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ADF Project Final Report Format

1. **Project title and ADF file number.**
   Soil health and nutrient uptake among diverse canola lines – added value to crop phenotyping, 20170182.

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4. **Abstract/ Summary**: *An outline on overall project objectives, methods, key findings and conclusions for use in publications and in the Ministry database (Maximum of 500 words or one page in lay language).*

This project provides added value to the aboveground and root microbiome phenotyping field trials established through funds from the Plant Phenotyping and Imaging Research Centre (P^2IRC) developed from the Canada First Research Excellence Fund awarded to the Global Institute for Food Security (GIFS). The field trials have been designed to test advanced imaging (e.g., drone-based multispectral), molecular (e.g., microbial metagenomics, synchrotron), and computational tools (e.g., machine learning) to link crop phenotype to genotype, a significant bottleneck limiting the advancement of crop breeding. The goal of this specific project was to characterize soil characteristics and crop nutrient uptake to get a better understanding of how these factors influence crop productivity, especially as assessed in breeder trials. Soil inorganic N and extractable S were most responsive to differences in B. napus genotype, while available P and soil pH were unaffected. Variation in soil properties was strongest within the growing season and changes in nutrients were relatively consistent among site-years, with some exceptions. In a more focused investigation of soil nitrogen cycling and nitrogen use efficiency, there was significant relationships between soil microbial community composition, soil inorganic N, and crop NUE. Further research in identifying the drivers of genotype-specific differences in soil nutrient cycling and uptake are warranted.

5. **Extension Messages:** *key outcomes and their importance for producers/industry (3-5 bullet points in lay language).*

   - *Brassica napus* genotype affected soil inorganic nitrogen, extractable sulphur, and total carbon, but had no effect on available P and pH, but genotype effects on soil nutrients were not consistent among three field sites across Saskatchewan
   - Soil nutrients varied more strongly over the course of the growing season
   - Root traits and soil inorganic nitrogen was related to microbial diversity and community composition and crop nitrogen use efficiency

6. **Introduction:** *Brief project background and rationale.*

Roots are critical for plant functioning; they act as a sink for soil resources including nutrients and water. Roots also provide energy and resources to soil microbial communities, which in turn maintain soil health and promote plant productivity (Bardgett et al., 2014). Physical traits such as root architecture determine the spatial extent of soil exploration for resources (Kuijken et al., 2015) and microbial-nutrient interactions (Spohn and Kuzyakov, 2013). Root exudates are critical for mobilizing relatively unavailable plant nutrients, particularly phosphorus through changes in soil pH and chelation (Richardson et al., 2011), and for stimulating microbial activity that accelerates organic nutrient mineralization to release nutrients available for root uptake (Kuzyakov and Xu, 2013). Moreover, roots and microbes can release enzymes that enhance nutrient uptake. For example, a maize genotype with high nitrogen use efficiency (NUE) supported higher enzyme activity and a

microbial community differing in structure, as compared to a genotype with lower NUE (Pathan et al., 2015). Rhizosphere nutrient cycling processes and the functioning of microorganisms in this root-influenced soil zone are shaped by the plant genotype (G) and the soil environment (E), which reflects inherent soil properties, and their interaction (G x E) (Schmidt et al., 2016). Therefore, beneficial effects of microbiomes on any particular crop genotype may not be the same in soils that differ in properties such as pH or organic matter content and quality, which varies across Saskatchewan's soil zones.

Crops that can readily exploit soil nutrients and utilize those nutrients more efficiently require less fertilizer inputs, offsetting significant input costs for producers and reducing potential losses to the environment (Baligar and Fageria, 2015). Developing crops with high nutrient uptake capacity and nutrient use efficiency, however, requires improved understanding of root structural traits that enable roots to explore soil, and of the root-microbe and root-mineral interactions that enhance soil nutrient availability (Hunter et al., 2014; Lynch, 2014; York et al., 2013). These physical and biotic traits can vary among crop genotypes, and therefore identifying phenotypes that confer improved nutrient uptake could be exploited in crop breeding programs if these phenotypes can be linked to genotype (Kuijken et al., 2015). In addition, such efforts can be further advanced by aboveground high-throughput phenotyping based on aerial- or ground-based imaging that can be related to plant nutrient content and can predict plant nutritional status. This research will enable us to make stronger linkages between crop phenotype and genotype, advancing breeding efforts to develop profitable crops for producers with minimal environmental impact for all.

The work described in this project seeks to understand how canola interacts with soil properties to affect crop nutrient uptake and productivity. We are focused on using a diverse panel of Nested Association Mapping (NAM) population of canola. The project is platformed on field trials established as part of the P^2IRC. In this specific project, we are working towards addressing: (1) whether crop nutrient uptake profiles and soil nutrient dynamics differ with canola genotype; and (2) how dynamic soil properties such as pH and soil carbon that govern nutrient availability interact with genotype to influence nutrient uptake and crop productivity. Underlying these objectives is also the need to understand within site soil variability (part of the environmental component needed to understand genotype by environment interactions) and how this variability may affect crop productivity and interpretations of phenotypic information across crop genotypes. This soil and plant nutrient data will be related to the rhizosphere, root, and endophyte (shoots and leaves) microbiome data as well as aboveground phenotyping data being collected as part of the P^2IRC initiative across a diverse set of canola genotypes. In this report, we related the rhizosphere microbiome to soil nitrogen and crop N uptake and NUE in one site-year.

7. Objectives and the progress towards meeting each objective.

Objectives **(Please list the original objectives and/or revised objectives if Ministry-approved revisions have been made to original objective. A justification is needed for any deviation from original objectives)**	**Progress** **(e.g. completed/not completed)**
a) Identify nutrient uptake patterns among diverse canola lines at multiple field sites in Saskatchewan	Modified. We focused on nitrogen uptake patterns only, due to labour constraints during the COVID-19 pandemic and damage to plant samples, further nutrient analyses could not be completed.
b) Quantify soil properties and plant- available nutrients in different soils under diverse canola lines	Completed.
c) Identify nutrient uptake patterns and quantify soil	Modified. We were not able to sample the trial due to labour constraints, but were able to sample a canola

nutrients among canola hybrids	nitrogen use efficiency trial using a subset of the NAM lines and hybrids.
d) Provide supporting soil and plant nutrient data to aboveground phenotyping data to strengthen a field-based phenotyping package	Completed.

Please add additional lines as required.

8. **Methodology:** *Specify project activities undertaken during entire project period. Include approaches, experimental design, methodology, materials, sites, etc.*

This project involves characterizing soil properties and nutrient availability and crop nutrient uptake under a set of *Brassica napus* parental lines from a subset of genotypes from a nested association mapping (NAM) population grown in field trials conducted over seven site-years from 2016-2018 as part of the P^{2}IRC (Table 1). Due to labour constraints, we were not able to collect soil and plant samples from the hybrid trials. An additional trial through the P^{2}IRC project was established in 2018, however, to evaluate the nitrogen fertilizer response of two NAM lines from the main experiment as well as two hybrids developed from these NAM lines. Soil and plant sampling and analyses was enabled by the PhD work of Shanay Williams-Johnson. This field trial enabled us to directly test the effects of nitrogen availability on canola belowground phenotypes with the same platform and approach as the main field experiments. All of the field research trials were established and maintained by AAFC field staff through direction by Dr. Sally Vail.

Table 1 Summary of *Brassica napus* genotypes sampled and analyzed for soil properties in the field trials and field seasons as well as additional data collected

Site-Year	Nested Association Mapping (NAM) *B. Napus* Genotypes	Growth stage sampled (days after sowing in brackets)	Additional data collected
Llewellyn – 2016	0, 13, 14, 17, 23, 30, 32, 37, 43, 46, 48, 5, 72, 76, 79, 94	1. 6-9 leaf stage (32) 2. start of flowering (39) 3. mid flowering (53) 4. end of flowering (67) 5. harvest maturity (81)	• Rhizosphere bacterial microbiomes (DNA sequencing)[1] • Root morphology • Nitrogen use efficiency
Llewellyn, Melfort - 2017	0, 13, 14, 17, 23, 30, 32, 37, 43, 46, 48, 5, 72, 76, 79, 94	1. early vegetative 2. flowering 3. pod-filling	• Rhizosphere bacterial microbiomes (DNA sequencing)[1], root morphology, nd soil nitrogen cycling processes for 6 of the 16 genotypes
Llewellyn, Scott, Melfort - 2018	0, 13, 14, 17, 23, 30, 32, 37, 43, 46, 48, 5, 72, 76, 79, 94	1. early vegetative 2. flowering 3. pod-filling	
Llewellyn – 2018	NAM-0, NAM-17 Experimental hybrids: H151816 and H151857	1. early vegetative 2. flowering 3. pod-filling	• Soil nitrogen cycling processes • Nitrogen use efficiency

[1]Root and rhizosphere microbiome data collection funded through P^{2}IRC

Detailed information of the NAM genotypes grown for the main field trials are summarized in Table 2. These genotypes were selected because they vary in seed characteristics including seed colour, fiber content, and seed erucic acid; and they exhibited differences in both aboveground and root characteristics (Figure 1). We

hypothesized that variation in seed quality characteristics would also result in variation in the chemical composition of root exudates, thus affecting the recruitment of the root and rhizosphere microbiomes. Further, these genotypes represent a diversity of origins and we hypothesized that morphological differences in their root systems would influence root-microbial-soil interactions, including soil nutrient availability.

Table 2 Seed quality traits of the 16 diverse B. napus genotypes grown in the main experiments. Table adapted from Taye et al. (2020)

B. napus genotype	Description	Country of origin	Seed colour	Acid detergent lignin (% of seed)	Seed glucosinolates (μmol)	Seed erucic Acid (% Oil)
NAM-0	Breeding Line	Canada	Black	3.8	8.8	0.44
NAM-13	Cultivar	Germany	Black	7.5	9.5	0.26
NAM-14	Cultivar	Sweden	Black	3.2	91	37.81
NAM-17	Breeding Line	Canada	Black	3.7	11.3	0.23
NAM-23	Accession	North Korea	Black	5.8	10.4	1.1
NAM-30	Cultivar	European	Black	8.7	8.6	0.35
NAM-32	Accession	South Korea	Black	6.6	114.4	0.18
NAM-37	Cultivar	Australia	Black	6.9	49.9	0.32
NAM-43	Accession	Bangladesh	Black	6.1	92.7	10.14
NAM-46	Accession	South Korea	Black	4.5	103.5	47.06
NAM-48	Breeding Line	Canada	Yellow	NA	NA	NA
NAM-5	Accession	India	Black	4.2	62.1	9.75
NAM-72	Breeding Line	Canada	Yellow	0.8	9.9	0.08
NAM-76	Cultivar	Canada	Black	6.6	14.3	2.18
NAM-79	Accession	Pakistan	Black	NA	NA	NA
YN04-C1213	Breeding Line	Canada	Yellow	3.7	119.9	40.08

Experimental design

Main field experiments comparing 16 B. napus *genotypes in 2016, 2017, and 2018*

In 2016, the genotypes were grown at Llewellyn Research Farm of the Saskatoon Research Centre of AAFC and in 2017 and 2018 at Llewellyn, Scott, and Melfort AAFC farms. The soils at Melfort are a Black Chernozem, with silty clay texture, pH 6.4, and 8.2% organic matter. The soils at Scott are a Dark Brown Chernozem, loam texture, pH 5.7, and 5.8% organic matter. The soils at Llewellyn are a Dark Brown Chernozem, clay loam texture, pH 7.5, and 5.1% organic matter.

Figure 1 Llewellyn field plots, 2016. Photos from P²IRC Theme 1.3 Plant Belowground Phenotype (Steve Siciliano and Bobbi Helgason).

The 16 genotypes were grown in a randomized complete block design with three replicates. Each plot was 6.1 m long and 1.8 m wide, with six rows. Each year, fertilizer application was guided by pre-seeding soil nutrient testing.

NAM line and hybrid fertilizer nitrogen response trial at Llewellyn in 2018

This field study conducted at Llewellyn farm in 2018 was comprised of four *B. napus* genotypes: two parental genotypes and two experimental hybrids. Parental genotypes included NAM-0 and NAM-17, both from the AAFC canola breeding program and that were also part of the 16 genotypes grown in the main field experiments. The two genotypes were breeding lines selected for production in Western Canadian but had genetically different germplasms. The experimental hybrid combinations for this study included H151816 (where parental genotype NAM-17 was the male crossed with and a female tester) and H151857 (NAM-0 crossed with the same female tester).

The experiment was laid out in a randomized split-plot design with four replications. The size of each main plot was 1.2 m in width and 5.94 m in length and each plot contained four rows per plot, with rows spaced at 0.3 m. Four N treatment rates (0, 50, 100, and 150 kg ha^{-1}) were assigned to the main plots and the four canola genotypes to the sub-plots. The treatments were replicated four times to give a total of 64 experimental units. Nitrogen was applied as urea (46% N) and was mid-row banded. Control plots that did not receive urea (0 kg N ha^{-1}) were included. Fertilizers for sulfur and phosphorus were added pre-seeding as 23.3 kg ha^{-1} ammonium sulfate and 39.8 kg ha^{-1} mono ammonium phosphate. Edge granular herbicide was applied to the field in spring at 20.5 kg ha^{-1}.

Sample collection and processing summary across all sites and years

Sample collection in 2016 and 2017 was completed as part of the P²IRC field research campaign and in 2018 as part of the current ADF project. In 2016, plant and soil samples were collected more intensively (five times total for N analyses; a subset of three collections analyzed for other soil properties), while in 2017 and 2018 plant and soil samples were collected three times to target specific growth stages (Table 1).

Soils in association with the plant roots were collected in all years and field sites. Three canola plants were selected at random within the plot, the plant clipped at the soil surface, and soil and root samples were collected using sterilized trowels or soil cores to a 10-cm depth with 5 cm diameter centred around the plant stem. Once back in the lab, roots were shaken to collect the loosely adhering soil for nutrient analyses. The soils that strongly adhered to the roots were washed, the soil-slurry collected, centrifuged and the soil pellet analyzed for rhizosphere microbial community composition and diversity in 2016. The loosely adhering soils were sieved (2 mm) and then separated into three subsamples for the following purposes: 1) air-dried for soil pH and soil available P, extractable S, and soil total carbon (C) determination; 2) stored frozen (-20C) for soil inorganic N analyses; and 3) oven-dried for determination of gravimetric moisture content. Aboveground plant tissue samples were dried and ground in preparation for nutrient analysis. In 2016, root material was also analyzed for root morphological traits.

Soil and plant analyses

Soil nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N) were determined using 2 M KCl extraction (Carter and Gregorich, 2008). Soil samples were weighed to 5 g and extracted with 50 ml of 2 M KCl. Soil available P was determined on air-dried soils using the modified Kelowna extraction method. Soil extractable S was determined by extracting 20 g of air-dried soil with 40 mL of 0.01 M $CaCl_2$. A subsample of soil was oven-dried (105°C) for 24 h to determine gravimetric moisture content. The filtered extracts were analyzed using a Technicon Auto-Analyzer. Soil pH was determined by suspending 1:2 of air-dried soil weight to 0.01 M $CaCl_2$ solution volume (Carter and Gregorich, 2008). Air-dried soil samples were finely ground and then analyzed for total C on a LECO S-832 analyzer for the Llewellyn and Melfort field sites in 2017, targeting the flowering time period. Sample C analyses were limited due to labour constraints during the COVID-19 pandemic. Quality control was conducted after every 20 samples, and field duplicates and experimental duplicates were included in the analysis. In the N rate trial conducted at Llewellyn in 2018, soils were also analyzed for urease activity, as a measure of microbial activity, and potential ammonium oxidation as a measure of nitrification.

Aboveground plant material was separated from the plant roots and dried, before being ground into a fine powder. Ground leaf and stem tissue as well as the whole seeds were analyzed for N content following the Dumas combustion protocol, using a LECO TruMac analyzer. Crop N use efficiency (NUE) was determined by dividing seed N by fertilizer N rate (Martinez-Feria et al., 2018).

In 2016 at Llewellyn, root morphology was assessed using root samples collected to a 10-cm depth. Roots were gently washed to remove soil and debris, and biomass was determined by weighing roots directly from the field. Roots could not be dried prior to weighing since they were to be analyzed for morphology. Roots were stored in a 10% v/v ethanol solution, then analyzed for root length, surface area and root average diameter using the WinRHIZO 2013 software.

Rhizosphere bacterial community composition was assessed by the P^2IRC team led by Steve Siciliano and Bobbi Helgason based on amplicon sequencing following Bazghaleh et al. (2020). Briefly, ~1.0 g of rhizosphere soil was recovered from each sample and the DNA of the soil bacteria were extracted using a MOBIO Power Soil DNA extraction kit, and DNA from the root bacteria were extracted using a MOBIO Power Plant extraction kit. Standard PCR analyses were conducted on rhizosphere soil (5ng/uL) and root samples (1.5ng/uL) for 16S rRNA for bacteria (342F- 806R primer). PCR amplifications were conducted and the products sequenced for bioinformatics analyses.

Statistical analyses

The effect of canola genotype and growth stage (early vegetative, flowering, pod-filling) on soil properties and plant nutrients were tested using mixed effects ANOVA, with block as a random effect and growth stage and genotype as fixed effects. Data from each site-year were analyzed separately as sampling across field sites did not exactly match growth stage—though fell generally within the same stage (e.g., vegetative, flowering, pod-filling).

Where necessary, data were log-transformed to meet the assumptions of the ANOVA. Tukey's post-hoc analyses evaluated differences among treatment means. All statistical analyses were conducted in R.

Rhizosphere bacterial microbiome data were analyzed in R using the phyloseq package v. 1.22.3, where amplicon sequence variants (ASVs) were filtered to remove chloroplast, mitochondria, archaea, and zero sum taxa. The ASV richness (the number of observed ASVs), and evenness (Simpson's evenness) in each sample was determined by scaling the raw proportions of the ASVs to the read count of the smallest libraries (10358 reads for the rhizosphere microbiome). Pearson's correlation coefficient test was used to assess the relationships between plant and soil parameters and microbial diversity (richness and evenness). At the community level, zeros in the microbial data were replaced using the Geometric Bayesian Multiplicative method for the rhizosphere and root microbial communities. The datasets were transformed using a centered-log ratio transformation using the CoDaSeq package v. 0.99.1], and the Aitchison distance was estimated. Permutational multivariate analysis of variance (PERMANOVA) using the "adonis" function in the vegan package was used to test the effect of canola genotype and days after sowing (DAS) on rhizosphere microbial community structures. Redundancy analysis (RDA) was used to correlate soil N, soil moisture, soil pH, root morphology, and aboveground plant biomass with rhizosphere microbial structure to determine their association with the soil and plant variables. The significance of the RDA models for the rhizosphere microbiomes was tested by PERMANOVA and the R^2 values generated by the "RsquareAdj" function in the R base package.

9. **Results and discussion:** *Describe results accomplished during the entire project period under each objective listed under section 6. The results need to be accompanied with tables, graphs and/or other illustrations. Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.*

Canola plant nitrogen and soil nitrogen cycling processes: Llewellyn 2016

In 2016, 16 canola genotypes and associated soils were intensively sampled (five times) throughout the growing season at the Llewellyn field site, with a focus on soil nitrogen dynamics, the soil microbiome, and crop nitrogen uptake and NUE.

Soil NO_3^--N and NH_4^+-N concentrations differed among genotypes and changed over time (days after sowing), as expected, but the effect of genotype on soil inorganic N did not vary with time (Table 4). Soil NO_3^--N was highest at 32 DAS (6-9 leaf stage; Table 5), likely because of the initial fertilizer N application and because plant biomass, and consequently N uptake, at this point in the season was low. Similar to Gan et al. (2010), soil NO_3^--N under canola generally increased from flowering (39-53 DAS) to pod-filling (67 DAS) before finally decreasing at harvest (81 DAS); soil NH_4^+-N followed a similar trend (Table 5). Increased soil inorganic N concentrations over the growing season indicates a high N supplying power from the soil. Rhizodeposition and root exudation could have stimulated soil N mineralization and nitrification later in the growing season, like trends observed under pea (Sawatsky and Soper, 1991) and wheat (Jensen, 1996). Indeed, canola contributes a high proportion of its plant N to rhizodeposits (Arcand et al., 2013). This was evident at 67 DAS when soil inorganic N was highest. Among the 16 genotypes, soil NO_3^--N was highest under NAM-79 and lowest under NAM-94, NAM-46, NAM-72 and NAM-23 (Table 2). Differences among genotypes indicates varying ability to acquire N; for example, lower soil NO_3^--N under certain genotypes may be reflective of high N uptake. Indeed, soil NO_3^--N was negatively correlated with aboveground plant biomass ($P < 0.05$). Further, soil NO_3^--N concentrations tended to be negatively correlated with root surface area ($P < 0.1$); as root surface area increased, there was more surface area for NO_3^--N absorption. In contrast to N, soil available P did not vary with genotype, but did change with time, decreasing between the early vegetative stage (32 DAS) and flowering (53 DAS; see Appendix). Note that P and S were analyzed on three of the five sampling times that N was. Similarly, extractable S varied with time ($P < 0.0001$), declining between the early

vegetative stage (32 DAS) and flowering (53 DAS), but increasing again at pod-filling (67 DAS). Genotype also did not affect extractable S (*P* = 0.0774).

Table 3 Analysis of variance *P* values of root traits and soil properties across 16 diverse canola genotypes sampled five times between 32 – 81 days after sowing (DAS). Bolded and italicized *P* values are significant at *P* < 0.05 and *P* < 0.10, respectively.

Factor	Root traits			Soil properties		
	Root length (cm)	Root surface area (cm^2)	Root diameter (cm)	NO_3^--N (mg kg $soil^{-1}$)	NH_4^+-N (mg kg $soil^{-1}$)	pH
Genotype	*0.0695*	0.1417	*0.0663*	**0.0053**	0.6302	**<0.0001**
DAS	**<0.0001**	**<0.0001**	**0.0039**	**<0.0001**	**<0.0001**	**<0.0001**
Genotype * DAS	0.4929	0.2476	0.7789	0.5047	0.9936	0.9829

Root traits varied with canola genotype and time. Root length was significantly affected by DAS (Table 4) and tended to be affected by genotype (*P* < 0.10). As expected, root length increased over the growing season, except when it decreased at 81 DAS, at the end of the growing season (Table 5). Increased root length could be attributed to plant roots needing to acquire mobile nutrients such as NO_3^--N, which may have been distributed farther from the roots in the soil profile with time. Because roots must explore the soil to acquire N and other nutrients and water, it is beneficial to have longer roots at flowering and physiological maturity as canola will require higher amounts of nutrients for seed formation. Notably, root length was positively correlated with root surface area (*p* < 0.05), and root length and surface area both increased over time until harvest maturity. Specifically, NAM-37 had the longest roots of all genotypes, while NAM-72 had the shortest (Table 5), potentially giving NAM-37 a greater advantage for nutrient absorption. Root diameter was smallest at 39 and 67 DAS, and largest at 81 DAS (Table 5). Root diameter was significantly negatively correlated with root length; as plants approached end of flowering and beginning and ripening, they appeared to prioritize root length over diameter, possibly aiming to absorb mobile NO_3^--N. For example, NAM-37 had the longest roots and smallest root diameter, while other genotypes like NAM-17 had larger root diameter (Table 5).

Table 4 Root traits and soil properties of 16 canola genotypes across five sampling points over the 2016 growing season. Means ± SD (n=48) followed by the same letter are not significantly different at *P* < 0.05.

DAS	Root traits			Soil properties		
	Root length (cm)	Root surface area (cm^2)	Root average diameter (cm)	NO_3^--N (mg kg $soil^{-1}$)	NH_4^+-N (mg kg $soil^{-1}$)	pH
32	114.21 ± 40.74^{a}	32.54 ± 10.18^{a}	0.95 ± 0.20^{a}	15.98 ± 6.85^{b}	3.40 ± 1.15^{b}	7.01 ± 0.24^{b}
39	202.67 ± 73.06^{b}	57.18 ± 23.18^{b}	0.91 ± 0.21^{a}	8.84 ± 4.76^{b}	3.10 ± 0.50^{b}	6.89 ± 0.21^{a}
53	228.80 ± 90.60^{bc}	67.48 ± 21.22^{bc}	0.99 ± 0.24^{ab}	8.57 ± 4.70^{c}	4.29 ± 1.00^{c}	7.04 ± 0.20^{bc}
67	304.68 ± 139.39^{c}	77.92 ± 23.65^{c}	0.97 ± 0.65^{a}	11.50 ± 7.27^{c}	4.50 ± 0.60^{c}	7.13 ± 0.27^{c}
81	206.04 ± 90.13^{b}	66.49 ± 22.58^{bc}	1.16 ± 0.50^{b}	4.82 ± 3.04^{a}	1.46 ± 0.50^{a}	7.09 ± 0.19^{bc}

Table 5 Root traits and soil properties of 16 canola genotypes across five sampling points over the 2016 growing season. Means ± SD (n=15) followed by the same letter are not significantly different at $P < 0.05$; bolded values are statistically highest within each variable.

Genotype	Root traits				Soil parameters		
	Root length (cm)	Root surface area (cm^2)	Root diameter (mm)	Root biomass (kg ha^{-1})	NH_4^+-N (mg kg^{-1} soil)	NO_3^--N (mg kg^{-1} soil)	soil pH
NAM-0	255.9 ± 137.3 ab	63.2 ± 26.8 ab	1.0 ± 0.3 ab	285.6 ± 122.9 ab	3.32 ± 1.15	9.99 ± 5.34 ab	7.13 ± 0.19 b
NAM-13	209.2 ± 109.0 ab	60.0 ± 22.7 ab	1.0 ± 0.3 ab	288.1 ± 96.6 ab	3.25 ± 1.14	11.96 ± 10.93 ab	7.07 ± 0.24 b
NAM-14	210.1 ± 98.5 ab	62.0 ± 26.2 ab	1.0 ± 0.3 ab	316.1 ± 122.9 ab	3.36 ± 1.38	9.15 ± 6.17 ab	6.99 ± 0.24 b
NAM-17	186.6 ± 93.8 ab	62.5 ± 32.6 ab	**1.2 ± 0.5 b**	314.1 ± 176.6 ab	3.05 ± 1.18	11.05 ± 3.68 ab	6.66 ± 0.15 a
NAM-23	204.5 ± 98.2 ab	65.3 ± 22.2 ab	1.2 ± 0.9 ab	**454.1 ± 339.9 b**	3.61 ± 1.24	7.84 ± 3.91 ab	7.08 ± 0.22 b
NAM-30	224.9 ± 75.3 ab	67.8 ± 30.8 ab	1.0 ± 0.3 ab	**357.8 ± 120.8 b**	3.27 ± 1.44	9.64 ± 8.32 ab	7.00 ± 0.17 b
NAM-32	273.7 ± 202.4 ab	70.7 ± 41.1 ab	0.9 ± 0.2 ab	281.4 ± 144.9 ab	2.68 ± 1.16	11.98 ± 5.43 ab	7.01 ± 0.13 b
NAM-37	**283.6 ± 140.1 b**	64.9 ± 24.2 ab	0.8 ± 0.1 a	255.3 ± 128.8 ab	3.44 ± 1.40	8.37 ± 5.30 ab	7.11 ± 0.15 b
NAM-43	195.9 ± 80.3 ab	51.7 ± 18.8 ab	0.9 ± 0.3 ab	290.1 ± 146.9 ab	3.69 ± 1.53	10.35 ± 4.19 ab	7.07 ± 0.27 b
NAM-46	178.8 ± 106.4 ab	55.5 ± 27.0 ab	1.1 ± 0.3 ab	250.2 ± 134.3 ab	3.60 ± 1.40	**6.36 ± 3.49 a**	6.94 ± 0.15 b
NAM-48	238.7 ± 101.6 ab	68.8 ± 25.3 ab	0.9 ± 0.2 ab	253.3 ± 163.0 ab	3.14 ± 1.20	11.45 ± 9.11 ab	7.12 ± 0.20 b
NAM-5	214.8 ± 153.9 ab	52.4 ± 27.8 ab	0.8 ± 0.2 ab	181.2 ± 89.2 a	3.20 ± 1.29	9.96 ± 5.73 ab	7.09 ± 0.17 b
NAM-72	166.5 ± 71.1 a	52.7 ± 22.7 ab	1.0 ± 0.4 ab	305.1 ± 232.8 ab	3.46 ± 1.35	**8.58 ± 6.39 a**	7.12 ± 0.16 b
NAM-76	202.1 ± 110.9 ab	60.1 ± 24.1	1.2 ± 0.7 ab	300.4 ± 232.9 ab	3.40 ± 1.75	8.66 ± 5.03 ab	7.03 ± 0.18 b
NAM-79	196.8 ± 100.7 ab	54.2 ± 25.0	0.9 ± 0.3 ab	257.9 ± 164.6 ab	3.58 ± 1.37	**14.03 ± 6.63 b**	7.14 ± 0.30 b
YN04-C1213	185.6 ± 106.5 ab	53.9 ± 23.4	1.0 ± 0.3 ab	260.0 ± 154.3 ab	3.46 ± 1.63	**9.43 ± 9.43 a**	6.92 ± 0.32 b

Table 6 Crop N uptake and NUE across 16 canola genotypes at harvest maturity (81 DAS), means followed by the same letter are not significantly different at *P* > 0.05.

Genotype	Straw N uptake (kg ha $^{-1}$)	Seed N uptake (kg ha $^{-1}$)	NUE
NAM-0	67.39 ± 13.90	107.40 ± 11.64 ^{bc}	0.60 ± 0.04 ^{bc}
NAM-13	58.27 ± 14.10	115.89 ± 3.06 ^{c}	0.64 ± 0.04 ^{c}
NAM-14	53.03 ± 40.72	117.40 ± 12.26 ^{c}	0.65 ± 0.04 ^{c}
NAM-17	43.63 ± 7.08	93.26 ± 1.79 ^{abc}	0.52 ± 0.04 ^{abc}
NAM-23	64.01 ± 5.46	124.38 ± 5.28 ^{c}	0.69 ± 0.04 ^{c}
NAM-30	67.76 ± 18.71	75.79 ± 6.18 ^{ab}	0.42 ± 0.04 ^{ab}
NAM-32	27.01 ± 22.44	94.94 ± 5.68 ^{abc}	0.53 ± 0.04 ^{abc}
NAM-37	42.82 ± 5.76	109.19 ± 4.91 ^{bc}	0.61 ± 0.04 ^{bc}
NAM-43	62.30 ± 14.19	91.43 ± 8.32 ^{abc}	0.51 ± 0.04 ^{abc}
NAM-46	56.61 ± 11.09	104.05 ± 26.67 ^{abc}	0.58 ± 0.04 ^{abc}
NAM-48	56.47 ± 18.90	93.26 ± 13.88 ^{abc}	0.52 ± 0.04 ^{abc}
NAM-5	52.63 ± 10.02	89.55 ± 6.37 ^{abc}	0.50 ± 0.04 ^{abc}
NAM-72	64.65 ± 17.87	102.73 ± 12.94 ^{abc}	0.57 ± 0.04 ^{abc}
NAM-76	45.02 ± 3.15	110.45 ± 27.27 ^{bc}	0.61 ± 0.04 ^{bc}
NAM-79	71.44 ± 11.99	69.42 ± 7.28 ^{b}	0.39 ± 0.04 ^{a}
YN04-C1213	44.65 ± 15.55	110.05 ± 11.24 ^{bc}	0.61 ± 0.04 ^{bc}

At harvest maturity (81 DAS), seed N uptake differed among genotypes ($P < 0.001$; Table 6). NAM-23, NAM-13, and NAM-14 had the highest seed N uptake (and yield; data not shown) and NAM-79 had the lowest (Table 7). NAM-23 had significantly lower soil NO_3^--N and significantly higher straw and root biomass. Nitrogen use efficiency also differed among genotypes ($P < 0.001$), with NAM-23, NAM-13, and NAM-14 having the highest NUE, while NAM-79 had the lowest (Table 6). In general, seed N uptake and NUE were both correlated with seed yield ($P < 0.0001$, r = 0.98) and negatively with soil NO_3^--N ($P = 0.011$, r = -0.61). The negative correlation between soil NO_3^--N and seed N uptake is likely due to depletion of soil nutrients from crop uptake.

The rhizosphere soil microbiome was also assessed in the 16 genotypes and five growth stages and related to root traits and soil inorganic N data. A total of 2680 rhizosphere ASVs were generated representing a diverse community of Bacteria across the 16 canola genotypes. The five most abundant taxa at the phyla level included Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi and Bacteroidetes. Microbial evenness and richness were both related to soil NH_4^+-N ($p = 0.0027$ and $p = 0.030$, respectively), and aboveground plant biomass ($P = 0.006$ and $P = 0.043$, respectively (Table 7). There was a negative relationship between NH_4^+-N and both diversity indices, signifying that as soil NH_4^+-N concentrations decreased microbial diversity increased.

Table 7 One-way ANOVA testing the relationship between soil and plant parameters with alpha diversity (Simpson's evenness and Richness) for the rhizosphere microbiomes across 16 diverse genotypes over the growing season. Significant *P*-values are bolded.

Variables	Simpson Evenness			Richness		
	R^2	*F*	*P*	R^2	*F*	*P*
Soil NO_3^--N	0.055	0.750	0.387	0.011	0.033	0.856
Soil NH_4^+-N	-0.188	9.199	**0.0027**	-0.136	4.763	**0.030**
Root length	0.048	0.577	0.448	0.039	0.390	0.533
Root surface area	0.033	0.267	0.606	0.020	0.105	0.746
Root diameter	-0.003	0.003	0.956	0.008	0.018	0.894
Aboveground plant biomass	0.172	7.666	**0.006**	0.127	4.128	**0.043**

Rhizosphere bacterial community composition was significantly different between genotypes ($P = 0.031$) and DAS ($P = 0.002$), but no significant interaction was observed ($P = 0.678$). These results indicated that time had a stronger effect on rhizosphere microbial community compositions than genotype. We used RDA to assess the relationships between the rhizosphere microbiomes with soil (inorganic N concentrations, moisture, and pH) and plant (root morphology, aboveground plant biomass) parameters. Genotype ($P = 0.027$) and DAS ($P = 0.007$) significantly affected rhizosphere microbial community structure; indicating that the drivers for rhizosphere bacterial community change is primarily linked to changes in plant growth stages and to plant genotypic differences. Soil pH is a known universal predictor of bacterial community structure, regulating bacterial composition and diversity across different soil types (Fierer and Jackson, 2006); hence it is important to note that pH moderately affected rhizosphere bacterial community structure in this study ($P = 0.098$).

Brassica napus genotype and environmental variation on soil properties

Soil nutrients and pH were analyzed across Llewellyn, Melfort, and Scott sites in 2017 and 2018 for all 16 *B. napus* NAM genotypes. Data from each site and year were analyzed separately due to slight differences in plant growth stage when sampling—though we have broadly categorized the three sample collection times within each growing season and site according to early vegetative, flowering, and pod-filling stages.

Sample collection time, coinciding broadly with the three growth stages, had the strongest effect on soil properties under *B. napus* (Table 8). Soil inorganic N concentrations were dynamic during the growing season, but patterns were inconsistent between the site-years. For example, soil NH_4^+-N declined sharply between the early vegetative

stage and flowering after which it remained stable in Melfort and Scott in 2017, while it remained high until flowering at Llewellyn in 2018 (Figure 2). Initially high nutrient concentrations are likely due to fertilizer and limited nutrient uptake while the plants are still relatively young; the discrepancy between sites may be due to more advanced growth, and consequently nutrient uptake, relative to sampling time at Llewellyn in 2017 and Melfort and Scott in 2018. A similar temporal pattern was observed for soil NO_3^--N (Figure 3), though the decline in NO_3^--N was not as stark between the vegetative and flowering stages at Melfort, possibly reflecting relatively higher N mineralization potential at this site due to higher soil organic matter content (8.2% at Melfort compared to 5.8% at Scott and 5.1% at Llewellyn. Available P also followed a similar temporal pattern, declining over time and following the same trend as observed at Llewellyn in 2016 (Figure 4). The reverse pattern was observed for extractable S, where extractable S tended to increase over time at all sites in 2017 and Melfort in 2018, whereas it declined between the vegetative stage and flowering and increased again between flowering and pod-filling (Figure 5). Soil pH varied with growth stage for five of the seven site-years (Table 8), with pH tending to increase at pod-filling compared to the early vegetative stage and flowering (Figure 6). The effect of growth stage on soil available P was less pronounced, and there was no significant effect at Scott in 2018.

The effect of genotype was greatest for soil inorganic N and extractable S, but there were no differences in soil pH or available P among *B. napus* genotypes (Table 8). Soil NH_4^+-N differed among genotypes across all sites except for at Scott. Soil NO_3^--N was affected by genotype, however, in the Scott 2017 site-year (Table 8). NAM-46 stood out as supporting higher NH_4^+-N in two site-years, while NAM-17 had high NH_4^+-N at Melfort in 2018 and high NO_3^--N at Scott in 2017. NAM-13, NAM-30, and NAM-76 had the lowest NH_4^+-N at Llewellyn in 2018. Extractable S differed across genotypes at Llewellyn in both years and Melfort in 2018 only. Although the ANOVA indicated genotype was a significant factor in explaining variation in total C at Llewellyn, post-hoc tests did not indicate statistical significance among means (see Appendix); higher statistical power with greater plot replication may have revealed differences. A summary of soil property means for each genotype are presented in the Appendix. The effect of genotype on soil NH_4^+-N and NO_3^--N depended on growth stage at Melfort in 2017. There was a tendency for a similar interactive effect on extractable S in that same year as well as in Llewellyn in 2017 and Scott in 2018.

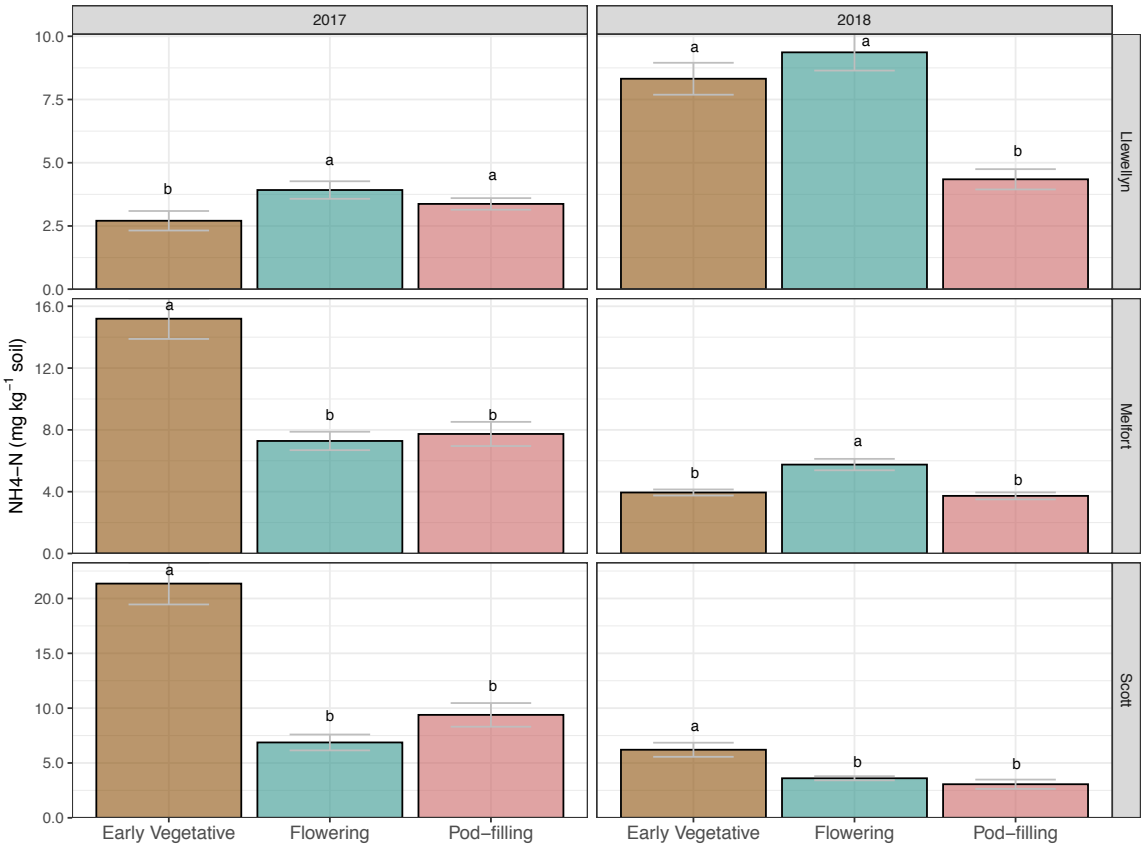

Figure 2 Soil NH_4^+-N concentrations in root-associated soils averaged across 16 *B. napus* genotypes within the 2017 and 2018 growing seasons for three sites (n=48).

Table 8 Analysis of variance *P* values on soil properties as affected by *B. napus* genotype and growth stage in 2017 and 2018 at three sites

Factor	NH_4^+-N (mg kg^{-1} soil)	NO_3^--N (mg kg^{-1} soil)	Modified-Kelowna P (mg kg^{-1} soil)	Extractable S (mg kg^{-1} soil)	pH
			Llewellyn - 2017		
Genotype	*0.0572*	0.2999	0.4648	*0.0547*	0.3930
Growth Stage	**0.0002**	**0.0158**	**<0.0001**	**<0.0001**	**<0.0001**
Genotype x Growth Stage	0.7821	0.1509	*0.0950*	0.0832	0.9411
			Llewellyn – 2018		
Genotype	**0.0096**	0.5634	0.7187	**0.0240**	0.1668
Growth Stage	**<0.0001**	**<0.0001**	**0.0170**	**<0.0001**	0.2721
Genotype x Growth Stage	0.6344	0.5530	0.9974	0.2224	0.2646
			Melfort -2017		
Genotype	*0.0509*	**0.0137**	0.2230	0.1227	0.5130
Growth Stage	**<0.0001**	**0.0098**	**<0.0001**	**0.0034**	**<0.0001**
Genotype x Growth Stage	**0.0111**	**0.0057**	0.3446	*0.0713*	0.2725
			Melfort -2018		
Genotype	**0.0064**	0.1697	0.2711	*0.0564*	0.3173
Growth Stage	**<0.0001**	**0.0079**	*0.0507*	**0.0108**	**0.0005**
Genotype x Growth Stage	0.8759	0.4856	0.9764	0.6875	0.5532
			Scott - 2017		
Genotype	0.3785	**0.0006**	0.9738	0.7651	0.1368
Growth Stage	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0166**
Genotype x Growth Stage	0.8280	0.1770	0.2790	0.8928	0.6805
			Scott - 2018		
Genotype	0.3794	0.8593	0.7789	0.1482	0.5136
Growth Stage	**<0.0001**	**<0.0001**	0.5601	**<0.0001**	**0.0289**
Genotype x Growth Stage	0.4587	0.4971	0.4932	*0.0959*	0.5718

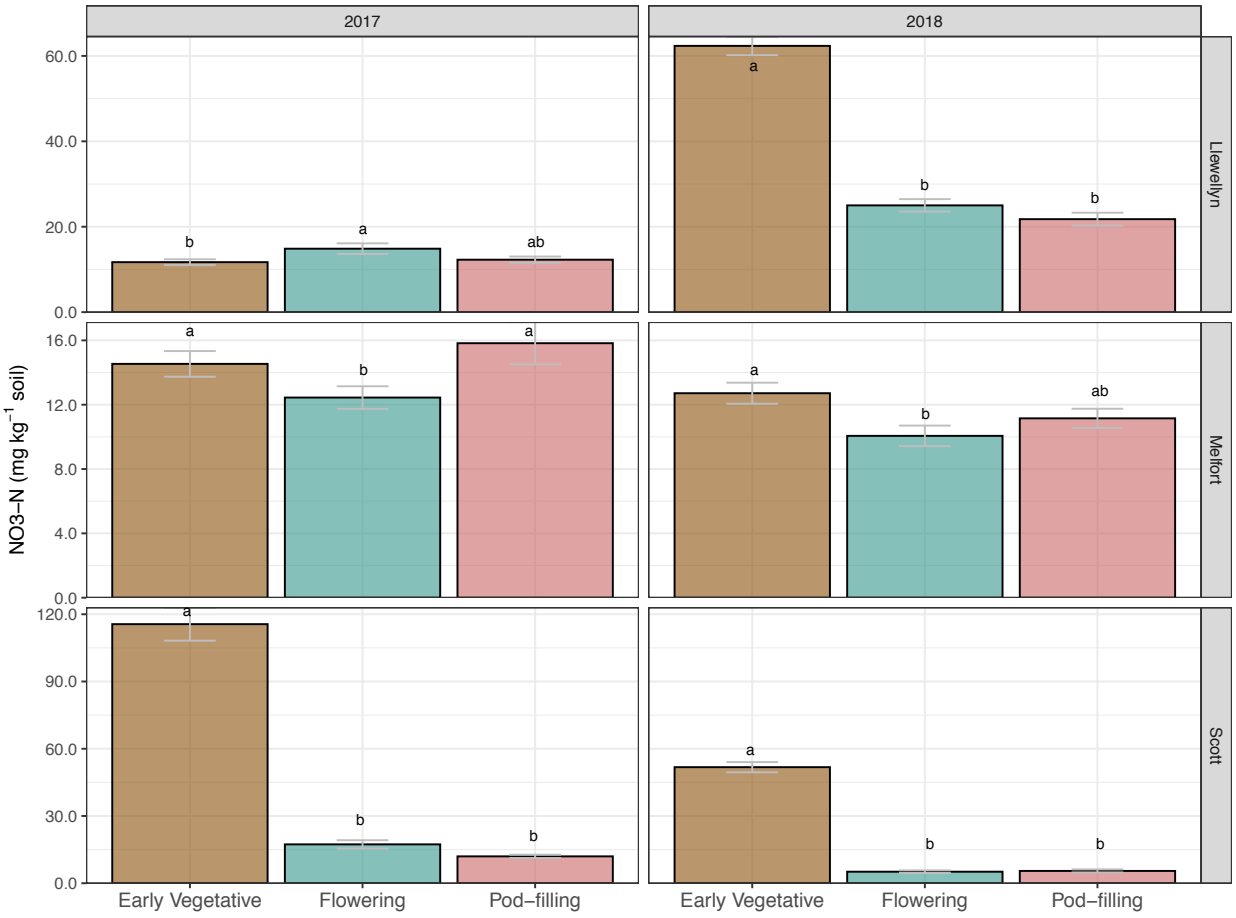

Figure 3 Soil NO_3^--N concentrations in root-associated soils averaged across 16 *B. napus* genotypes within the 2017 and 2018 growing seasons for three sites (n=48).

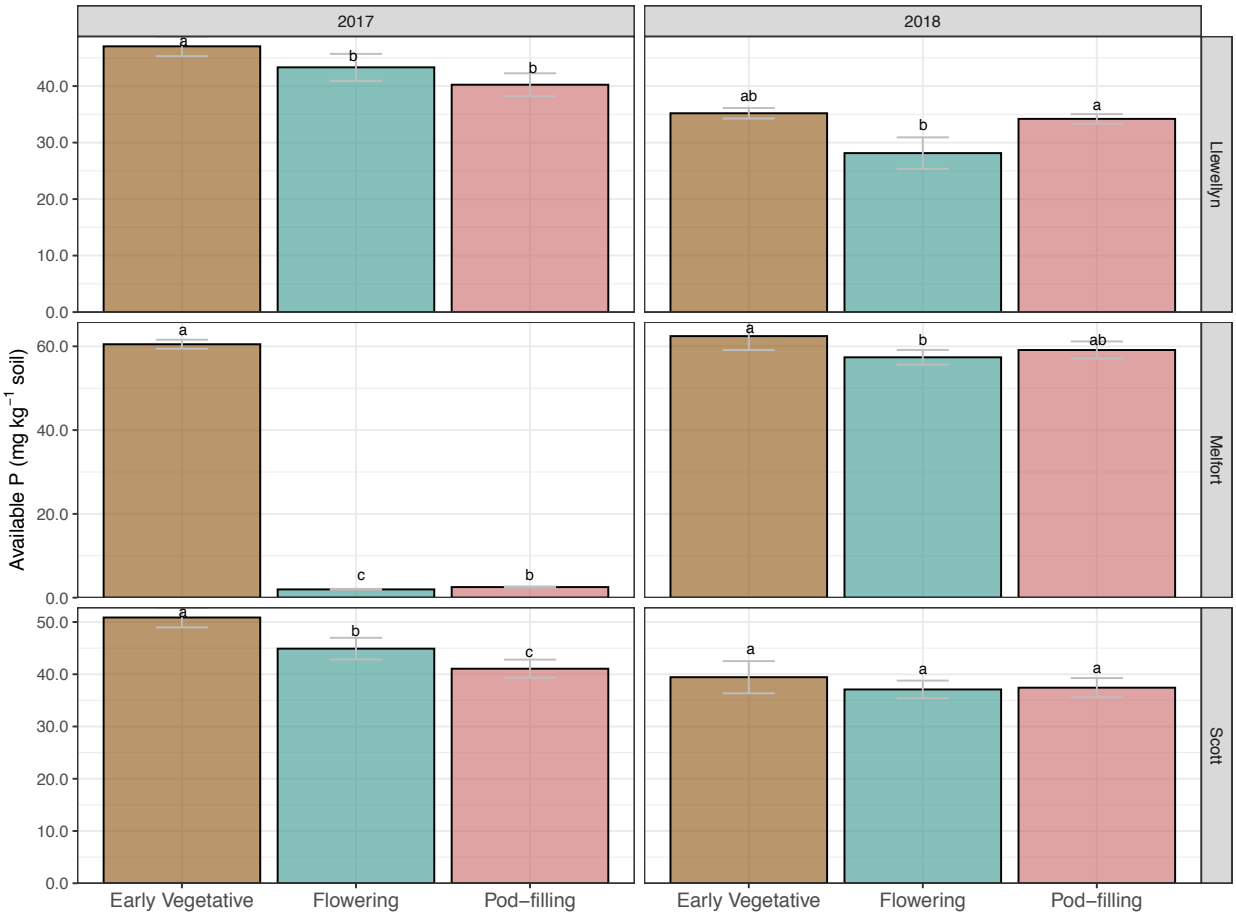

Figure 4 Soil available P (modified-Kelowna) concentrations in root-associated soils averaged across 16 *B. napus* genotypes within the 2017 and 2018 growing seasons for three sites (n=48).

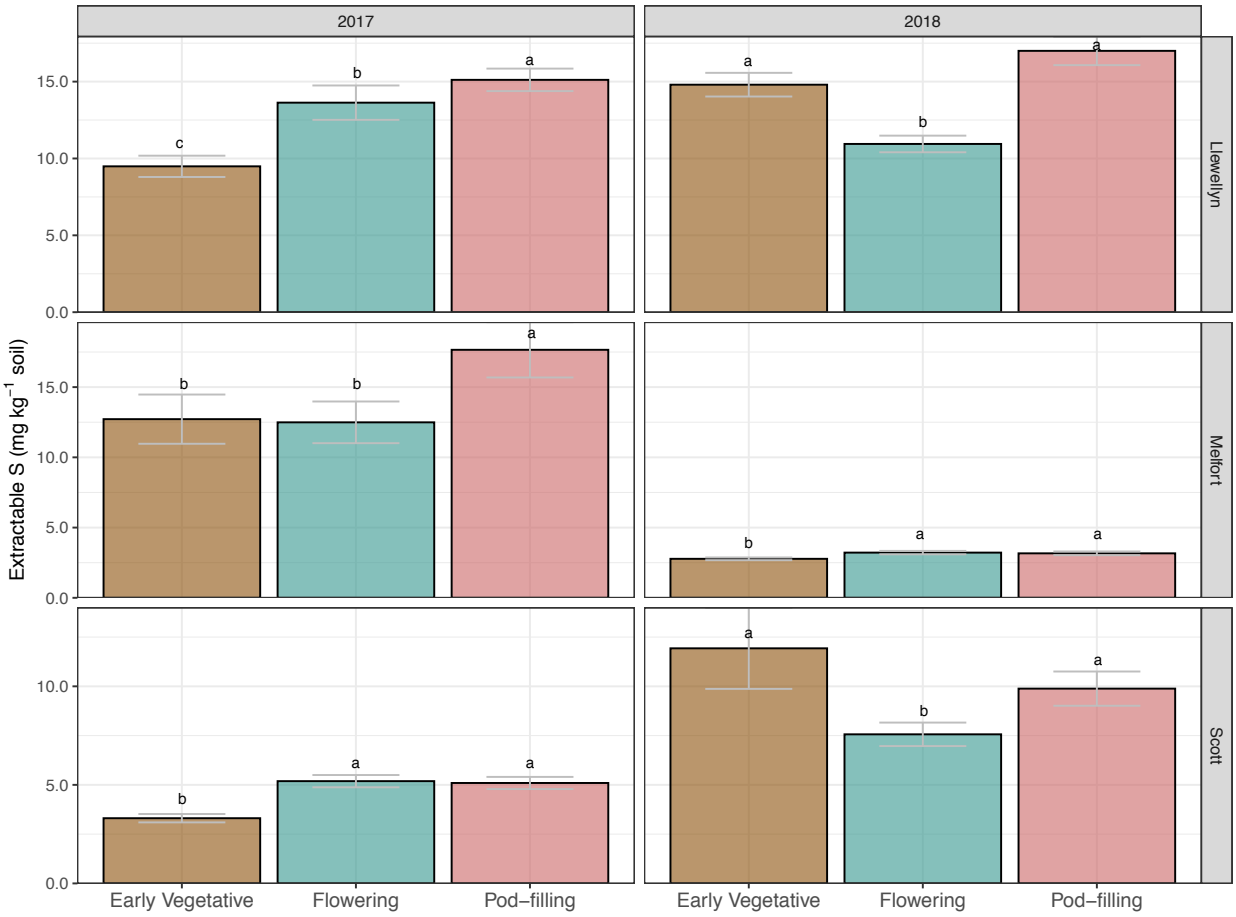

Figure 5 Soil extractable S concentrations in root-associated soils averaged across 16 *B. napus* genotypes within the 2017 and 2018 growing seasons for three sites (n=48).

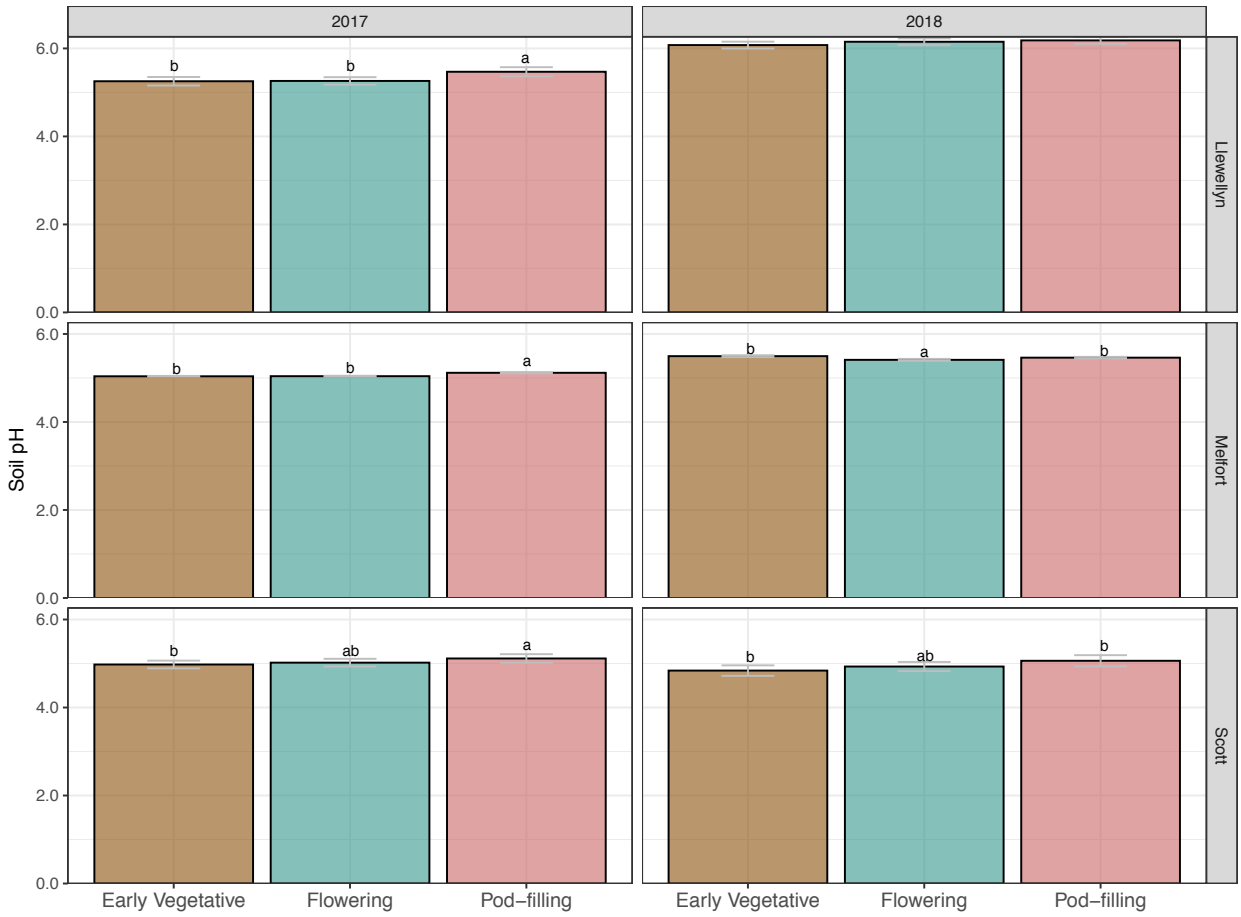

Figure 6 Soil pH in root-associated soils averaged across 16 *B. napus* genotypes within the 2017 and 2018 growing seasons for three sites (n=48).

Parental line and hybrid canola response to varying rates of nitrogen fertilizer: belowground processes and crop NUE

In 2018, a field trial was established at Llewellyn as part of the P^{2}IRC phenotyping project to examine soil nitrogen dynamics and crop nitrogen use efficiency of two NAM parental lines, studied in 7 site-years previously described, and two associated hybrids.

Soil NH_4^+-N varied with the interaction between N fertilizer rates and growth stage (P = 0.0072). Soil NH_4^+-N was highest under the 150 kg ha^{-1} N rate at both growth stages, and lowest in the control plots at the 5-6 leaf stage (Figure 7). There was also a two-way interaction between canola genotype and growth stage on soil NO_3^--N (P = 0.0055), with greater genotype differences at flowering. At flowering, soil NO_3^--N was similar under hybrid H151816 and its parental genotype NAM-17, and similar under parent genotype NAM-0 and hybrid H151857, which exhibited the lowest soil NO_3^--N (Figure 7). Soil NO_3^--N concentrations also increased with increasing N treatment rates at both growth stages (P = 0.0010). Urease activity varied with growth stage (P = 0.001); it was 1.3 times higher at flowering compared to the 5-6 leaf stage (Figure 8). However, urease activity was not affected by either canola genotype (P = 0.4652) or fertilizer N rate (P = 0.7557). Similarly, ammonium oxidation activity was not affected by canola genotype (p = 0.6585) nor N rate (P = 0.5350), but was affected by growth stage (P = 0.0003). In contrast to urease, the trend for ammonium oxidation activity was reversed, being 1.3 times greater at the 5-6 leaf

stage compared to flowering (Figure 8). Urease and ammonium oxidation activities were both positively correlated with soil pH (r = 0.72 and 0.67, respectively).

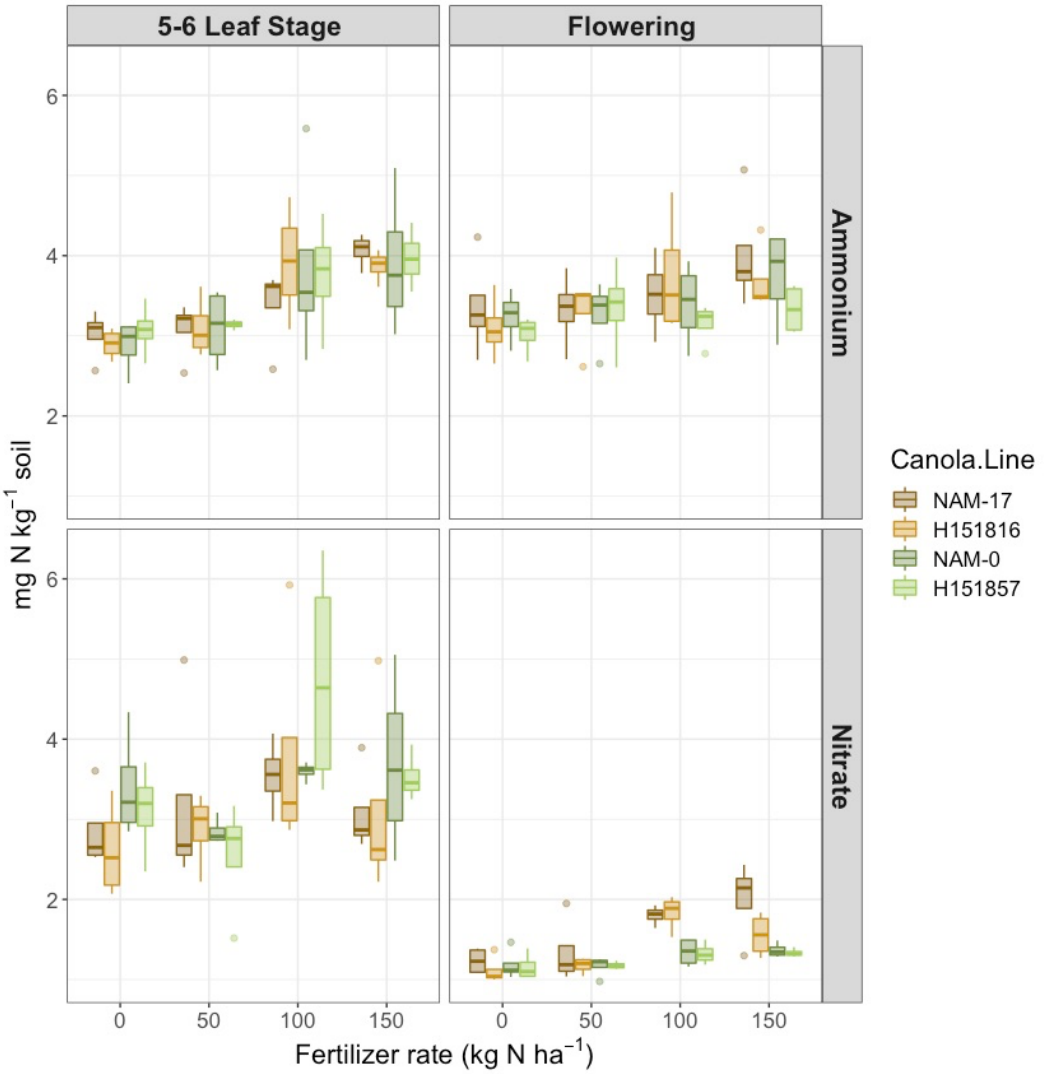

Figure 7 Soil ammonium-N and nitrate concentrations under four diverse canola genotypes and four fertilizer N rates at two phenological growth stages (5-6 leaf stage and flowering). Error bars represent standard error of the mean (n=4)

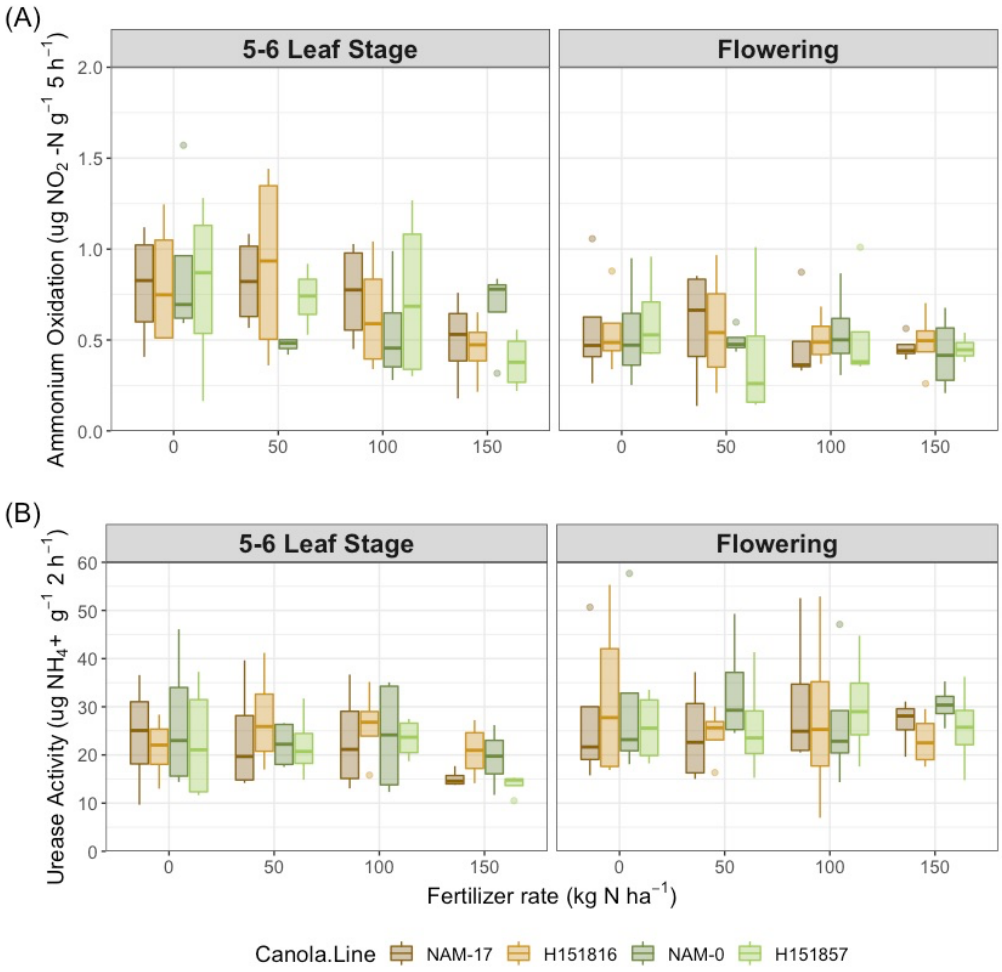

Figure 8 Ammonium oxidase activity (A) and urease activity (B) under four diverse canola genotypes and four fertilizer N rates at two phenological growth stages (5-6 leaf stage and flowering). Error bares represent standard error of the mean (n=4)

Canola yield differed among genotypes ($P < 0.0001$) and with N fertilizer rate ($P = 0.0149$). There was no significant interaction between genotype and N rate. Yield was lowest under both parental genotypes, and highest under both hybrids. N fertilizer increased yield at all application rates relative to the control, but there were no differences among N rates (Figure 9). Similarly, seed N uptake differed among canola genotypes ($P < 0.0001$) and N fertilizer rate ($P = 0.0006$), but not by the interaction between these two factors (Table 2). Like yield, seed N uptake was lowest under both parental genotypes, highest under both hybrids and increased in all fertilizer N-applied plots relative to the control plot, but not among the N rates. There was high variability in seed N uptake under the four genotypes, and like seed yield, parental genotype NAM-17 plateaued after the 50 kg ha^{-1} N rate. Additionally, NUE varied among canola genotypes ($p < 0.0001$) and N treatment rates ($p < 0.0001$), but there was no interaction between these two factors. Canola NUE was negatively correlated with N fertilizer rate ($r=-0.94$, $P < 0.0001$). Canola NUE was higher under both hybrids, as shown in previous studies, and lower under the parental genotypes, though the ranking of the parental genotypes did not align with their corresponding hybrids.

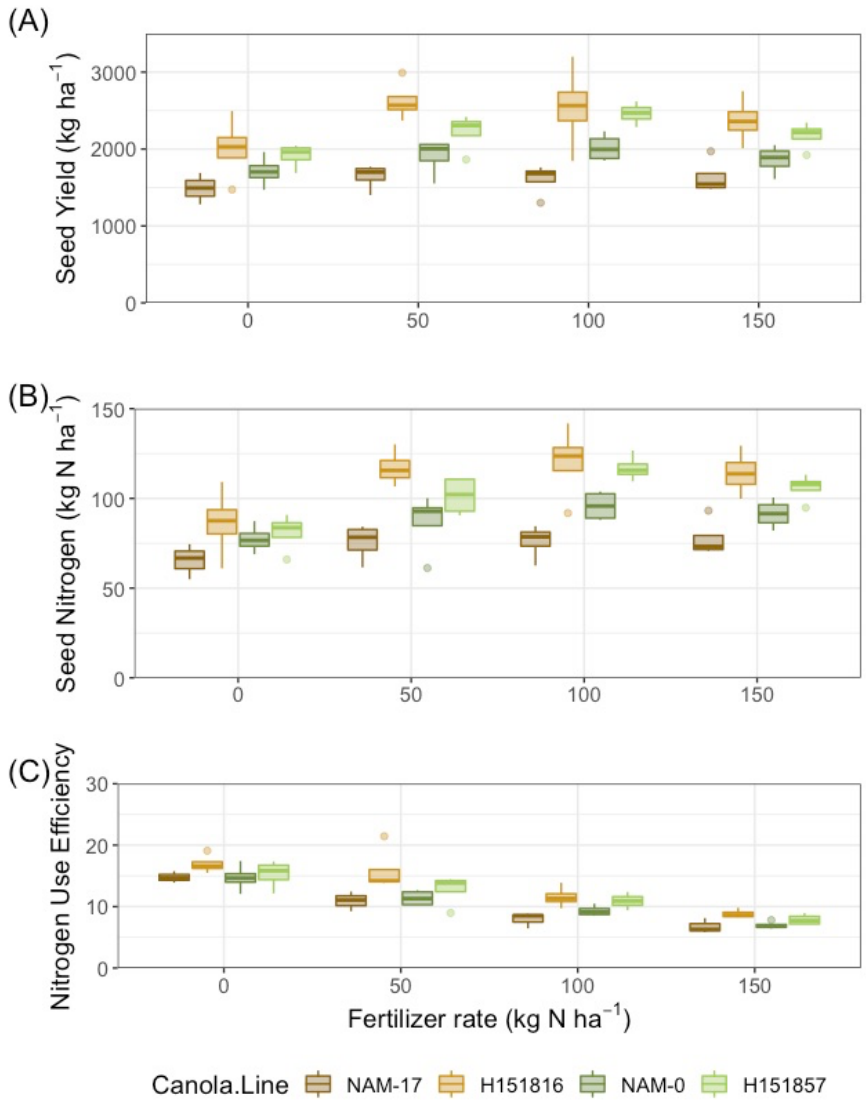

Figure 9 Yield, seed N uptake, and nitrogen use efficiency of four diverse canola genotypes and four fertilizer N rates at two phenological growth stages (5-6 leaf stage and flowering). Error bars represent standard error of the mean (n=4).

Relationships between soil and plant parameters were tested using pairwise Pearson's correlations. Soil NO_3^--N was negatively correlated with crop N uptake (r=-0.87, *P* = 0.00023). Soil NH_4^+-N was negatively correlated with ammonium oxidation rates (r=-0.85, *P* = 0.00048), urease activity (r=-0.77, *P* = 0.0033), and soil pH (r=-0.89, *P* = 0.00011), and positively correlated with N fertilizer rate (r=0.82, *P* = 0.0011). Soil pH was positively correlated with ammonium oxidation rates (r=0.83, *P* = 0.00086), and urease activity (r=0.91, *P* < 0.0001), and negatively correlated with N fertilizer rate (r=-0.65, *P* = 0.022). Urease activity was correlated with ammonium oxidation rates (r=0.60, *P* = 0.04). Nitrogen fertilizer rate was negatively correlated with urease activity (r=-0.59, *P* = 0.042) and ammonium oxidation rates (r=-0.67, p = 0.017).

10. **Conclusions and Recommendations:** Highlight significant conclusions based on the findings of this project, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project findings.

This project highlighted differences in soil properties, including soil inorganic N, extractable S, and pH under a diverse panel of *B. napus* genotypes. Crop nitrogen use efficiency was related to soil inorganic N, while soil inorganic N was correlated to soil microbial community diversity and composition. There were certain genotypes that had showed differences in soil inorganic N in more than one site-year, but what has caused these differences is unclear and warrants further investigation. The results from this work also indicate a strong sampling time effect on soil properties, driven by differences in crop growth stage that varied among genotypes due to differences in growth rates, but also likely due to seasonal changes in environmental factors such as soil moisture and temperature.

11. **Is there a need to conduct follow up research?** Detail any further research, development and/or communication needs arising from this project.

The *B. napus* genotypes utilized in this research varied greatly in seed quality characteristics and were chosen for the broader P^2IRC study as it was hypothesized that biochemical differences in the seed would translate to differences in the root exudate profiles, driving differences in the soil microbiome. However, the genotypes also varied in aboveground and belowground morphologies. As a result, the drivers of variations in soil properties underlying these genotypes, and feedbacks to crop productivity, were difficult to parse out. This initial survey demonstrated genotypic differences in certain soil properties, which lays the groundwork for future research. For example, future research includes directly characterizing root exudate profiles under varying crop genotypes in order to evaluate the relationship between root exudates and the soil microbial community. Additional research should test variations in root system architecture and crop nutrient uptake and yields under different nutrient availabilities (e.g., N fertilizer rates) to determine to what extent belowground plant traits explain variation in crop growth.

12. **Patents/ IP generated/ commercialized products:** List any products developed from this research.

13. **List technology transfer activities:** Include presentations to conferences, producer groups or articles published in science journals or other magazines.

Oral presentations

Arcand, M.M. (2020). Understanding soil-root interactions to maximize efficient use of soil resources and build soil health. Nutrien Agronomy Meeting. Saskatoon, SK, February 26, 2020.

Williams-Johnson, S., Vail, S. and **Arcand, M**. (2019). Determining canola nitrogen use efficiency in response to varying nitrogen rates across four select canola genotypes. Canola Innovation Day. Saskatoon, SK, December 5, 2019.

Williams-Johnson, S., Vail, S, and Arcand, M.M. (2019). Soil enzymatic nitrogen transformation in response to varying N treatments across four diverse Brassica napus (canola) lines. Canadian Society of Soil Science. Saskatoon, SK, July 11, 2019.

Williams-Johnson, S., Vail, S. and Arcand, M.M. (2019). Determining soil nitrogen transformation using enzymology in response to varying N treatments across four diverse Brassica napus (canola) lines. Soils and Crops Workshop. Saskatoon, SK, March 5, 2019.

Williams-Johnson, S., Vail, S, Helgason, B., Siciliano, S.D., Shirtliffe, S. and Arcand, M. (2018). Investigating the relationship between soil microbial communities and nitrogen cycling and crop nitrogen uptake among diverse canola lines throughout the growing season. Canadian Society of Soil Science Annual Meeting. Niagara Falls, ON, June 11, 2018.

Poster presentations

Williams-Johnson, S., Vail, S., Helgason, B., Siciliano, S., and Arcand, M.M. (2019). Nitrogen cycling in root associated soils at bolting, flowering and seed pod filling across eight diverse Brassica napus (canola) lines. Canadian Society of Soil Science, Annual Meeting. Saskatoon, SK, July 11, 2019.

Williams-Johnson, S., Vail, S., and **Arcand, M.M.** (2019). Nitrogen cycling in root associated soils at bolting, flowering and seed pod filling across eight diverse *Brassica napus* (canola) lines in Saskatoon, SK. Rhizosphere5, International Conference. Saskatoon, SK, July 10, 2019.

Williams, S., Vail, S. and Arcand, M. (2019). Soil enzymatic nitrogen transformation in response to varying N treatments across four diverse Brassica napus (canola) lines. Rhizosphere5. Saskatoon, SK. July 2019.

Williams, S., Vail, S., Helgason, B., Mamet, S., Siciliano, S., Shirtliffe, S. and Arcand, M. (2019). Nitrogen cycling in root associated soils at bolting, flowering and seed pod filling across eight diverse Brassica napus (canola) lines. Poster presentation at Canadian Society of Soil Science. Saskatoon, SK. July 7-10, 2019.

Williams-Johnson, S., Vail, S, Helgason, B., Siciliano, S.D., and Arcand, M. (2019). Nitrogen cycling in root associated soils at bolting, flowering and seed pod filling across eight diverse Brassica napus (canola) lines. Soils and Crops Workshop. Saskatoon, SK, March 5, 2019.

Williams-Johnson, S., Vail, S, Helgason, B., Siciliano, S.D., Shirtliffe, S. and Arcand, M. (2018). Linking soil nitrogen cycling to microbial community composition and function to differentiate nitrogen uptake among diverse canola (Brassica napus) genotypes. Ecology of Soil Microorganisms 2018. Helsinki, Finland, June 17, 2018.

Williams-Johnson, S., Vail, S, Helgason, B., Siciliano, S.D., Shirtliffe, S. and Arcand, M. (2018). From soil to seed: Nitrogen uptake and soil N under diverse canola (Brassica napus) lines. Soils and Crops Workshop. Saskatoon, SK, March 6, 2018.

14. List any industry contributions or support received.

This work was jointly funded through ADF and the Saskatchewan Canola Development Commission. Funding for establishment of the field trials was provided through Agriculture and Agri-Food Canada. Soil microbial analyses, soil N cycling process analyses, and some labour costs were supplemented through the Theme 1.3 Plant Pedological Phenotype of the Plant Phenotyping and Imaging Research Centre (P^2IRC) developed from the Canada First Research Excellence Fund awarded to the Global Institute for Food Security. A Robert P. Knowles Scholarship awarded to Shanay Williams-Johnson supplemented labour costs.

15. Acknowledgements. Include actions taken to acknowledge support by the Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bilateral agreement (for projects approved during 2013-2017) or Canadian Agriculture Partnership (For projects approved beyond 2017).

The Saskatchewan Ministry of Agriculture, the Canadian Agriculture Partnership, and SaskCanola logos were featured in the acknowledgements section of all poster and oral presentations.

16. Appendices: Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited.

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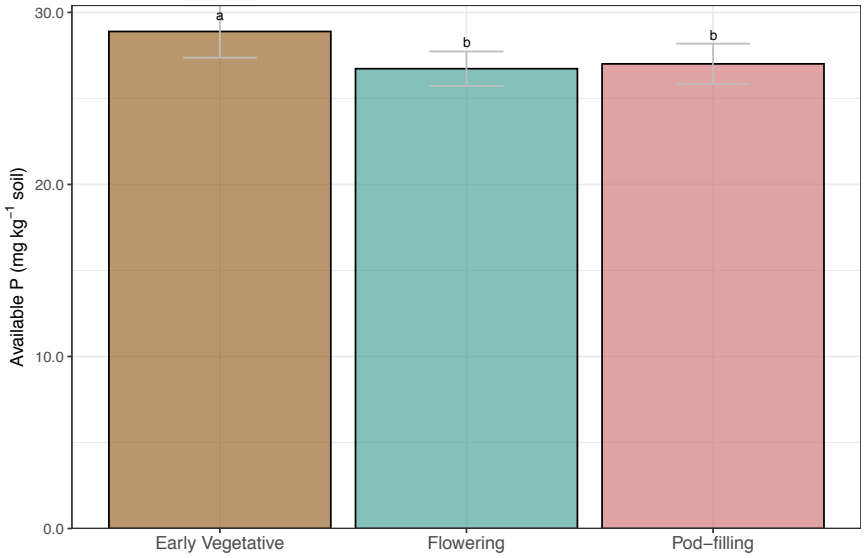

Figure 10 Soil available P (modified Kelowna) averaged across 16 *B. napus* genotypes over the growing season at Llewellyn site in 2016

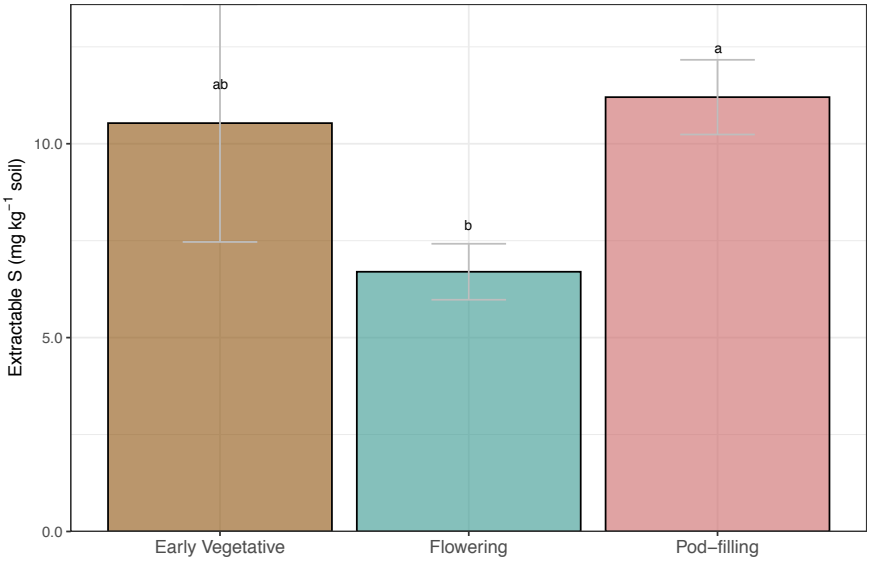

Figure 11 Soil extractable S averaged across 16 *B. napus* genotypes over the growing season at Llewellyn site in 2016

Table 9 Effect of *B. napus* genotype on soil properties averaged across three sampling times at three field sites in 2017

	Soil pH			NH_4^+-N (mg kg^{-1} soil)			NO_3^--N (mg kg^{-1} soil)			Modified-Kelowna P (mg kg^{-1} soil)			Extractable S (mg kg^{-1} soil)		
Genotype	**Llewellyn**	**Melfort**	**Scott**	**Llewellyn**	**Melfort**	**Scott**	**Llewellyn**	**Melfort**	**Scott**	**Llewellyn**	**Melfort**	**Scott**	**Llewellyn**	**Melfort**	**Scott**
NAM-0	5.2 ± 0.24	5.1 ± 0.02	5.5 ± 0.25	3.3 ± 0.74	9.9 ± 1.37	4.8 ± 0.93	11.8 ± 1.97	16.3 ± 3.59	33.3 ± 11.77	43.9 ± 5.98	20.0 ± 8.84	37.6 ± 5.43	12.1 ± 0.99	10.0 ± 2.33	4.6 ± 0.83
NAM-13	5.6 ± 0.33	5.1 ± 0.03	4.9 ± 0.12	2.6 ± 0.36	5.3 ± 0.84	11.6 ± 4.19	14.1 ± 2.22	9.9 ± 0.53	41.6 ± 16.71	45.3 ± 6.46	21.4 ± 9.73	45.8 ± 4.38	12.2 ± 1.75	7.2 ± 0.64	3.7 ± 0.58
NAM-14	5.1 ± 0.11	5.1 ± 0.02	5.0 ± 0.26	3.7 ± 0.57	13.3 ± 2.57	13.1 ± 2.00	12.2 ± 1.61	15.1 ± 1.74	35.4 ± 12.96	46.0 ± 3.67	21.7 ± 9.87	46.8 ± 5.01	13.3 ± 2.29	10.1 ± 1.48	4.6 ± 0.91
NAM-17	5.2 ± 0.08	5.1 ± 0.02	5.1 ± 0.26	4.6 ± 1.11	9.2 ± 2.79	21.4 ± 7.02	18.6 ± 3.49	13.3 ± 1.41	72.0 ± 27.49[a]	41.2 ± 2.74	22.7 ± 10.59	46.1 ± 6.12	13.4 ± 2.19	14.1 ± 3.25	5.5 ± 0.54
NAM-23	5.2 ± 0.13	5.1 ± 0.03	5.2 ± 0.26	3.5 ± 0.75	9.9 ± 3.58	13.0 ± 3.43	15.4 ± 3.98	15.4 ± 1.5	44.8 ± 15.31	43.7 ± 6.98	20.9 ± 9.67	39.0 ± 4.12	14.7 ± 1.69	18 ± 5.44	4.7 ± 0.60
NAM-30	5.8 ± 0.34	5.0 ± 0.03	4.6 ± 0.06	2.9 ± 0.28	6.8 ± 1.15	14.9 ± 3.06	14.8 ± 2.48	11.0 ± 0.83	59.7 ± 19.06	45.8 ± 5.20	22.6 ± 9.94	45.6 ± 1.44	10.1 ± 1.42	12.7 ± 2.48	4.1 ± 0.65
NAM-32	6.0 ± 0.26	5.1 ± 0.03	5.5 ± 0.23	1.6 ± 0.31	10.9 ± 2.06	10.5 ± 3.43	9.7 ± 1.18	15.7 ± 1.38	38.4 ± 13.43	28.9 ± 4.58	20.2 ± 9.23	39.5 ± 5.02	10.1 ± 1.34	17.1 ± 2.93	3.7 ± 0.55
NAM-37	5.2 ± 0.24	5.1 ± 0.05	4.6 ± 0.07	2.4 ± 0.39	8.0 ± 1.39	14.9 ± 3.81	10.8 ± 1.34	10.4 ± 0.72	43.3 ± 16.22	40.6 ± 5.18	22.0 ± 9.73	54.8 ± 6.07	9.6 ± 1.08	12.4 ± 3.10	5.1 ± 1.19
NAM-43	5.2 ± 0.11	5.1 ± 0.02	4.7 ± 0.06	3.7 ± 0.77	11.3 ± 2.75	11.8 ± 3.10	14.2 ± 1.97	15.8 ± 2.53	40.0 ± 16.56	44.4 ± 4.72	24.2 ± 10.65	52.0 ± 4.80	13.3 ± 2.42	13.6 ± 2.42	3.2 ± 0.47
NAM-46	4.9 ± 0.06	5.0 ± 0.02	4.7 ± 0.05	5.5 ± 1.25	10.7 ± 2.84	15.2 ± 3.40	13.5 ± 1.7	13.5 ± 1.54	63.9 ± 29.53	53.8 ± 4.17	21.8 ± 9.90	54.4 ± 3.13	11.0 ± 1.63	23.4 ± 5.25	4.7 ± 0.71
NAM-48	5.8 ± 0.29	5.0 ± 0.02	4.8 ± 0.05	4.1 ± 1.08	9.2 ± 2.22	13.9 ± 2.89	16.2 ± 1.9	12.3 ± 1.34	42.6 ± 14.65	44.0 ± 4.98	23.8 ± 10.87	49.9 ± 3.34	20.3 ± 2.42	10.5 ± 2.69	4.6 ± 0.98
NAM-5	5.5 ± 0.13	5.1 ± 0.03	5.1 ± 0.18	3.2 ± 0.54	8.7 ± 1.45	10.5 ± 2.71	11.4 ± 1.08	12.3 ± 1.69	48.5 ± 18.33	39.9 ± 2.89	19.0 ± 8.77	45.2 ± 4.29	13.7 ± 2.21	10.2 ± 1.71	4.1 ± 0.35
NAM-72	5.4 ± 0.36	5.0 ± 0.03	5.2 ± 0.25	2.6 ± 0.62	15.4 ± 5.34	8.9 ± 4.71	10.1 ± 0.62	17.2 ± 3.55	52.2 ± 21.78	44.5 ± 4.06	23.6 ± 10.71	46.2 ± 4.71	8.2 ± 1.33	20.2 ± 6.52	5.0 ± 0.62
NAM-76	5.2 ± 0.08	5.1 ± 0.02	5.3 ± 0.23	2.4 ± 0.53	10.4 ± 1.59	15.5 ± 5.54	11.0 ± 1.83	14.3 ± 1.4	49.0 ± 22.43	41.5 ± 3.00	21.4 ± 9.72	39.6 ± 4.36	15.5 ± 3.45	24.5 ± 9.00	5.0 ± 0.77
NAM-79	5.0 ± 0.07	5.1 ± 0.03	5.2 ± 0.27	4.5 ± 1.00	13.0 ± 2.20	9.3 ± 3.45	11.8 ± 1.05	22.2 ± 4.45	55.0 ± 23.92	47.7 ± 5.88	21.1 ± 9.42	42.9 ± 3.74	15.5 ± 2.99	13.3 ± 2.80	4.7 ± 0.66
YN04-C1213	5.0 ± 0.06	5.1 ± 0.03	4.9 ± 0.11	2.7 ± 0.51	9.3 ± 1.82	14.2 ± 3.93	11.0 ± 2.49	13.6 ± 2.19	84.3 ± 29.76	45.3 ± 3.19	20.1 ± 9.07	52.1 ± 2.72	10.3 ± 0.98	11.3 ± 1.82	5.1 ± 0.76

Table 10 Effect of *B. napus* genotype on soil properties averaged across three sampling times at three field sites in 2018

	Soil pH			NH_4^+-N (mg kg^{-1} soil)			NO_3^--N (mg kg^{-1} soil)			Modified-Kelowna P (mg kg^{-1} soil)			Extractable S (mg kg^{-1} soil)		
Genotype	**Llewellyn**	**Melfort**	**Scott**	**Llewellyn**	**Melfort**	**Scott**	**Llewellyn**	**Melfort**	**Scott**	**Llewellyn**	**Melfort**	**Scott**	**Llewellyn**	**Melfort**	**Scott**
NAM-0	6.1 ± 0.11	5.5 ± 0.05	5.5 ± 0.43	6.6 ± 1.31 [ab]	3.7 ± 0.55 [ab]	2.7 ± 0.65	29.3 ± 8.19	9.3 ± 0.85	17.3 ± 7.62	31.6 ± 5.07	48.6 ± 3.75	31.3 ± 3.86	13.0 ± 1.32	2.9 ± 0.19	7.1 ± 0.49
NAM-13	5.9 ± 0.12	5.5 ± 0.05	5.0 ± 0.4	4.7 ± 0.52 [b]	3.6 ± 0.65 [ab]	3.8 ± 0.78	35.3 ± 8.24	9.7 ± 1.11	22.2 ± 8.71	35.4 ± 4.62	62.9 ± 3.18	42.3 ± 6.59	11.6 ± 1.05	2.6 ± 0.18	8.5 ± 0.46
NAM-14	5.9 ± 0.14	5.5 ± 0.04	5.0 ± 0.35	9.8 ± 2.1 [ab]	3.6 ± 0.3 [ab]	3.6 ± 0.56	32.5 ± 6.28	9.4 ± 0.82	13.0 ± 6.51	37.3 ± 4.28	54.8 ± 2.83	39.1 ± 1.69	15.3 ± 1.55	2.9 ± 0.36	7.3 ± 0.34
NAM-17	6.4 ± 0.19	5.5 ± 0.04	4.8 ± 0.16	7.5 ± 1.48 [ab]	6.3 ± 1.06 [a]	3.8 ± 0.56	39.8 ± 7.3	12.4 ± 2.02	14.4 ± 7.19	34.7 ± 4.94	60.8 ± 5.09	39.6 ± 4.96	16.2 ± 1.86	3.0 ± 0.26	7.0 ± 0.3
NAM-23	5.8 ± 0.09	5.4 ± 0.02	4.7 ± 0.08	6.5 ± 1.38 [ab]	3.4 ± 0.54 [ab]	4 ± 0.56	40.5 ± 5.92	8.7 ± 0.55	14.8 ± 7.22	33.2 ± 3.91	59.5 ± 4.64	38.5 ± 3.42	14.5 ± 1.95	2.4 ± 0.19	20.5 ± 7.18
NAM-30	6.1 ± 0.21	5.3 ± 0.03	5.3 ± 0.39	4.9 ± 0.78 [b]	3.7 ± 0.38	3.9 ± 1.21	34.7 ± 5.93	11.0 ± 0.89	16.4 ± 6.64	32.6 ± 4.28	53.5 ± 4.15	45.4 ± 13.24	12.0 ± 0.95	2.5 ± 0.16	9.8 ± 2.39
NAM-32	6.2 ± 0.1	5.5 ± 0.03	5.2 ± 0.3	8.5 ± 2.34 [ab]	3.2 ± 0.48 [b]	3.0 ± 0.58	41.2 ± 11.46	11.3 ± 1.64	17.0 ± 8.31	27.4 ± 3.28	79.3 ± 11.65	30.0 ± 3.69	15.2 ± 1.57	3.2 ± 0.21	8.3 ± 0.69
NAM-37	6.3 ± 0.22	5.5 ± 0.05	4.6 ± 0.04	7.5 ± 1.65 [ab]	4.2 ± 0.48 [ab]	7.1 ± 1.6	34.5 ± 6.92	10.5 ± 1.43	17.7 ± 6.90	33.1 ± 4.00	59.9 ± 5.45	44.4 ± 3.24	14.3 ± 1.43	2.9 ± 0.26	22.4 ± 8.01
NAM-43	5.8 ± 0.07	5.5 ± 0.04	5.5 ± 0.45	8.6 ± 2.32 [ab]	4.5 ± 0.45 [ab]	2.2 ± 0.46	38.5 ± 6.92	14.6 ± 2.21	15.8 ± 7.82	37.0 ± 5.05	62.2 ± 6.05	31.8 ± 5.43	14.6 ± 1.20	3.2 ± 0.32	7.7 ± 0.89
NAM-46	5.7 ± 0.08	5.4 ± 0.05	5.1 ± 0.17	11.8 ± 1.44 [a]	6.0 ± 0.73 [a]	3.4 ± 0.75	35.7 ± 5.26	10.9 ± 2.17	17.7 ± 9.70	32.5 ± 4.33	59.5 ± 5.37	30.8 ± 4.20	17.5 ± 1.66	3.7 ± 0.36	7.5 ± 0.33
NAM-48	6.3 ± 0.26	5.6 ± 0.08	4.6 ± 0.09	6.1 ± 1.11 [ab]	5.0 ± 0.82 [ab]	4.8 ± 1.22	29.6 ± 6.49	11.3 ± 0.90	17.1 ± 9.00	27.7 ± 3.84	55.9 ± 5.48	38.6 ± 3.65	10.5 ± 0.90	3.3 ± 0.33	8.8 ± 1.33
NAM-5	6.3 ± 0.21	5.4 ± 0.02	4.6 ± 0.07	7.4 ± 1.53 [ab]	4.8 ± 0.61 [ab]	4.8 ± 0.9	34.2 ± 7.09	11.4 ± 1.21	19.0 ± 8.28	30.3 ± 5.52	59.2 ± 6.37	44.8 ± 3.23	12.7 ± 1.40	3.2 ± 0.26	8.7 ± 0.86
NAM-72	6.6 ± 0.22	5.4 ± 0.04	5.1 ± 0.33	7.3 ± 0.92 [ab]	4.6 ± 0.52 [ab]	4.3 ± 1.09	34.6 ± 7.31	11.3 ± 1.63	18.7 ± 10.21	34.4 ± 4.25	58.0 ± 4.31	36.2 ± 4.34	12.1 ± 1.20	3.1 ± 0.26	8.9 ± 1.47
NAM-76	6.4 ± 0.2	5.5 ± 0.06	4.7 ± 0.11	5.8 ± 1.61 [b]	4.1 ± 0.42 [ab]	4.5 ± 1.64	36.1 ± 9.97	12.6 ± 1.51	16.2 ± 7.16	26.2 ± 3.46	60.4 ± 5.82	36.2 ± 4.09	13.5 ± 2.87	3.0 ± 0.23	7.6 ± 0.64
NAM-79	6.4 ± 0.15	5.4 ± 0.05	4.7 ± 0.04	7.6 ± 1.14 [ab]	5.8 ± 0.86 [ab]	3.7 ± 0.67	40.7 ± 7.1	15.2 ± 1.45	16.9 ± 8.82	34.6 ± 4.39	64.8 ± 5.97	37.0 ± 1.35	18.5 ± 3.13	3.9 ± 0.30	8.3 ± 0.51
YN04-C1213	6.0 ± 0.17	5.5 ± 0.04	4.6 ± 0.10	6.1 ± 1.41 [ab]	5.0 ± 1.11 [ab]	5.4 ± 1.73	28.5 ± 8.57	11.5 ± 1.53	18.4 ± 9.17	30.6 ± 4.32	55.1 ± 4.66	41.8 ± 6.58	16.7 ± 3.82	3.1 ± 0.17	7.9 ± 0.64

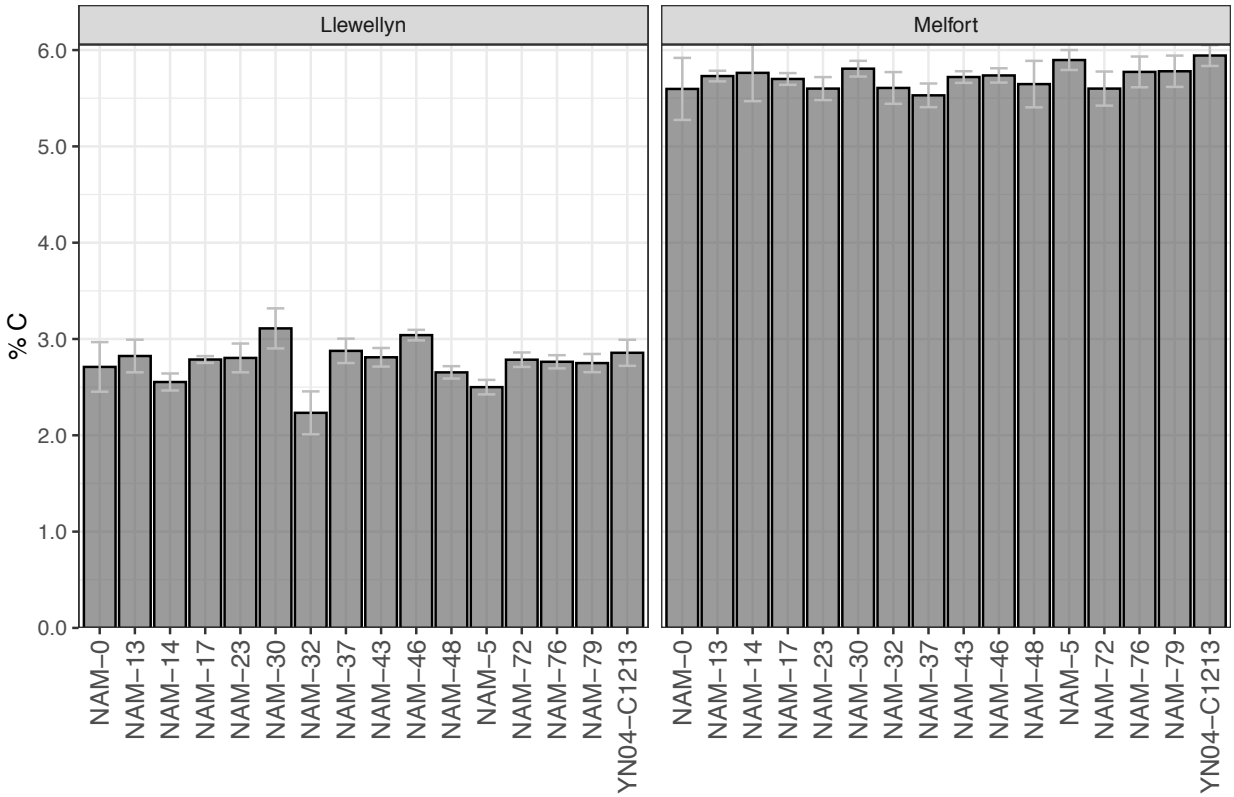

Figure 12 Soil total carbon (%) in root-associated soils at flowering (n=3) in 16 B. napus genotypes at the Llewellyn and Melfort field sites in 2017