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RESEARCH ARTICLE

Plant species are more important to bacterial community composition than seeding ratio in a pea-canola intercropping system

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Societal Impact Statement

Demand for agricultural land has increased due to rising demand for food, potentially leading to expansion into previously untouched habitats, which threatens refugia of native plants. We aimed to provide insight into how variation in seeding ratio may impact soil bacterial communities in a pea-canola intercropping system, potentially altering crop efficiency. We found a high level of variation in bacterial communities across seeding ratios, demonstrating that changes to aboveground diversity can unpredictably impact belowground communities. Information on how management practices impact bacterial communities may be used by agronomists to design efficient intercropping systems, reducing the need for agricultural expansion.

Summary

- The pea-canola (*Pisum sativum–Brassica napus*) intercropping system (peaola) is a high yielding mixed intercropping system that is being studied to improve agricultural sustainability and productivity. Plant-microbe interactions have been considered important to intercrop success, but it is unknown how these dynamics change based on crop seeding ratios. The goal of this study was to determine how seeding ratio and cropping system impact the diversity, structure, and predicted function of the bacterial community in peaola. We also determined how nitrogen availability in soil and pea and canola leaf nitrogen content were impacted across seeding ratios and cropping systems.
- We collected soil, rhizosphere, and mature leaf samples from peaola, with nine different seeding ratios, and monoculture pea and canola controls. Bacterial communities in soil and rhizosphere were characterized using 16S rRNA, and soil and leaf nitrogen content were measured.
- Intercropping increased the nitrogen content of mature canola leaves, suggesting changes to nitrogen availability. Plant influence on the bacterial community was determined to be the primary contributor to community structure and function.

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Overall, no clear patterns were observed between seeding ratios and the bacterial communities.

- High variability in our results highlights that changes to aboveground diversity may have unpredictable impacts on belowground diversity, making it difficult to make management decisions. Our observations may also be due to additional factors beyond the ratio of pea:canola planted, or a result of sampling variation.

KEYWORDS
facilitation, intercropping, nitrogen, plant-microbe interactions, rhizosphere, seeding ratio, soil bacterial communities

1 | INTRODUCTION

Intercropping, the practice of growing two or more crops with spatial and temporal overlap, has been found to improve the productivity of agricultural land (Dowling et al., 2021; Parks & Friesen, 2025). Recently, there has been an increase in focus on intercropping systems to improve agricultural sustainability and productivity (Homulle et al., 2022). Increases in land productivity have been shown, with studies on intercropping systems reporting land equivalent ratios (LER) greater than the baseline of 1 (Dowling et al., 2021; Homulle et al., 2022). Intercropping success can be impacted by biotic and abiotic factors, particularly nutrient availability and plant–plant interactions (competition or facilitation).

The productivity of intercropping systems has been found to change with varying seeding rates and ratios (Dowling et al., 2021). These results varied with intercropping system, showing that there is not one universal ratio to use when optimizing crop management (Bakhtiyari et al., 2020; Blaser et al., 2006; Yilmaz et al., 2015). Some approaches to finding optimal system yields have included inoculation of plant growth promoting microorganisms (Pourjani et al., 2022; Rezaei-Chiyaneh et al., 2021). To our knowledge, however, few studies have investigated how the seeding ratio within an intercropping system alters the bacterial community and if these changes could contribute to changes in crop productivity.

Alterations to the seeding ratio of included crops in an intercropping system have the potential to alter plant–plant interactions. Intercropping alone can impact how essential plant nutrients, such as nitrogen, are distributed throughout the intercropping system due to plant–plant competition for resources, which can impact the bacterial community (Parks et al., 2025). The exclusion of competitors, such as weeds, can have beneficial impacts on both crops involved in the intercropping system (Hauggaard-Nielsen et al., 2001). In situations where crops are planted with increased density, competition for resources can increase, altering how plants acquire nutrients within the system (Andersen, 2005; Hauggaard-Nielsen et al., 2006; Zhu et al., 2022, 2023). We know that plants alter their soil bacterial communities in order to cope with biotic and abiotic stressors, largely through the production of root exudates, but it is not clear the degree and direction to which these changes could be occurring in an intercropping system at various seeding ratios (Bakker et al., 2018; Ulbrich et al., 2022; Wang et al., 2021). Understanding how seeding ratios impact the bacterial community in intercropping systems is necessary in order for us to design more efficient and productive intercropping systems in the future. Therefore, the goal of this study was to determine how seeding ratio impacts the bacterial community in a mixed pea-canola (*Pisum sativum–Brassica napus*) system (peaola).

To answer this overarching question, we aimed to determine how variation in seeding ratios and cropping system (i.e., intercropped vs. monoculture) impacted (1) the diversity and structure of soil and rhizosphere bacterial communities; (2) predicted functional diversity, with a specific focus on nitrogen cycling genes, of rhizosphere and soil bacterial communities; and (3) soil nitrogen availability and pea and canola nutrient uptake. We expected to see an increase in the diversity of the rhizosphere bacterial community of pea in treatments where the pea:canola seeding ratio was smaller as canola is known to be a more competitive crop (Dowling et al., 2021). We expected there would be an increase in predicted functional diversity of the pea rhizosphere bacterial community in treatments where the pea:canola seeding ratio was smaller, specifically with increases in genes predicted to be involved in nitrogen cycling, as a result of increased competition for resources. We expected treatments with larger pea:canola seeding ratios would see a decrease in the predicted functional diversity of the canola rhizosphere bacterial community, specifically in the presence of genes predicted to be involved in nitrogen cycling as facilitation between pea and canola could decrease the need for canola to seek its own nitrogen resources. Facilitation and complementation has been observed between pea and canola depending on the seeding ratio and intercropping system used (Dowling et al., 2021; Fletcher et al., 2015). Consequently, we expected that soil nitrogen availability and plant uptake would be highest in treatments with larger pea:canola seeding ratios due to their relationship with rhizobia and other nitrogen fixing bacteria.

2 | MATERIALS AND METHODS

2.1 | Field plot design

Plots (1.7 × 9 m) were seeded in late August of 2021 at a WSU research farm near Davenport, WA (47.652333, −118.135750) into

no-till winter wheat chemical fallow using a no-till Cross Slot drill. The winter pea variety Goldenwood (ProGene Plant Research, LLC) and the canola variety Plurax (Rubisco Seeds) were used. Peas and canola were planted in the same row at the same time, resulting in variable spacing between pea and canola for the intercropping system. Treatments were replicated across four blocks. Seeding rate was varied within the intercropping system, resulting in nine different pea:canola seeding ratios (Table S1). At planting, 67 kg N ha^{-1} was applied along with 34 kg P_2O_5 ha^{-1} to all plots. No other fertilizer was applied between planting and sample collection.

2.2 | Field sampling and rhizosphere harvesting

Sampling of the plots occurred on May 11, 2022, which corresponded with early flowering. On the day of sampling, the high temperature was 13.9°C and the low temperature was −1.6°C. There was 0 cm of precipitation. Three sampling locations were identified across the center of each plot resulting in 12 replicates per treatment (Table S1; Figure S1). Some treatments had plots with dead zones resulting in fewer rhizosphere samples for those groups, which is shown in Table S1 under "Rhizosphere Replicates" (Figure S1). Field sampling and rhizosphere harvest were performed as described in Parks et al. (2025, 2026). For each plot, three soil cores were collected at three different locations from the center of the plot (Figure S1). A maximum depth of 10 cm was used. These samples are referenced as bulk soil throughout the manuscript. Root samples were collected from the same locations as soil cores (Figure S1). In monoculture plots, one root was taken per sampling site, while in peaola plots, one pea root and one canola root were taken per sampling site (Figure S1). Samples were placed in a cooler and transported to the lab where soil samples were stored at −20°C until DNA extraction. Rhizosphere was harvested from roots into rhizosphere buffer (10.765 g NaH_2PO_4, 17.32 g $NaHPO_4$, 700-mL dH_2O, pH 7, dH_2O to bring final volume to 1 L, and 1 mL of 50% tween-20) as described in Parks et al. (2025, 2026) within 24 h of sample collection. Twenty milliliters of rhizosphere buffer was used for collection. Samples were vortexed at maximum speed for 1 min then sonicated for 1 min to ensure rhizosphere soil was removed from the roots. Roots were removed, and rhizosphere samples were stored at −80°C until DNA extraction.

2.3 | DNA extraction and sequencing

DNA extraction and sequencing were performed as described in previous studies (Madsen et al., 2022; Parks et al., 2025). The QIAGEN® MagAttract® PowerSoil® DNA KF Kit was used for DNA extraction using the Kingfisher Flex system following the Earth Microbiome Project's protocol (Schwartz et al., 2018). No-sample blanks were included on each plate. A high-sensitivity dsDNA kit from Qubit was used for DNA quantification following the manufacturer's protocol. Test amplification of the 16S V4 region was done using the primers 515F: 5′-GTGCCAGCMGCCGCGGTAA-3′ and 806R: 5′-GGACTACHVGGGTWTCTAAT-3′ with Thermo Scientific DreamTaq DNA Polymerase following the manufacturer's protocol (Caporaso et al., 2011). Thermocycler parameters were denaturation at 95°C for 3 min; 30 cycles of 95°C for 45 s, 50°C for 60 s, and 72°C for 90 s; final elongation at 72°C for 10 min; and an infinite hold at 15°C matching the protocol used by the Michigan State University Research Technology Support Facility. Agarose gel electrophoresis was performed to ensure amplicons of the correct size (~300 bp) were yielded. Illumina amplicon sequencing of the 16S V4 region was done at the Michigan State University Research Technology Support Facility. The MiSeq v2 Standard platform was used to generate 250-bp paired-end reads. Sequencing depth and accuracy were tested by the inclusion of a microbial standard (ZymoBIOMICS Microbial Community Standard II [Log Distribution]). Across sequencing runs, four microbial community standards were included and showed we were able to accurately and consistently detect bacteria down to a theoretical abundance of 0.069%. A negative control was added per plate by the sequencing center to detect contamination equating to 32 negative controls across all sequencing runs. Thirteen of the included negative controls were found to contain reads, with 54 total reads being produced from our negative controls. Therefore, out of the 6,706,352 reads produced, reads from our negative controls accounted for 0.00081% of our reads. amplicon sequencing variants (ASVs) found in negative controls were not removed from our samples and did not appear in any of our results for differentially abundant ASVs.

2.4 | Bacterial community analysis

QIIME2 version 2023.7 was used for sequence analysis on the Kamiak High Performance Computing Cluster at Washington State University as described in previous studies (Bolyen et al., 2019; Parks et al., 2025). In some cases, samples were resequenced with sequencing runs combined using "qiime feature-table group" using the mode sum in order to improve sequencing depth and quality. ASVs identified as mitochondria or chloroplasts were filtered out of all samples. Soil samples were analyzed at a read depth of 9292, and rhizosphere samples were analyzed at a read depth of 9722 to determine the diversity metrics of the population. Alpha-rarefaction plots were generated using a maximum read depth of 19,000 for soil samples, and 16,809 for rhizosphere samples showing that the depth of our analyses were sufficient to represent the diversity within our samples (Figure S2a,b). Analysis was done as described in previous studies (Parks et al., 2025). RStudio version 2022.12.0 + 353 with R version 4.2.1 was used for further analyses throughout this paper. The packages tibble version 3.3.0, tidyverse version 2.0.0, and glue version 1.8.0 were used to manage datasets for figure production. MetBrewer version 0.2.0 was used to create color palettes. Boxplots and divergent barplots were generated using ggplot2 version 3.4.1 with ggpubr version 0.6.1 and patchwork version 1.3.1 being used to create multipanel graphs. Bray–Curtis dissimilarity matrices were made using QIIME2, with cmdscale and ggplot2 being used in RStudio to perform

Principal Coordinate Analysis (PCoA). Using ALDEx2 version 1.28.1 in RStudio, differential abundance analyses were performed at the genus level. Comparisons were made between monoculture pea and canola, intercropped pea and canola from the same seeding ratio, intercropped pea and monoculture pea with the same seeding rate of pea, intercropped canola and monoculture canola with the same seeding rate of canola, intercropped canola from different seeding ratios with the same canola seeding rate, and intercropped pea from different seeding ratios with the same pea seeding rate.

2.5 | Functional predictions analysis

Methods described in Parks et al. (2025, 2026) were used to make functional predictions first using PICRUSt2 in QIIME2 version 2023.7 (Douglas et al., 2020). Diversity metrics were generated for soil using a depth of 13,634,091 for Kyoto Encyclopedia of Genes and Genomes orthologs metagenome predictions (KOMP) and 15,371,548 for KOMPs in the rhizosphere. The full PICRUSt2 pipeline was run using PICRUSt2 version 2.5.2 on Miniconda3 version 3.10 on the Kamiak High Performance Computing Cluster. Functional predictions were added with the script "add_description.py" from PICRUSt2. Outputs from the full PICRUSt2 pipeline were used for differential abundance analysis using ALDEx2 version 1.28.1. The same comparisons were made as for differential abundance of genera. Differentially abundant genes were filtered for the inclusion of "nif," "nitrification," "fixation," and "nitrogen" in their descriptions to aid in the identification of nitrogen cycling genes.

2.6 | Statistical analysis: Bacterial community and functional predictions

Statistical analyses were performed as described in previous studies (Parks et al., 2025, 2026). Statistics related to alpha and beta-diversity metrics were generated using QIIME2. For alpha-diversity metrics, Kruskal–Wallis with Benjamini–Hochberg false discovery rate (FDR) were used. For beta-diversity metrics, pairwise permutational multivariate analysis of variance (PERMANOVA) with Benjamini–Hochberg FDR were used in QIIME2 using "qiime diversity adonis" with the formula "crop_system + crop_type + treatment + block" for rhizosphere samples. Crop system referred to monoculture or intercropped plots; crop type referred to canola, pea, intercropped pea, or intercropped canola; treatment referred to the seeding ratio used; and block referred to which block the samples were in (i.e., 1, 2, 3, or 4). Pea and canola rhizosphere samples from peaola plots were analyzed as separate groups (i.e., as intercropped canola and intercropped pea) for all statistical analyses due to them being significantly different from each other. Sample sizes are shown in Table S1. Statistical analyses performed for differential abundance analyses included Kruskal–Wallis with Benjamini–Hochberg-adjusted *p*-values. As recommended by documentation for ALDEx2, a corrected *p*-value less than 0.1 was used to determine if genera and KOMPs were differentially abundant (Gloor, 2018).

2.7 | Soil ammonium, nitrate, and nitrite extraction and analysis

Analysis of soil ammonium, nitrate, and nitrite was performed on bulk soil samples that were collected using the same sampling methods as described for bacterial community analysis. These samples were collected at the same time. Methods described for extractions in Parks et al. (2025, 2026) were used. For ammonium, these included methods from Sinsabaugh et al. (2000). Standards ranging from 0 to 2 ppm NH_4^+ were made by diluting 100 ppm NH_4^+ (0.23585-g ultrapure $[NH_4^+]_2SO_4$ and 500 mL 0.5 M K_2SO_4). A check standard (1 ppm NH_4^+) to ensure the accuracy of the calibration curve was made from a fresh 100-ppm NH_4^+ solution using the previously described reagents. A negative control of only 0.5 M K_2SO_4 was added to each plate. Samples were diluted 1:3 and analyzed for ammonium content as described in Parks et al. (2025, 2026). This ensured our samples would be within the detection limit. Forty microliters of each component (samples, calibration curve samples, check standard, and negative control) were added to a 96-well plate in analytical triplicates. Reagent packets marketed for water testing kits (salicylate reagent—Fisher part no. 09-053-242; cyanurate reagent—Fisher part no. 09-053-241) were dissolved in 5-mL nanopure H_2O (npH_2O) for spectrophotometric quantification. Forty microliters of salicylate reagent were added to each well and allowed to react for 3 min with 40 µL of the cyanurate reagent added after. Samples were allowed to react for 20 min before being measured on a SpectraMax M2e microplate reader measuring an absorbance of 670 nm.

Methods described in NECi Method N07-0003 (2014) were used for the analysis of nitrate and nitrite. Standards ranging from 0 to 2 ppm NO_3^- were created from a 100-ppm NO_3^- stock (0.3609-g ultrapure KNO_3, 500-mL npH_2O). A check standard (1 ppm NO_3^-) to ensure the accuracy of the calibration curve was made from a fresh 100 ppm NO_3^- solution using the previously described reagents. All standards were protected from light and kept at −20°C until use. A negative control of only 0.5 M K_2SO_4 was added to each plate. Samples were diluted 1:4 and analyzed as described in Parks et al. (2025, 2026) to ensure our samples would be within the detection limit. Ten microliters of each component (samples, calibration curve samples, check standard, and negative control) were added to a 96-well plate in analytical triplicates. Following the manufacturer's protocol, nitrate reductase enzyme from NECi Superior Enzymes (AtNaR2, EC 1.7.1.1) was reconstituted. Ninety microliters of nitrate reductase working solution (1-mL concentrated nitrate reductase enzyme and 9-mL phosphate buffer pH 7.5) was added to each well. Twenty-four microliters of NADH working solution (1 mL 0.2% NADH in 9-mL phosphate buffer pH 7.5) was added to each well. Phosphate buffer was made using 3.75 g KH_2PO_4, 1.4 g KOH, 1 L 25 mM Na_2EDTA. A 1-h incubation occurred on a shaker table at 37°C and 25 rpm. Parafilm was used to prevent evaporation. Fifty microliters of 1% SULF (5-g sulfanilamide, 150 mL 38% HCl, 350-mL npH_2O) were added after incubation. After 2 min, 50-µL NED solution was added (500 mg N-

[1-napthyl]etheylenediamine dihydrochloride, 500-mL npH2O) and allowed to react for 2 min. Using the same microplate reader as previously described, absorbances were measured at 545 nm.

Calculations and analyses were done as described in Parks et al. (2025, 2026). The equation of the linear curve ($y = mx + b$) was generated using the average absorbance for each known concentration of our standards with Y and X representing absorbance and concentration respectively. To correct for solvent absorbance, the average negative control absorbance was subtracted from each sample and accounted for in the equation. Original concentrations were calculated per sample using the generated equation and multiplied by the dilution ratio. As each plate contained its own calibration curve, calculations were performed per plate. The lm function (R stats package version 4.2.1) and the emmeans package version 1.8.9 were used to perform type two analysis of variance (ANOVA) and post hoc adjustment using Šidák correction, respectively, to explore significant differences across groups. Data were log transformed in order to achieve a normal distribution, with the residuals of each model being checked for normality using a Kolmogorov–Smirnov test through the olsrr package version 0.6.1. Two separate models were run each time, with the factors "crop" or "treatment" referring to if samples were from canola, pea, or peaola, and which pea:canola seeding ratio they belonged to. Boxplots were made using ggplot2 in RStudio.

2.8 | Elemental analysis

Samples were prepared for elemental analysis as described in Parks et al. (2026) on one mature leaf per shoot. Leaves were ground to a powder by placing leaves in 15-mL conical tubes with three 3-mm ball bearings into a paint shaker. Between 1.217 and 1.826 mg of powdered leaf tissue were tinned. Tin capsules with dimensions 5 × 3.5 mm (C11240.500P) from OEA labs were used. The Stable Isotope Core at Washington State University performed the C and N analyses, providing information on C and N percentages for each sample. The lm function (R stats package version 4.2.1) and the emmeans package version 1.8.9 were used to perform type two ANOVA and post hoc adjustment using Šidák correction, respectively. Data were log transformed to achieve a normal distribution, with the normality of residuals checked per model using a Kolmogorov–Smirnov test as part of the olsrr package version 0.6.1. To test changes caused in percent nitrogen content of leaves across crop types, the factor "crop" was included in the model referring to if leaves were from canola, pea, intercropped canola, or intercropped pea. To test if changes occurred in the percent nitrogen content of leaves across seeding rates in peaola, a subset of intercropped pea and a subset of intercropped canola were created. The model for both subsets was "c_seed * p_seed," with "c_seed" referring to the canola seeding rate and "p_seed" referring to the pea seeding rate. An additional model was also tested per subset which included the factor "seed_ratio" referring to the pea:canola seeding ratio. Boxplots were made using ggplot2 in RStudio.

3 | RESULTS

3.1 | Variation in pea:canola seeding ratio did not impact soil bacterial diversity and composition

Monoculture canola had an average of 722 ASVs. Monoculture pea had an average of 766 ASVs and peaola had an average of 716 ASVs. The relative abundance of taxa found at the class level is shown in Figure S3a. No significant differences were observed in the soil community using the alpha-diversity metric Shannon entropy nor Bray–Curtis dissimilarity to measure beta-diversity. No genera were identified as differentially abundant.

3.2 | Changes in seeding ratios caused some changes in the rhizosphere community diversity and structure

3.2.1 | No clear pattern was observed between changes in rhizosphere bacterial diversity and composition, and pea:canola seeding ratio

On average, 462 ASVs were observed in monoculture canola rhizosphere, while 457 were observed in intercropped canola. In monoculture pea, 396 ASVs were observed on average, while 356 were observed in intercropped pea. The relative abundance of taxa found at the class level is shown in Figure S3b. The rhizosphere bacterial community of intercropped canola was significantly more diverse than intercropped pea (Kruskal–Wallis, $H = 39.76$, $p < .0001$) and monoculture pea bacterial communities (Kruskal–Wallis, $H = 6.02$, $p = .028$; Figure 1). The monoculture canola bacterial community was more diverse than the intercropped pea bacterial community (Kruskal–Wallis, $H = 8.73$, $p = .0094$; Figure 1). Pea:canola seeding ratio alone did not significantly impact community diversity. When looking at the combined effects of crop type and seeding ratio, intercropped canola with a pea:canola seeding ratio of 33.59 was significantly more diverse than intercropped pea in all treatments except for pea:canola seeding ratios of 28.03 and 41.99 (Kruskal–Wallis, $p < .05$; Figure S4). Intercropped canola with pea:canola seeding ratios of 28.03 and 50.38 were significantly more diverse than intercropped pea in all treatments except for pea:canola seeding ratios of 22.42, 28.03, and 41.99 (Kruskal–Wallis, $p < .05$; Figure S4). Intercropped pea with a pea:canola seeding ratio of 33.59 was significantly less diverse than monoculture canola (Kruskal–Wallis, $H = 8.80$, $p = .032$; Figure S4). No significant differences were found between intercropped canola and canola and intercropped pea and pea regardless of treatment.

Crop type was the largest contributing factor to the variance observed within the data accounting for 16% (Table 1). Variation across blocks and seeding ratio both accounted for 4% of the variance, while crop system accounted for less than 1% (Table 1). Pea and canola had significantly different community compositions regardless of crop type (PERMANOVA, $p < .05$; Figure 2a). No significant differences were observed between like crops (i.e., intercropped pea and pea).

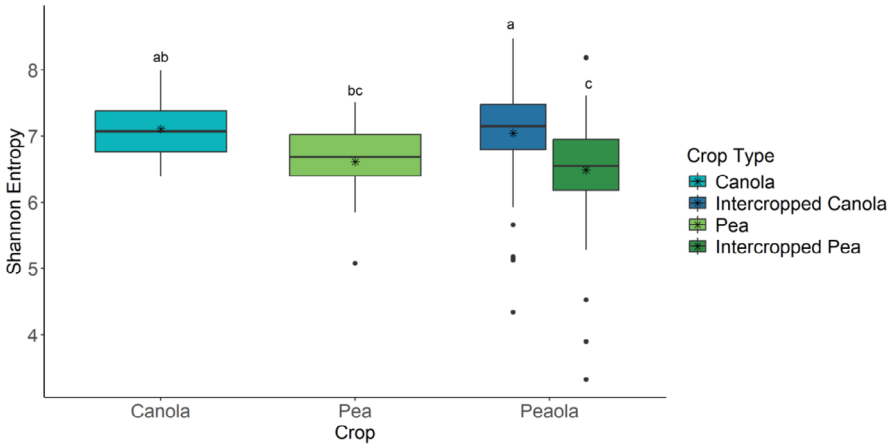


FIGURE 1 Boxplots showing the alpha-diversity (Shannon entropy) of the rhizosphere bacterial community across crop types. Different letters indicate significant differences. The bacterial community of monoculture canola was significantly more diverse than intercropped pea (Kruskal–Wallis, $H = 8.73$, $p = .0094$). Intercropped canola had a significantly more diverse bacterial community than intercropped pea (Kruskal–Wallis, $H = 39.76$, $p < .0001$) and monoculture pea (Kruskal–Wallis, $H = 6.02$, $p = .028$).

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Crop system	1.00	0.17	0.17	1.00	0.00	0.41
Crop type	2.00	7.57	3.79	22.74	0.16	0.00
Seeding ratio	8.00	1.88	0.24	1.41	0.04	0.00
Block	3.00	1.86	0.62	3.72	0.04	0.00
Residuals	217.00	36.14	0.17	NA	0.76	NA
Total	231.00	47.61	NA	NA	1.00	NA

Abbreviation: NA, not applicable.

TABLE 1 PERMANOVA table showing the impact of cropping system, crop type, seeding ratio, and block on variance amongst rhizosphere samples.

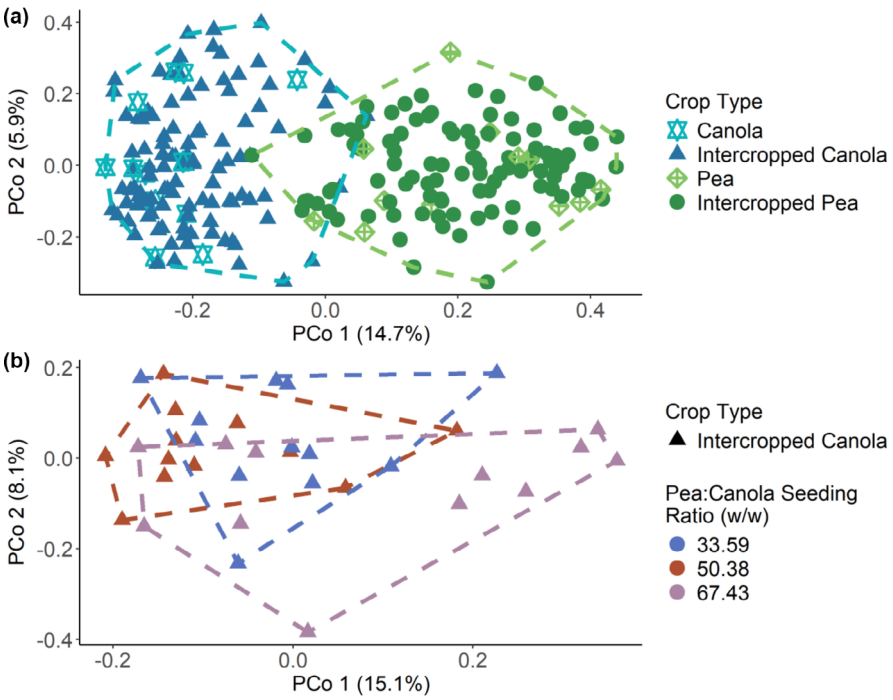


FIGURE 2 PCoAs showing the beta-diversity (Bray–Curtis dissimilarity) of the rhizosphere bacterial communities (a) across crop types, and (b) across pea:canola seeding ratios in canola only. In panel a, blue dashed lines outline canola and intercropped canola while green dashed lines outline pea and intercropped pea. Significant differences were observed in the community compositions of the pea and canola bacterial communities regardless of crop type or treatment (PERMANOVA, $p < .05$). In panel b, the blue, red, and purple dashed lines outline canola with pea:canola seeding ratios of 33.59, 50.38, and 67.43, respectively. Intercropped canola with a pea:canola seeding ratio of 50.38 was found to have a significantly different community composition than intercropped canola with pea:canola seeding ratios of 33.59 (PERMANOVA, $F = 1.51$, $p = .019$) and 67.43 (PERMANOVA, $F = 1.99$, $p = .013$).

Considering seeding ratio alone, the bacterial community of monoculture pea had a significantly different community composition from all other seeding ratios except for pea:canola seeding ratios of 33.63, 41.99, and 101.14 (PERMANOVA, $p < .05$). The bacterial community of monoculture canola had a significantly different community composition from all other seeding ratios (PERMANOVA, $p < .05$).

When considering the combined effects of crop type and seeding ratios, significant differences were still observed in the community compositions of the pea and canola bacterial communities regardless of crop type or treatment (PERMANOVA, $p < .05$; Figure 2a). Intercropped canola with a pea:canola seeding ratio of 50.38 had a significantly different community composition than intercropped canola

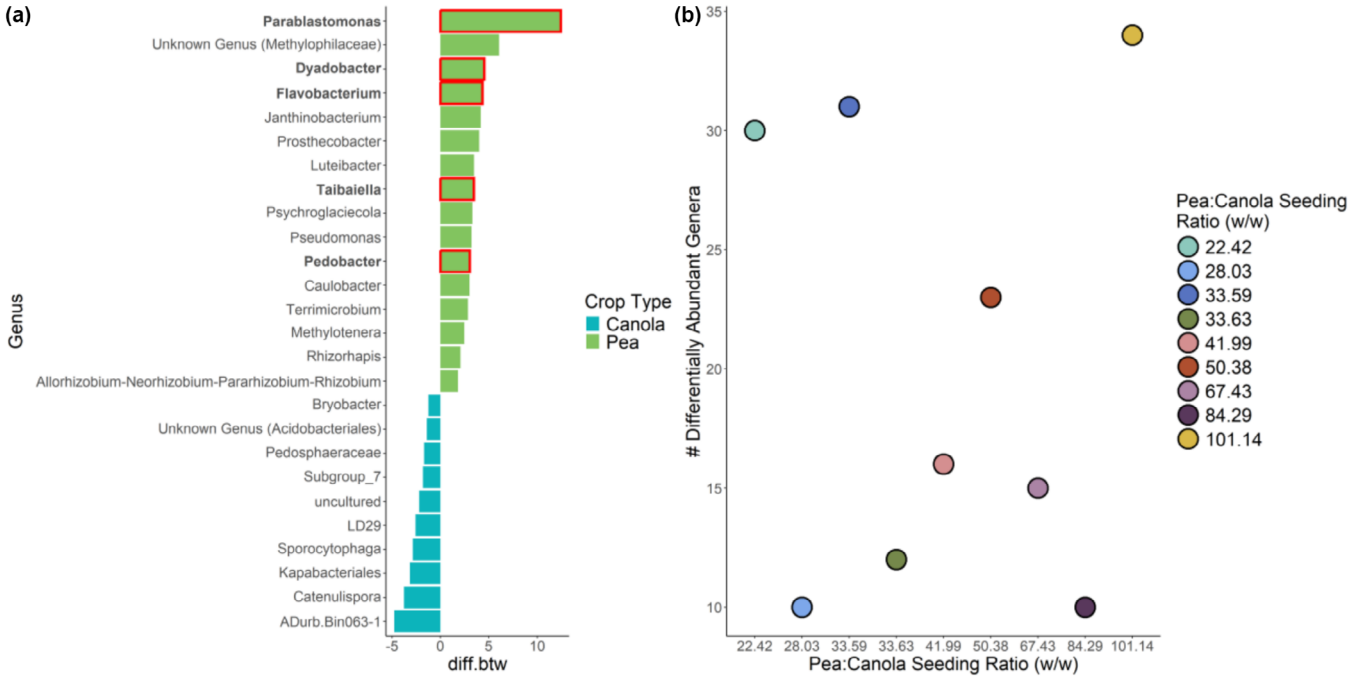


FIGURE 3 Differentially abundant genera in the rhizosphere communities between (a) monoculture pea and monoculture canola and (b) intercropped pea and intercropped canola across pea:canola seeding ratios. In panel a, labels on the *y*-axis represent the lowest taxonomic classification assigned to each genus. If an unknown genus, the lowest taxonomic classification assigned is given in the parentheses. The values for "diff.btw" on the *x*-axis in panel a represent the median difference in the centered log ratios between the compared groups. Values on the *x*-axis that are negative show genera that are more abundant in canola. Positive values on the *x*-axis indicate genera that are more abundant in pea. Genera found to be consistently differentially abundant between pea and canola (across all 10 comparisons) are highlighted in red and their taxonomic classification bolded. Panel b gives the number of differentially abundant genera found between intercropped pea and intercropped canola across pea:canola seeding ratios.

with pea:canola seeding ratios of 33.59 (PERMANOVA, $F = 1.51$, $p = .019$) and 67.43 (PERMANOVA, $F = 1.99$, $p = .013$; Figure 2b).

3.2.2 | Genera were differentially abundant across different pea:canola seeding ratios

When comparing the communities of monoculture pea and monoculture canola, 26 genera were differentially abundant (Figure 3a). The number of differentially abundant genera between pea and canola changed with seeding ratio (Figure 3b). Across all crop types and seeding ratios, the genera identified as *Dyadobacter*, *Flavobacterium*, *Parablastomonas*, *Pedobacter*, and *Taibaiella* were more abundant in pea than canola (Figure S5). No genera were found to be differentially abundant across seeding ratios with the same seeding rate for intercropped bacterial communities (i.e., intercropped canola with pea:canola seeding ratios 67.43 and 84.29).

3.3 | Changes in pea:canola seeding ratio led to some changes in the predicted function of the bacterial community

Differences in pea:canola seeding ratio and cropping system caused no significant differences in the KOMPs of the soil bacterial communities

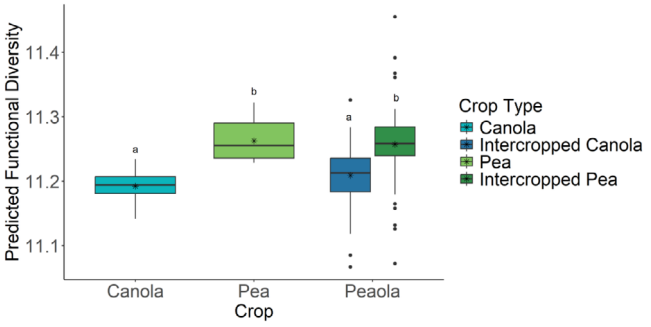


FIGURE 4 Boxplots showing the alpha-diversity (Shannon entropy) of KOMPs in the rhizosphere bacterial communities across crop types. Different letters indicate significant differences. KOMPs in the pea bacterial community were more diverse than KOMPs in the canola (Kruskal–Wallis, $H = 14.52$, $p = .00021$) and intercropped canola (Kruskal–Wallis, $H = 20.18$, $p = .000021$) bacterial communities. Likewise, KOMPs in the intercropped pea bacterial community were more diverse than KOMPs in the canola (Kruskal–Wallis, $H = 16.27$, $p = .00011$) and the intercropped canola (Kruskal–Wallis, $H = 54.28$, $p = 1.04 \times 10^{-12}$) bacterial communities. No significant differences were observed between like crops (i.e. pea and intercropped pea).

using Shannon entropy. The composition of the KOMPs present in the soil bacterial communities was not significantly impacted by seeding ratio nor crop type. No KOMPs were found to be differentially abundant across crop types and seeding ratios in bulk soil.

KOMPs in the pea rhizosphere bacterial community regardless of cropping system were more diverse than canola regardless of crop type (Kruskal–Wallis, $p < .05$; Figure 4). No significant differences in the diversity of KOMPs were found between the rhizosphere communities of like crops. Between different treatments, KOMPs in the rhizosphere bacterial community of monoculture canola were less diverse than KOMPs in the rhizosphere bacterial communities of treatments with pea:canola seeding ratios of 33.63 (Kruskal–Wallis, $H = 12.05$, $p = .0095$), 84.29 (Kruskal–Wallis, $H = 13.63$, $p = .0061$), and monoculture pea (Kruskal–Wallis, $H = 14.52$, $p = .0061$). With the combined effects of crop type and seeding ratio, generally KOMPs in the pea rhizosphere bacterial community were more diverse than the canola rhizosphere bacterial communities (Kruskal–Wallis, $p < .05$; Figure S6). KOMPs in the pea rhizosphere bacterial communities with pea:canola seeding ratios of 67.43 and 101.14 were only observed to be more diverse than KOMPs in the rhizosphere bacterial community of monoculture canola (Kruskal–Wallis, $p < .05$; Figure S6). Overall, no significant differences were observed in the diversity of KOMPs in the rhizosphere bacterial communities of like crops (i.e., pea and intercropped pea) regardless of pea:canola seeding ratio. KOMPs in the rhizosphere bacterial community of canola with a pea:canola seeding ratio of 84.29 were more diverse than the KOMPs in the rhizosphere bacterial community of monoculture canola (Kruskal–Wallis, $H = 5.88$, $p = .049$; Figure S6).

KOMPs in the pea rhizosphere bacterial community were significantly different in their composition from KOMPs in the canola rhizosphere bacterial community regardless of crop type (PERMANOVA, $p < .05$; Figure 5a). Between different seeding ratios, the composition of KOMPs in the rhizosphere bacterial community of monoculture canola was significantly different than the composition of KOMPs in the rhizosphere bacterial communities of monoculture pea (PERMANOVA, $F = 8.54$, $p = .028$) and all peaola communities (PERMANOVA, $p < .05$) except for pea:canola seeding ratios of 28.03, 33.59, and 41.99. When looking at the combined effects of crop type and treatment, the composition of KOMPs in the rhizosphere bacterial communities of pea and canola regardless of crop type and pea:canola seeding ratio were significantly different (PERMANOVA, $p < .05$), except for between pea with a pea:canola seeding ratio of 28.03 and canola with a pea:canola seeding ratio of 33.63. No significant differences were found between KOMPs in the pea rhizosphere bacterial communities regardless of crop type and treatment (Figure 5a). KOMPs in the rhizosphere bacterial community of monoculture canola had a significantly different composition than KOMPs in the canola rhizosphere bacterial communities with pea:canola seeding ratios of 22.42, 33.63, 67.43, and 84.29 (PERMANOVA, $p < .05$; Figure 5b).

Between pea and canola rhizosphere communities, KOMPs were differentially abundant regardless of crop type or seeding ratio. Comparing monoculture pea and monoculture canola, 1955 KOMPs were

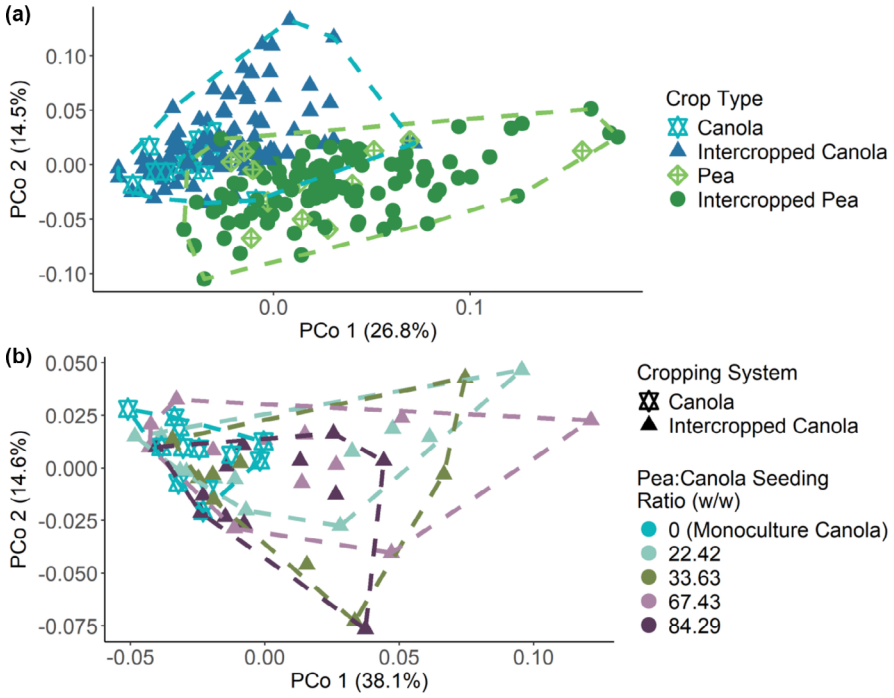


FIGURE 5 PCoA showing the beta-diversity (Bray–Curtis dissimilarity) of KOMPs in the rhizosphere bacterial communities (a) across crop types, and (b) across pea:canola seeding ratios in canola only. In panel a, green dashed lines outline pea and intercropped pea, and blue dashed lines outline canola and intercropped canola. The composition of KOMPs in the bacterial community of pea were significantly different from those in the canola bacterial community (PERMANOVA, $p < .05$) except for between pea with a pea:canola seeding ratio of 28.03 and canola with a pea:canola seeding ratio of 33.63. In panel b, teal dashed lines outline monoculture canola, while light blue, green, purple, and dark purple dashed lines outline canola with pea:canola seeding ratios of 22.42, 33.63, 67.43, and 84.29, respectively. The bacterial community of monoculture canola had a significantly different composition of KOMPs than canola with pea:canola seeding ratios of 22.42 (PERMANOVA, $F = 3.44$, $p = .020$), 33.63 (PERMANOVA, $F = 3.67$, $p = .0061$), 67.43 (PERMANOVA, $F = 2.98$, $p = .022$), and 84.29 (PERMANOVA, $F = 3.37$, $p = .0061$). No other significant differences were observed between like crops (i.e. pea and intercropped pea).

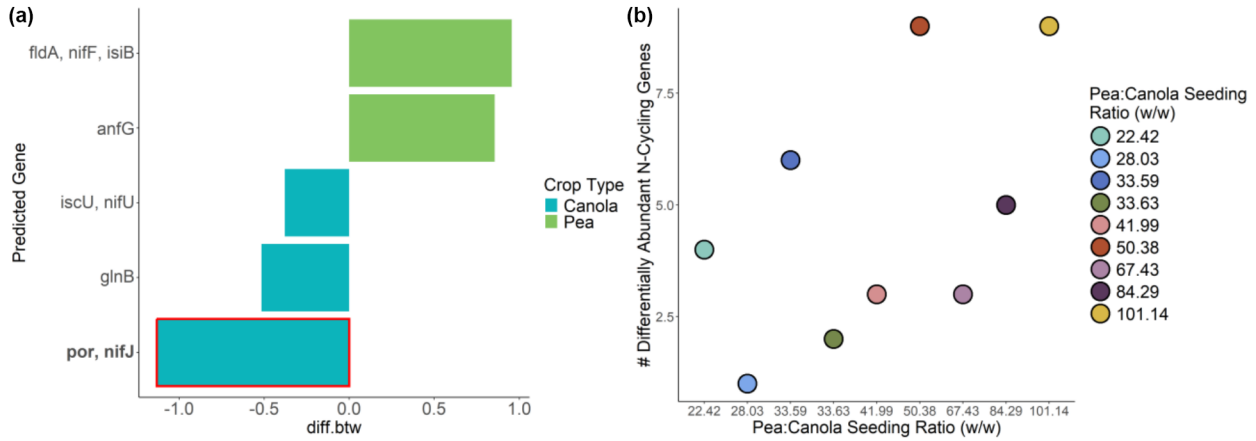


FIGURE 6 Differentially abundant predicted functional genes involved in nitrogen cycling processes in the rhizosphere communities between (a) monoculture pea and monoculture canola, (b) intercropped pea and intercropped canola across different pea:canola seeding ratios. The values for "diff.btw" on the *x*-axis in panel a represent the median difference in the centered log ratios between the compared groups. Values on the *x*-axis that are negative show predicted genes that are more abundant in canola. Positive values on the *x*-axis indicate predicted genes that are more abundant in pea. Genes found to be consistently differentially abundant between pea and canola (across all 10 comparisons) are highlighted in red and bolded. Panel b gives the number of differentially abundant nitrogen cycling genes found between intercropped pea and intercropped canola across different pea:canola seeding ratios.

differentially abundant. The monoculture canola rhizosphere bacterial community had 1112 KOMPs that were more abundant than monoculture pea. Monoculture pea had 833 KOMPs that were more abundant than monoculture canola. When comparing KOMPs in the rhizosphere bacterial communities of intercropped pea and intercropped canola across pea:canola seeding ratios, 1814 KOMPs were differentially abundant on average. On average, 902 KOMPs were more abundant in the rhizosphere bacterial community of intercropped canola than intercropped pea, while 912 were more abundant in the rhizosphere bacterial community of intercropped pea. KOMPs were not differentially abundant between like crops within the same seeding rate (i.e. pea and intercropped pea with a pea:canola seeding ratio of 84.29). Genes associated with nitrogen cycling processes were differentially abundant between the pea and canola rhizosphere bacterial communities regardless of crop type or pea:canola seeding ratio (Figure 6a,b). Crop type and pea:canola seeding ratio did lead to variation in which genes were differentially abundant with only *por/nifJ* being consistently more abundant in canola across all 10 comparisons (Figure S7).

3.4 | Intercropping increased nitrogen leaf content of canola

The leaf tissue of pea had significantly greater ($t[228] < -2$, $p < .05$) percent *N* than canola regardless of crop type (Figure 7). Intercropped with pea, canola had significantly greater ($t[228] = -2.88$, $p = .026$) percent *N* in its leaf tissue than monoculture canola (Figure 7). No significant differences were observed between pea and intercropped pea (Figure 7). No significant differences were observed across different seeding rates and ratios in peaola regardless of crop type (Figure S8). No significant differences were found in the concentration of ammonium, nitrate, and nitrite in the soil.

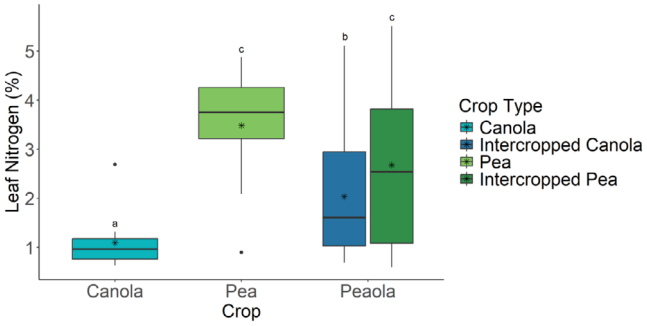


FIGURE 7 Boxplots showing the percent N content of leaf tissue across crop types. Different letters indicate significant differences. The percent N content of pea leaves was consistently significantly greater ($t[228] < -2$, $p < .05$) than in canola leaves regardless of crop type. Intercropped canola leaves had a significantly greater ($t[228] = -2.88$, $p = .026$) percent *N* content than monoculture canola leaves. No significant differences were observed between pea and intercropped pea leaves.

4 | DISCUSSION

4.1 | Intercropping and variation in pea:canola seeding ratio altered the composition of the rhizosphere bacterial community

Our results show, the canola rhizosphere bacterial community as more diverse than the pea rhizosphere bacterial community, matching results from our previous study (Figure 3; Parks et al., 2025). Different pea:canola seeding ratios may have a small impact on overall community diversity as intercropped canola with a pea:canola seeding ratio of 28.03 was no longer more diverse than intercropped pea despite being canola dominated (Figure S4). This could be due to differences

in plant–plant interactions within this treatment, thus altering the influence each plant had on their own rhizosphere bacterial communities (Dowling et al., 2021). Microbial spillover from the canola to the pea bacterial community may also be influencing this result; however, other pea:canola seeding ratios did not show this pattern, making it difficult to draw any definitive conclusions and may suggest results are due to sampling variation (Ulbrich et al., 2022).

Consistent with our previous studies, pea and canola rhizosphere bacterial communities had significantly different community compositions regardless of cropping system (Parks et al., 2025, 2026; Figure 2a). When considering the impact of pea:canola seeding ratio independently, monoculture canola had a significantly different community composition from monoculture pea and all pea:canola seeding ratios. Meanwhile, monoculture pea did not have a significantly different community composition from three of the pea:canola seeding ratios (33.63, 41.99, and 101.14). Again, no clear pattern is seen across seeding ratios. It is possible that these results differ due to an increase in competition for resources, which would prompt plants to alter their bacterial recruitment to meet their needs (Andersen, 2005; Hauggaard-Nielsen et al., 2006; Ulbrich et al., 2022; Wang et al., 2021; Zhu et al., 2022, 2023). This would make sense with crop type being the main contributing factor to variance within the data, accounting for 16% (Table 1). With the combined effects of crop type and pea:canola seeding ratio, intercropped canola with a pea:canola seeding ratio of 50.38 was found to have a significantly different community composition than intercropped canola with pea:canola seeding ratios of 33.59 and 67.43 (Figure 2b). This could be due to alterations in plant–plant interactions driving changes in the community composition within these groups, potentially due to resource competition or other forms of plant–plant competition (Andersen, 2005; Dowling et al., 2021; Hauggaard-Nielsen et al., 2006; Ulbrich et al., 2022; Wang et al., 2021; Zhu et al., 2022, 2023). However, with a lack of clear pattern observed, no definitive conclusions can be made.

Changes to the composition of the rhizosphere bacterial community across pea:canola seeding ratios can be seen in our differential abundance analyses as the number and identities of differentially abundant genera varied by seeding ratio (Figure 3b; Figure S5). Overall, this shows that there are fine scale changes in the rhizosphere bacterial communities of pea and canola depending on pea:canola seeding ratio. Treatments with pea:canola seeding ratios of 101.14, 33.59, and 22.42 had the largest numbers of differentially abundant genera, 34, 31, and 30, respectively (Figure 3b). The largest and smallest ratios tested (101.14 and 22.42) being amongst this group makes sense considering they are either pea or canola dominated, leaving the potential for one crop to have a greater overall influence. The pea:canola seeding ratios of 28.03 and 84.29 had the smallest number of differentially abundant genera (both 10; Figure 3b). These seeding ratios are the second smallest and second largest seeding ratios we tested, which could indicate variation in the strength each plant species has on the bacterial community based on the pea:canola seeding ratio. For instance, belowground processes such as root exudate production could be altered therefore changing bacterial recruitment, or plant–plant interactions may be altered (i.e., increased or decreased competition; Dowling et al., 2021; Ulbrich et al., 2022). Additionally, it should be noted that the group *Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium* was only more abundant in the pea rhizosphere community when comparing monoculture pea and canola, and intercropped pea and intercropped canola with seeding ratios of 22.42, 33.59, 50.38, and 101.14 (Figure S5). The lack of consistency for this observation can likely be explained in part by the application of nitrogen fertilizer. It is known that under conditions where nitrogen is abundant, legumes like pea will not favor forming relationships with rhizobia in addition to moderating the relationship based on nutrient availability (Friel & Friesen, 2019).

Despite variation in differentially abundant genera across pea:canola seeding ratios, *Dyadobacter*, *Flavobacterium*, *Parablastomonas*, *Pedobacter*, and *Taibaiella* were consistently more abundant in canola than pea across all comparisons (Figure 3a; Figure S5). *Parablastomonas* has been observed to be a prominent genus in studies characterizing soil microbial communities in agricultural systems, yet there is not much information about how it interacts with plants (Shi et al., 2021; Zhiyong et al., 2024). Conversely, *Dyadobacter* and *Flavobacterium* are known plant growth promoters showing diazotrophic properties and aiding with abiotic stressors, respectively (Kumar et al., 2018; Seo et al., 2024). Future work should determine how these genera are interacting with plants in this system to improve and validate our understanding of their roles. Additionally, future studies should include analyses of fungal communities, which have been found to experience larger shifts in their community composition and structure in some legume nonlegume intercropping systems (Parks & Friesen, 2025). Specifically, a focus on arbuscular mycorrhizal fungi (AMF) should be emphasized considering that canola is nonmycorrhizal and has been shown to cause decreases in fungal and mycorrhizal communities when planted in monoculture (Hansen et al., 2019).

4.2 | Intercropping and variation in pea:canola seeding ratio led to changes in the predicted function of the rhizosphere bacterial community

We hypothesized that we would see changes to the predicted functional diversity of the bacterial community due to connections observed between overall bacterial diversity and bacterial functional diversity (Tian et al., 2022; Zak et al., 1994). This pattern was observed in our study, although some variation was observed. When determining the impact of crop type on predicted functional diversity, we saw that pea and intercropped pea had greater functional diversity than canola and intercropped canola, contradicting what we observed for overall bacterial diversity (Figure 4). Intercropping itself had no significant impact on predicted functional diversity of pea and canola bacterial communities, matching our prior observations (Parks et al., 2025). When combined with the impacts of pea:canola seeding ratio, the predicted functional diversity of pea remained greater than that of canola except for treatments with pea:canola seeding ratios of 67.43 and 101.14, which were only more diverse than monoculture canola. These seeding ratios were some of the largest tested. KOMPs

in the bacterial community of canola with a pea:canola seeding ratio of 84.29 (the second highest ratio tested) were also more diverse than monoculture canola. With these treatments being pea dominated, this suggests that there may be shifts in community dynamics in pea dominated systems.

The pattern we observed for community composition of predicted KOMPs matched what we observed for overall bacterial community composition with significant differences between pea and canola, also matching our prior observations (Parks et al., 2025, 2026). This makes sense in the context that crop type was the primary contributor of variance (16%) making it predominantly influential to community composition (Table 1). When accounting for the combined effects of crop type and pea:canola seeding ratio, monoculture canola had a significantly different composition than intercropped canola in treatments with pea:canola seeding ratios of 22.42, 33.63, 67.43, and 84.29. Although no clear pattern is present, variation in the composition of KOMPs from monoculture to intercropped canola could suggest alterations in plant–plant interactions potentially resulting from changes in plant needs (Dowling et al., 2021; Fletcher et al., 2015). Specifically, interactions with pea may be altering how canola is interacting with its bacterial community due to competition for resources or facilitation. In the more canola dominated systems, interspecific and intraspecific competition may both be contributing to the observed results.

Although untested in our study, changes in plant–plant interactions, such as increased competition for resources, have led to changes in bacterial recruitment by plants in order to alter overall microbiome function (Friel & Friesen, 2019; Omae & Tsuda, 2022). Differing from our differential abundance analysis of the overall rhizosphere bacterial community, monoculture canola and monoculture pea did not have the most differentially abundant KOMPs. Instead, intercropped pea and intercropped canola with pea:canola seeding ratios of 33.59, 50.38, and 101.14 had the most differentially abundant KOMPs with 2296, 2741, and 2379, respectively. The pea:canola seeding ratios of 50.38 and 101.14 also had the most differentially abundant KOMPs predicted to be involved in nitrogen cycling processes (nine each; Figure 6b). Treatments with pea:canola seeding ratios of 28.03, 33.63, and 67.43 had the fewest differentially abundant KOMPs predicted to be involved in nitrogen cycling processes with one, two, and three, respectively (Figure 6b). No distinct pattern between the number of differentially abundant functional genes and pea:canola seeding ratios was observed both overall and for genes predicted to be involved in nitrogen cycling processes. Combined with our results for soil and leaf nitrogen content, these results further support that changes are occurring in the bacterial community that may be contributing to the observed changes in plant leaf nitrogen content. Further work should be done to determine how plant–plant interactions are being altered across seeding ratios, and to perform a more in-depth study on the function of the bacterial community that is not based on functional predictions.

Across all comparisons of KOMPs involved in nitrogen cycling processes, *por/nifJ* was consistently differentially abundant between pea and canola (Figure 6b). The gene *por/nifJ* (K03737) encodes for the pyruvate-ferredoxin/flavodoxin oxidoreductase per the KEGG database and couples the oxidation of pyruvate to *nifF*, thus contributing an electron to dinitrogen reductase (Vicente & Dean, 2017). A study conducted on pea-maize intercropping found that the abundance of *por/nifJ* was changed by intercropping, showing that this gene can vary in abundance based on cropping system; however, no studies to our knowledge have explored differences in its abundance in different plant associated bacterial communities (Lu et al., 2025). Given its higher abundance in canola, bacteria associated with this prediction could be free-living nitrogen fixers. Alternatively, this result may be due to biases introduced by the predictive nature of PICRUSt2, which assume the presence of functional genes based on bacterial identity. Further work should aim to validate this finding and determine the identities of the community members associated with this gene. Additionally, the presence, abundance, and expression of all the nitrogen cycling genes discussed within this study should be experimentally validated and determined.

4.3 | Intercropping led to changes in leaf nitrogen content despite there being no detectable changes in soil nitrogen content

Other studies that have measured nitrogen uptake through percent nitrogen content of plant leaves have seen increases in the nitrogen content of leaves with intercropping, especially with nitrogen fertilizer application (Dordas & Lithourgidis, 2011; Luo et al., 2021; Nasar et al., 2020). In our study, intercropped canola had greater leaf nitrogen content than monoculture canola (Figure 7). This may suggest improved nitrogen availability to canola when intercropped with pea, although this is not reflected in soil nitrogen content.

Soil ammonium, nitrate, and nitrite concentrations were unaffected by intercropping and pea:canola seeding ratios matching our results from previous studies focused on other management practices (Parks et al., 2025, 2026). Other studies that have looked at soil nitrogen content in intercropping systems have also found no significant differences (Oelbermann et al., 2015; Sun, Dong, et al., 2022). Studies reviewed by Parks and Friesen (2025) mostly found increasing soil nitrogen content with intercropping, with one study by Sun, Zhang, et al. (2022) reporting a decrease in soil nitrogen content with intercropping. A possible explanation for why we did not observe changes in soil nitrogen content could be due to the time at which samples were collected. With samples collected during early flowering, nitrogen resources could have been depleted. Alternatively, the activity of plant recruited microorganisms involved in nutrient cycling processes could be providing nitrogen to the system. Microorganisms have been observed to scale their productivity based upon the needs of the plants they are associated with (Ehrmann & Ritz, 2014; Friel & Friesen, 2019; Omae & Tsuda, 2022; Parks & Friesen, 2025; Ulbrich et al., 2022). Therefore, even if differences in pea:canola seeding ratios are altering competition for resources such as nitrogen, the bacterial community could be responding to differing demands of each system and plant–plant dynamics. Future studies focused on this system should test both soil and leaf nitrogen content at different time

points throughout crop production to fully understand how nitrogen content is impacted by peaola and variation in seeding rates.

5 | CONCLUSIONS

Overall, crop type was the primary determining factor structuring the bacterial community, matching previous findings. Pea:canola seeding ratio does impact the bacterial community of peaola, but no clear patterns were seen between pea:canola seeding ratios and the bacterial community. Evidence for shifts in the function of the bacterial community was seen, with changes observed across some seeding ratios in the predicted abundance of nitrogen cycling genes. Intercropped canola leaves contained higher nitrogen content than monoculture canola, providing evidence that the observed changes in composition and predicted function of the intercropped canola bacterial community could improve the availability of nitrogen when intercropped with pea. This could be due to shifts in plant recruitment of bacteria in response to changes in resource availability, with changes in the predicted function of the bacterial community supporting this. Validation of these results is needed to draw more substantial conclusions in addition to further studies on how plant–plant interactions may be altered across seeding ratios. It is also possible that changes may be occurring in the fungal community, which could contribute to overall crop performance. Therefore, it is important that future studies also assess changes occurring in the fungal community. Regardless, these results highlight how changes in aboveground diversity can impact belowground diversity. Ultimately, this knowledge can be used by growers to help improve crop yields and agricultural sustainability by providing information about what practices may impact belowground community diversity and function the most. From the results of our study, plant species had the strongest impact on the bacterial community, highlighting that consideration needs to be given to which crops are included above seeding ratio when aiming to design productive intercropping systems. With the variability and unpredictability of these results, understanding why changes to aboveground diversity impact belowground diversity is exceedingly important and should be investigated.

AUTHOR CONTRIBUTIONS

Janice M. Parks conducted the experiments described in this manuscript along with the data analysis. Parks produced the figures and tables presented in the manuscript and wrote the manuscript. Braeden J. Stiffler performed the experiments for the bulk soil nutrient analyses under the mentorship and guidance of Parks. Maren L. Friesen conducted field sampling and mentored Parks throughout the production of this manuscript, which included providing constructive feedback on the experiments, analysis, writing, figures, and tables presented in the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Code used for data analyses is archived on the Open Science Framework, DOI 10.17605/OSF.IO/2ZF6B. Sequences are archived through NCBI under the reference number PRJNA1162884 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1162884). Some of the mock community and negative sequences are archived under the reference numbers PRJNA1162556 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1162556) and PRJNA1162582 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1162582) due to overlap in sequencing runs. These include SRA accession numbers SRR33471491, SRR33471501 through SRR33471505, SRR33471507 through SRR33471514, and SRR34231268 through SRR34231282.

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SUPPORTING INFORMATION

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

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