant compartment and growth stage but not between lines. *Appl. Environ. Microbiol.* **88**. doi:[10.1128/aem.00273-22](https://doi.org/10.1128/aem.00273-22). PMID: 35481756.
- Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver, E., Van Themaat, L., and Schulze-Lefert, P. 2013. Structure and functions of the bacterial microbiota of plants. *Annu. Rev. Plant Biol.* **64**: 807–838. doi:[10.1146/annurev-arplant-050312-120106](https://doi.org/10.1146/annurev-arplant-050312-120106). PMID: 23373698.
- Cardona, C., Weisenhorn, P., Henry, C., and Gilbert, J.A. 2016. Network-based metabolic analysis and microbial community modeling. *Curr. Opin. Microbiol.* **31**: 124–131. doi:[10.1016/j.mib.2016.03.008](https://doi.org/10.1016/j.mib.2016.03.008). PMID: 27060776.
- Chaparro, J.M., Badri, D. V., and Vivanco, J.M. 2014. Rhizosphere microbiome assemblage is affected by plant development. *ISME J.* **8**: 790–803. doi:[10.1038/ismej.2013.196](https://doi.org/10.1038/ismej.2013.196). PMID: 24196324.
- De Cáceres, M., and Legendre, P. 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology*, **90**: 3566–3574. *Ecology*. doi:[10.1890/08-1823.1](https://doi.org/10.1890/08-1823.1).
- De Cáceres, M., Legendre, P., Wiser, S.K., and Brotons, L. 2012. Using species combinations in indicator value analyses. *Methods Ecol. Evol.* **3**: 973–982. doi:[10.1111/j.2041-210X.2012.00246.x](https://doi.org/10.1111/j.2041-210X.2012.00246.x).
- Dixon, M.M., Afkairin, A., Manter, D.K., and Vivanco, J. 2024. Rhizosphere microbiome co-occurrence network analysis across a tomato domestication gradient. *Microorganisms*, **12**: 1756. doi:[10.3390/microorganisms12091756](https://doi.org/10.3390/microorganisms12091756). PMID: 39338431.
- Farwell, A.J., Vesely, S., Nero, V., Rodriguez, H., Shah, S., Dixon, D.G., and Glick, B.R. 2006. The use of transgenic canola (*Brassica napus*) and plant growth-promoting bacteria to enhance plant biomass at a nickel-contaminated field site. *Plant Soil*, **288**: 309–318. doi:[10.1007/s11104-006-9119-y](https://doi.org/10.1007/s11104-006-9119-y).
- Floc'h, J.B., Hamel, C., Harker, K.N., and St-Arnaud, M. 2020a. Fungal communities of the canola rhizosphere: keystone species and substantial between-year variation of the rhizosphere microbiome. *Microb. Ecol.* **1**–16. doi:[10.1007/s00248-019-01475-8](https://doi.org/10.1007/s00248-019-01475-8).
- Floc'h, J.-B., Hamel, C., Lupwayi, N., Harker, K.N., Hijri, M., and St-Arnaud, M. 2020b. Bacterial communities of the canola rhizosphere: network

- analysis reveals a core bacterium shaping microbial interactions. *Front. Microbiol.* **11**: 1587. doi:10.3389/fmicb.2020.01587.
- Foster, E.A., Franks, D.W., Morrell, L.J., Balcomb, K.C., Parsons, K.M., van Ginneken, A., and Croft, D.P. 2012. Social network correlates of food availability in an endangered population of killer whales, *Orcinus orca*. *Anim. Behav.* **83**: 731–736. doi:10.1016/j.anbehav.2011.12.021.
- Fuhrman, J.A. 2009. Microbial community structure and its functional implications. *Nature*, **459**: 193–199. doi:10.1038/nature08058. PMID: 19444205.
- Grady, E.N., MacDonald, J., Liu, L., Richman, A., and Yuan, Z.C. 2016. Current knowledge and perspectives of *Paenibacillus*: a review. *Microb. Cell Fact.* **15**: 1–18. doi:10.1186/s12934-016-0603-7.
- Hallam, S.J., and Mccutcheon, J.P. 2015. Microbes don't play solitaire: how cooperation trumps isolation in the microbial world. *Environ. Microbiol. Rep.* **7**: 26–28. doi:10.1111/1758-2229.12248. PMID: 25721597.
- Hardoim, P.R., van Overbeek, L.S., Berg, G., Pirttilä, A.M., Compant, S., Campisano, A., et al. 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev.* **79**: 293–320. doi:10.1128/MMBR.00050-14. PMID: 26136581.
- Hinsinger, P., Bengough, A.G., Vetterlein, D., and Young, I.M. 2009. Rhizosphere: biophysics, biogeochemistry and ecological relevance. *Plant Soil*, **321**: 117–152. doi:10.1007/s11104-008-9885-9.
- Hirano, H., and Takemoto, K. 2019. Difficulty in inferring microbial community structure based on co-occurrence network approaches. *BMC Bioinf.* **20**: 329. doi:10.1186/s12859-019-2915-1.
- Hirel, B., Le Gouis, J., Ney, B., and Gallais, A. 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot.* **58**: 2369–2387. doi:10.1093/jxb/erm097. PMID: 17556767.
- Kim, Y.C., Leveau, J., McSpadden Gardener, B.B., Pierson, E.A., Pierson, L.S., and Ryu, C.M. 2011. The multifactorial basis for plant health promotion by plant-associated bacteria. *Appl. Environ. Microbiol.* **77**: 1548–1555. doi:10.1128/AEM.01867-10. PMID: 21216911.
- Kraiser, T., Gras, D.E., Gutiérrez, A.G., González, B., and Gutiérrez, R.A. 2011. A holistic view of nitrogen acquisition in plants. *J. Exp. Bot.* **62**: 1455–1466. doi:10.1093/jxb/erq425. PMID: 21239377.
- Kuntal, B.K., Chandrakar, P., Sadhu, S., and Mande, S.S. 2019. 'NetShift': a methodology for understanding 'driver microbes' from healthy and disease microbiome datasets. *ISME J.* **13**: 442–454. doi:10.1038/s41396-018-0291-x. PMID: 30287886.
- Kuntal, B.K., Ghosh, T.S., and Mande, S.S. 2013. Community-analyzer: a platform for visualizing and comparing microbial community structure across microbiomes. *Genomics*, **102**: 409–418. doi:10.1016/j.ygeno.2013.08.004. PMID: 23978768.
- Lai, D., Lu, H., and Nardini, C. 2010. Enhanced modularity-based community detection by random walk network preprocessing. *Phys. Rev. E*, **81**: 066118. doi:10.1103/PhysRevE.81.066118.
- Lay, C., Bell, T.H., Hamel, C., Harker, K.N., Mohr, R., Greer, C.W., et al. 2018. Canola root-associated microbiomes in the Canadian Prairies. *Front. Microbiol.* **9**: 1188. doi:10.3389/fmicb.2018.01188. PMID: 29937756.
- Layeghifard, M., Hwang, D.M., and Guttman, D.S. 2017. Disentangling interactions in the microbiome: a network perspective. *Trends Microbiol.* **25**: 217–228. doi:10.1016/j.tim.2016.11.008. PMID: 27916383.
- Li, Y., Vail, S.L., Arcand, M.M., and Helgason, B. 2023. Contrasting nitrogen fertilization and *Brassica napus* (canola) variety development impact recruitment of the root-associated microbiome. *Phytobiomes J.* **7**: 125–137. doi:10.1094/PHYBIOMES-07-22-0045-R.
- Liu, C., Cui, Y., Li, X., and Yao, M. 2021. microeco: an R package for data mining in microbial community ecology. *FEMS Microbiol. Ecol.* **97**: 255. doi:10.1093/femsec/fiaa255.
- Louca, S., Parfrey, L.W., and Doebeli, M. 2016. Decoupling function and taxonomy in the global ocean microbiome. *Science* (1979), **353**: 1272–1277. doi:10.1126/SCIENCE.AAF4507/SUPPL_FILE/LOUCA.SM.PDF.
- Lu, T., Ke, M., Lavoie, M., Jin, Y., Fan, X., Zhang, Z., et al. 2018. Rhizosphere microorganisms can influence the timing of plant flowering. *Microbiome*, **6**: 231. doi:10.1186/s40168-018-0615-0. PMID: 30587246.
- Mamet, S.D., Helgason, B.L., Lamb, E.G., McGillivray, A., Stanley, K.G., Robinson, S.J., et al. 2022. Phenology-dependent root bacteria enhance yield of *Brassica napus*. *Soil Biol. Biochem.* **166**: 108468. doi:10.1016/j.soilbio.2021.108468.
- Mimmo, T., Hann, S., Jaitz, L., Cesco, S., Gessa, C.E., and Puschenreiter, M. 2011. Time and substrate dependent exudation of carboxylates by *Lupinus albus* L. and *Brassica napus* L. *Plant Physiol. Biochem.* **49**: 1272–1278. doi:10.1016/j.plaphy.2011.08.012. PMID: 22000050.
- Morris, J.J., Johnson, Z.I., Szul, M.J., Keller, M., and Zinser, E.R. 2011. Dependence of the cyanobacterium *prochlorococcus* on hydrogen peroxide scavenging microbes for growth at the ocean's surface. *PLoS ONE*, **6**: e16805. doi:10.1371/journal.pone.0016805. PMID: 21304826.
- Newman, M.E.J. 2006. Modularity and community structure in networks. *Proc. Natl. Acad. Sci.* **103**: 8577–8582. doi:10.1073/pnas.0601602103. PMID: 16723398.
- Nguyen, C. 2003. Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie*, **23**: 375–396. doi:10.1051/agro:2003011.
- Ortiz-Castro, R., Contreras-Cornejo, H.A., Macías-Rodríguez, L., and López-Bucio, J. 2009. The role of microbial signals in plant growth and development. *Plant. Signal. Behav.* **4**: 701–712. doi:10.4161/psb.4.8.9047.
- Panke-Buisse, K., Poole, A.C., Goodrich, J.K., Ley, R.E., and Kao-Kniffin, J. 2014. Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME J.* **9**: 980–989. doi:10.1038/ismej.2014.196.
- Philippot, L., Raaijmakers, J.M., Lemanceau, P., and van der Putten, W.H. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* **11**: 789–799. doi:10.1038/nrmicro3109. PMID: 24056930.
- Ren, D., Madsen, J.S., Sørensen, S.J., and Burmølle, M. 2015. High prevalence of biofilm synergy among bacterial soil isolates in cocultures indicates bacterial interspecific cooperation. *ISME J.* **9**: 81–89. doi:10.1038/ismej.2014.96. PMID: 24936766.
- Rosenberg, E. 2014. The Family Chitinophagaceae. In *The Prokaryotes: other major lineages of bacteria and the Archaea*. Edited by E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt and F. Thompson. Springer-Verlag, Berlin Heidelberg. pp. 493–495. doi:10.1007/978-3-642-38954-2_137/FIGURES/1.
- Schwager, E., Weingart, G., Bielski, C., and Huttenhower, C. 2014. CCPEPE : compositionality corrected by permutation and renormalization. *R*. 1–16.
- Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., and Firestone, M.K. 2016. The interconnected rhizosphere: high network complexity dominates rhizosphere assemblages. *Ecol. Lett.* **19**: 926–936. doi:10.1111/ele.12630. PMID: 27264635.
- Shobade, S.O., Zabolina, O.A., and Nilsen-Hamilton, M. 2024. Plant root associated chitinases: structures and functions. *Front. Plant Sci.* **15**: 1344142. doi:10.3389/fpls.2024.1344142. PMID: 38362446.
- Soil Classification Working Group. 1998. The Canadian system of soil classification. 3rd ed. Agriculture and Agri-Food Canada Publication 1646: 187.
- Taye, Z.M., Helgason, B.L., Bell, J.K., Norris, C.E., Vail, S., Robinson, S.J., et al. 2020. Core and differentially abundant bacterial taxa in the rhizosphere of field grown *Brassica napus* genotypes: implications for Canola breeding. *Front. Microbiol.* **10**: 2019.03007. PMID: 32010086.
- Taye, Z.M., Noble, K., Siciliano, S.D., Helgason, B.L., and Lamb, E.G. 2022. Root growth dynamics, dominant rhizosphere bacteria, and correlation between dominant bacterial genera and Root traits through *Brassica napus* development. *Plant Soil*, **473**: 441–456. doi:10.1007/s11104-022-05296-6.
- Town, J.R., Dumonceaux, T., Tidemann, B., and Helgason, B.L. 2023. Crop rotation significantly influences the composition of soil, rhizosphere, and root microbiota in canola (*Brassica napus* L.). *Environ. Microbiome*. **18**: 40. doi:10.1186/s40793-023-00495-9.
- Wagner, M.R., Lundberg, D.S., Coleman-Derr, D., Tringe, S.G., Dangl, J.L., and Mitchell-Olds, T. 2014. Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild *Arabidopsis* relative. *Ecol. Lett.* **17**: 717–726. doi:10.1111/ele.12276. PMID: 24698177.
- Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., et al. 2009. Three genomes from the phylum acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Appl. Environ. Microbiol.* **75**: 2046–2056. doi:10.1128/AEM.02294-08. PMID: 19201974.
- Wiegel, J., Tanner, R., and Rainey, F.A. 2006. An introduction to the Family clostridiaceae. In *The Prokaryotes*. Edited by M. Dworkin, S. Falkow,

- E. Rosenberg and K.-H. Schleifer. Springer, New York, NY. pp. 654–678. doi:[10.1007/0-387-30744-3_20](https://doi.org/10.1007/0-387-30744-3_20).
- Yang, Z., Algesheimer, R., and Tessone, C.J. 2016. A comparative analysis of community detection algorithms on artificial networks. *Sci. Rep.* **6**: 30750. doi:[10.1038/srep30750](https://doi.org/10.1038/srep30750). PMID: [27476470](https://pubmed.ncbi.nlm.nih.gov/27476470/).
- Zhalnina, K., Louie, K.B., Hao, Z., Mansoori, N., Da Rocha, U.N., Shi, S., et al. 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat. Microbiol.* **3**: 470–480. doi:[10.1038/s41564-018-0129-3](https://doi.org/10.1038/s41564-018-0129-3). PMID: [29556109](https://pubmed.ncbi.nlm.nih.gov/29556109/).