Nitrogen uptake by rapeseed varieties from organic matter and inorganic fertilizer sources

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Abstract

Aims Half of field crop nitrogen (N) is often derived soil organic matter (SOM) mineralization, yet we do not fully understand the extent to which plant genotypic differences influence SOM mineralization dynamics across different soil N contexts. We explored the effects of rapeseed (*Brassica napus*) genotypic diversity on N uptake from organic and inorganic N sources.

Methods In a greenhouse study, we applied dual ^{15}N labeled ammonium-nitrate fertilizer to examine N uptake patterns of rapeseed in different N environments. Ten varieties were grown in a full factorial experiment with four treatments, including combinations of high and low N fertilizer and SOM.

Results We found limited varietal differences in total biomass or N uptake across soil environments. Across all varieties, SOM was an important, additive N source even as N fertilizer availability increased. High SOM/High Fertilizer treatment plants obtained 64% of N from SOM, while plants grown with High SOM/Low Fertilizer obtained 89% of total N from SOM. Under low SOM availability, the high fertilizer addition increased overall N uptake from SOM by 42% relative to the low N fertilizer treatment.

Conclusions Integrating plant reliance on SOM-N sources into crop breeding and NUE estimates has potential to improve crop productivity and improve overall system N use efficiency.

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Introduction

Over the last half century, the application of mineral nitrogen (N) fertilizer has supported increased crop production while also more than doubling the availability of reactive N in the global environment (Zhang et al. 2015). Only about 50% of N fertilizer is taken up by crops in the year of application despite decades of research aimed at improving the delivery of fertilizer to plants (Yan et al. 2019) and large N losses from agricultural systems continue to impact water quality, contribute to greenhouse gas emissions, and negatively impact surrounding ecosystems (Sobota et al. 2015). Reducing reliance on synthetic N inputs and improving plant nitrogen use efficiency (NUE) are essential to improve agricultural sustainability.

Globally, N mineralized from soil organic matter (SOM) is estimated to provide about 50% of N taken up by global cereal crops even in intensive management systems (Chen et al. 2014; Gardner and Drinkwater 2009; Yan et al. 2019). While SOM is recognized as a source of plant available N in soil testing and fertilizer recommendations, there has been limited research focused on managing N mineralization processes through targeted crop management or crop breeding (although see recent examples such as Kelly et al. 2022; Mwafulirwa et al. 2021). As a result, we have a limited understanding of belowground activity that affects crop utilization of N derived from the mineralization of SOM. Most modern crops have been primarily bred for high grain yield and field agronomic performance in systems where N is not a limiting factor. While roots are known to mediate N uptake through physiological and morphological mechanisms (direct effect) and microbial activity (indirect effect), belowground traits are challenging to introduce into traditional breeding programs and have largely been ignored (Bouchet et al. 2016; Dawson et al. 2008; Jilling et al. 2018; Stahl et al. 2016).

Crop NUE is typically defined as the total plant biomass or seed yield divided by applied N. However, NUE is a complex trait that integrates the efficiency of plant N uptake from the soil environment as well as N utilization patterns within the plant (Bouchet et al. 2016; Kant et al. 2011; Perchlik and Tegeder 2017; Rathke et al. 2006). Due to the challenges of directly measuring soil N mineralization from SOM, N management and breeding efforts have primarily focused on either N fertilizer uptake efficiency (RE_N, the proportion of plant N uptake to N fertilizer applied) or the efficiency of internal N utilization (the proportion of N uptake translocated to produce seed at maturity) (Cassman et al. 2002; Kessel et al. 2012).

Root biomass and structure affect soil carbon (C) inputs and root exudates, which can have profound influences on the crop's ability to obtain nitrogen from diverse sources (Bouchet et al. 2016). Root morphology is also important for nutrient uptake and has been shown to have a significant effect on aboveground growth and vigor (Garnett et al. 2009). A number of studies support that root traits, such as root-shoot ratios, root length density, and root N transport and metabolism could contribute to higher NUE in crops (Bingham et al. 2012; Bowles et al. 2015; Garnett et al. 2009; Ju et al. 2015).

Belowground traits are recognized as important for N uptake and may be particularly important for improving utilization of N from SOM (Kelly et al. 2022).

The mineralization of soil N from SOM into plant available forms is highly complex and requires multiple steps. The depolymerization of SOM from more complex N-containing molecules, such as lignin and proteins, to more simple monomers, such as amino acids, has been proposed as the rate limiting step in most soil environments (Daly et al. 2021; Schimel and Bennett 2004). From these monomers, N is typically converted rapidly to the most common plant available mineral forms of ammonium and then, under aerobic environments via nitrification, to nitrate. Each of these processes is mediated by microbially-derived extracellular enzymes. Indirectly, roots can enhance or inhibit these processes via their effects on soil microbial communities and activity (Coskun et al. 2017a; Jilling et al. 2018). Root exudates can enhance microbial mineralization of C, N, and other essential nutrients, such as through increasing the availability of mineral-associated N-containing compounds for plant uptake (Faucon et al. 2017; Fontaine et al. 2003; Jilling et al. 2018). Plants can also influence the rate of SOM decomposition by stimulating or inhibiting microbial activity in general (Huo et al. 2017), or by inhibiting specific processes such as nitrification (Coskun et al. 2017b).

Rapeseed (*Brassica napus*), also known as canola and oilseed rape, is the second most important oilseed crop in the world. Rapeseed is highly valued for its widely used, high quality vegetable oil as well as high protein meal used in livestock feed (Stahl et al. 2016). Generally, rapeseed is considered a high N demanding crop with a low NUE compared to other field crops (Bouchet et al. 2016; Kessel et al. 2012; Rathke et al. 2006). The relatively low NUE is attributed primarily to a narrow acquisition efficiency of the crop (Rathke et al. 2006; Stahl et al. 2016). The focus on improving N management has led to more interest in breeding rapeseed varieties with increased NUE capacity. This research has demonstrated genetic variation in both RE_N and internal N utilization efficiency (Kessel et al. 2012; Stahl et al. 2016). Kessel et al. (2012) suggested that rapeseed RE_N showed more genetic variation at lower fertilizer N levels whereas N translocation dynamics within the plant contributed more to variation in NUE at high fertilizer N levels.

Understanding the belowground dynamics of N recovery from SOM, as well as applied N fertilizer, could be an important new direction for improving the sustainability of this globally important crop. Recent research has focused on root exudate effects on nitrification (Coskun et al. 2017b), but limited research has focused on plant mediation of upstream N mineralization processes such as depolymerization. We evaluated the effects of rapeseed genotypic diversity on N uptake from organic and inorganic N sources. We used ^{15}N enriched ammonium-nitrate (NH_4NO_3) fertilizer and soil enzyme activity to determine differences in N acquisition and microbially-mediated N cycling from organic and inorganic N pools as influenced by rapeseed varieties. We hypothesized that different rapeseed varieties would differ in N source uptake patterns and soil enzyme activity.

Materials and methods

Experimental design

A greenhouse pot study was conducted at the Colorado State University Plant Growth Facility in Fort Collins, CO (40.5717° N, 105.0812° W) from June to September 2016. Ten rapeseed varieties were selected from the 51 founder lines of the Parkin et al. (2017) project, that were selected for development of a germplasm resource to dissect complex traits in *B. napus*. This project collected diverse rapeseed lines from around the world to make a nested association mapping (NAM) population that could be used to introduce new diversity into breeding germplasm (Parkin et al. 2017). The ten varieties for this study were selected using 12,612 single nucleotide polymorphisms (SNP) markers to capture the widest possible general genetic diversity based on genetic distance, and geographic locations of the markers while controlling for common flowering time (Table 1, Fig. 1).

The rapeseed plants were grown in soil mixtures that represented 2 levels of SOM and 2 levels of N fertilizer for a total of 4 treatments (High SOM/High Fert, High SOM/Low Fert, Low SOM/High Fert, and Low SOM/Low Fert). To generate the SOM treatments, a high organic matter field soil was added to a non-soil mixture to create almost a 7-fold difference in soil organic matter levels across the high and low SOM treatments (Table 2). The field soil was a

Table 1 *Brassica napus* varieties selected for this study chosen from the founder lines of NAM project (Parkin et al. 2017) and their country of origin

NAM Founder Line ID	Variety Name	Origin
NAM-0	N99–508	Common line
NAM-1	Czyzowska	Poland
NAM-5	BN-1	India
NAM-26	Noiza 531	Argentina
NAM-28	Topas	Sweden
NAM-33	Dong Hae 3	S. Korea
NAM-43	PI433395	Unknown
NAM-73	Optima	Denmark
NAM-76	Ebony	Canada
NAM-82	Tribune	Australia

fine loamy Aridic Argiustoll with 67 g kg^{-1} organic matter (37 g kg^{-1} C, 3.8 g kg^{-1} N) collected from a farm near Fort Collins, Colorado, with a history of organic vegetable production. The field topsoil (0–10 cm) was collected and sieved to 8 mm. The non-soil mixture of 25% sand, 37.5% calcined clay, and 37.5% vermiculite by volume was homogenized. The field soil was mixed with this non-soil mixture at two different proportions by volume to create high SOM and low SOM treatments (Table 2) and homogenized using a clean cement mixer. Initial soil treatment properties were analyzed for texture, organic matter (loss on ignition), and extractable nutrients by Ward Lab (Lincoln, Nebraska) and results are presented in Table 2. The SOM levels were selected with the general assumption than 1–2% of total N in SOM would be mineralized during the short time period of the study, resulting in mineralization of approximately 50–100 mg N in high SOM and 10–20 mg N in low SOM treatments.

The plants were planted in randomized complete blocks with 5 blocks of each of the 4 treatments with each of the 10 varieties for a total of 200 pots. Each block was planted 1 week apart for 5 weeks. The planting was staggered to allow for staggered harvest and time-sensitive soil analyses to be completed for each block. Greenhouse conditions included 16 h of light per day and approximately 15.6 °C nighttime and 26.7 °C daytime temperatures.

Four seeds were planted into a 3.8-l pot (16.5 cm top diameter, 12.7 cm bottom diameter, 16.5 cm height) and thinned to one plant per pot one week

Fig. 1 Dendrogram of genetic relationships and distances of *Brassica napus* founder lines of Nested Association Mapping (NAM) project (Parkin et al. 2017). Genetic distances are presented on the Y axis and variety on the X axis. Blue text indicates varieties selected for this study
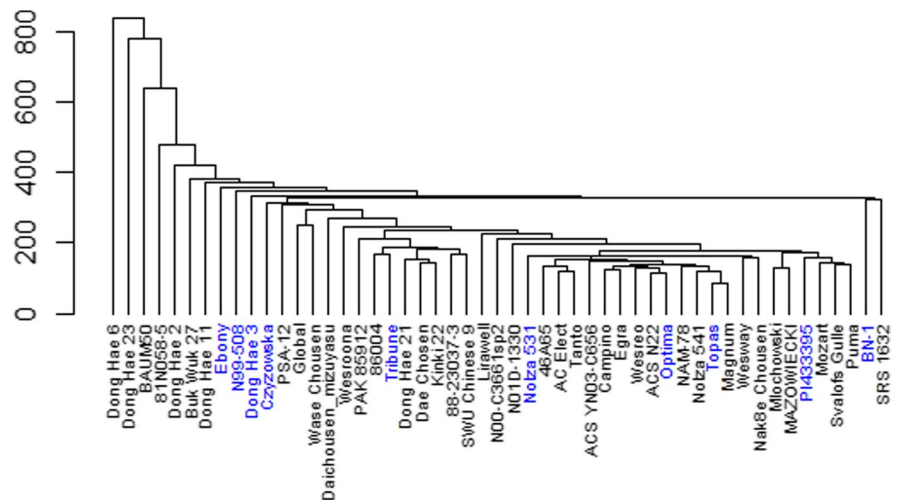
Table 2 Initial properties of the low and high soil organic matter (SOM) soil mixtures generated by mixing the same field soil with different proportions of a soilless mixture by volume

	High SOM	Low SOM
Field Soil (%)	60	10
Soilless Mix (%)	40	90
Sand (%)	64	87
Silt (%)	16	8
Clay (%)	20	5
pH	7.3	7.0
SOM (g kg^{-1})	35	6
Total N (g pot^{-1})	5.4	0.9
NO_3^- (g kg^{-1})[a]	42	12
P (g kg^{-1})[b]	107	77
K (g kg^{-1})	532	387

[a]Extractable nitrate in 2 *M* KCl; [b] Mehlich 3 extractable phosphorus

after planting. Fertilizer treatments were initiated at 2 weeks after planting when the first true leaves were beginning to emerge. Once per week, 50 ml of N-free Hoagland's nutrient solution was applied to each pot to ensure that nutrients other than N were not limiting across all treatments. A ^{15}N enriched N fertilizer solution using 98% ^{15}N enriched dual labeled NH_4NO_3, diluted down to 8% ^{15}N enrichment, was applied weekly as a liquid solution to the soil surface to obtain the specified total N additions for the high and low fertilizer rates (Balint and Rengel 2008; Damon et al. 2007). Fertilizer rates of 50 mg N pot^{-1} for Low Fert treatments and 150 mg N

pot^{-1} for High Fert treatments were chosen to provide sufficient N through vegetative growth based on estimates from Balint and Rengel (2008). Based on the treatment, supplemental ^{15}N enriched fertilizer was applied weekly to achieve desired N rates. The second week after planting, 100 ml of N-fertilizer solution was applied, and 50 ml was applied in all subsequent weeks for a total of 5 fertilizer applications. For the Low Fert treatment, 10 mg N were added each week for a total of 50 mg N. For the High Fert treatment, 50 mg N were added with the first application and 25 mg N was added in each subsequent week for a total of 150 mg N. Any liquid that ran through the pot was caught in the trays below the pot and added back into the pot to eliminate N loss by leaching.

The irrigation system was started three weeks after planting and water was applied for two minutes each day using drip emitters. No additional irrigation water was given on days when liquid N fertilizer was applied. Due to differences in soil texture across treatments, a soil moisture probe (Hydrosense II, Campbell Scientific, Logan, UT) was used in the pots twice a week to measure pot moisture and the irrigation amounts were adjusted to equalize volumetric water content to approximately 20% across all treatments and blocks.

Because our research question was about N acquisition from the soil environment and not internal N translocation patterns, we sampled plants at peak biomass. Each block was destructively harvested at 39–41 days after planting when about 75% of the plants in a block were at the elongation stage before flowering. Each individual pot in the block was

photographed, weighed, and notes were recorded for the number of leaves per plant and the growth stage (pre-bud, bud, or early flowering). The plant was clipped at the base of the stem. The clipped plant shoot was put in a paper bag and dried at 55 °C for at least 5 days and weighed for dry shoot biomass.

The pot of soil was turned upside down in a clean tub. The loose soil was gently brushed off leaving the root ball and the rhizosphere soil surrounding the roots. The rhizosphere soil and root ball and the bulk soil were placed in separate zip lock bags and placed in a cooler with ice until they were placed in cold storage for further processing. A subsample of the bulk soil from each pot was weighed and dried at 105 °C for a minimum of 48 h to determine soil gravimetric water content.

Enzyme activity

The activity of four soil enzymes involved in SOM decomposition and soil nutrient cycling were measured using fluorescence-based enzyme activity assays. We included enzymes involved in C, N, and P mineralization processes (Table 3). In particular, we focused on enzymes involved in catalyzing the conversion of more complex N-containing compounds to amino sugars or amino acids (NAG, LAP, Table 3). We included corresponding C and P cycling enzymes to evaluate whether enzyme activity effects were specific to N-cycling processes or broader shifts in soil microbial activity. Rhizosphere soil samples from each pot were analyzed using a microplate fluorescence-based MUB (4-methylumbelliferone) and MUC (7-amino-4-methylcoumarin) substrate protocol (Bell et al. 2013). Briefly, the day after the plants were harvested, 1.1–1.3 g of soil was weighed from the rhizosphere soil sample.

The soil was blended to homogenize sample with a 50 mM sodium acetate buffer solution, that had been adjusted to the average soil pH of 7.5 to make a soil slurry. Soil slurry was pipetted into black, 96-well microplates with compound-specific fluorescing substrates. Samples were analyzed using a Tecan Infinite M200 plate reader (Tecan Austria GmbH, Salzburg, Austria).

Inorganic nitrogen

A 10 g sample of the rhizosphere soil from each pot was extracted with 100 mL of a 2 M potassium chloride (KCl) solution to analyze levels of extractable ammonium (NH_4^+) and nitrate (NO_3^-) in the soil at the time of harvest using the microplate colorimetric method (Sims et al. 1995). The vanadium (III) chloride (VCl_3) protocol was used to determine soil NO_3^-, where 30 μL of the KCl extracted sample was pipetted into microplates with 0.4 M VCl_3 solution (Doane and Horwáth 2003). The salicylate-hypochlorite method was used to determine soil NH_4^+, where 70 μL of KCl extracted sample was used in each of the microplate wells. Both assay reactions were read on a microplate reader (BioTek Instruments, Winooski, VT). Inorganic N values are not dependent on dry plant biomass so, all 5 blocks of data were used for analysis.

Nitrogen source analysis

Isotopic values of the dried plant shoots were analyzed to determine the relative contributions of the fertilizer and SOM to plant N content. Dried plant samples were ground to 2 mm in a Wiley Mill and then roller ground until the sample was homogenized.

Table 3 Enzyme name, abbreviation, function in the soil (nutrient cycle indicator), and final product for the four soil enzymes assayed

Enzyme Name	Abbreviation	Function in Soil	Final product
B-1, 4-n-acetyl-glycosaminidase	NAG	Hydrolysis of chitin	N-acetyl-b-D-glucosamine (sugar)
Leucine amino peptidase	LAP	Hydrolysis of amino acid residues (N- terminus of peptides and proteins)	Leucine (other amino-acids)
B-1, 4-glucosidase	BG	Hydrolysis of cellulose	Glucose (sugar)
tobacco acid pyrophosphatase	TAP	Catalyze the hydrolysis of a phosphoric ester bond in a wide spectrum of molecules	Phosphate

All samples were analyzed for total C, total N and ^{15}N at EcoCore Analytical Services Lab, at Colorado State University, Fort Collins, CO, using an Elemental-Analyzer – Isotope Ratio Mass Spectrometry (Costech, Valencia, CA).

The contributions of the N from the labeled inorganic ^{15}N and the organic N acquired from the SOM were calculated by applying the isotopic mixing model (Hauck and Bremner 1976). The fraction of fertilizer-derived N ($f_{fertilizer}$) was calculated using Eq. 1:

$$f_{fertilizer} = \left(^{15}N_{sample} - {^{15}N_{soil}}\right) / \left(^{15}N_{fertilizer} - {^{15}N_{soil}}\right) \tag{1}$$

Where $^{15}N_{sample}$, $^{15}N_{soil}$, and $^{15}N_{fertilizer}$ represent the atom % ^{15}N of the total sample, natural abundance of the soil mixture, and fertilizer (8 atom % ^{15}N) respectively. The value for $^{15}N_{sample}$ was the sample value output from EA-IRMS analysis. The $^{15}N_{Soil}$ was the background atom % ^{15}N of the soil mixture, 0.3681 atom % ^{15}N for low SOM and 0.3699 atom % ^{15}N for High SOM treatments. The contribution of soil derived N was calculated using Eq. 2:

$$f_{SOM} = 1 - f_{fertilizer} \tag{2}$$

Where f_{SOM} is the fraction of plant aboveground N from SOM.

The RE_N was calculated using Eq. 3:

$$RE_N = (Total\ Plant\ Aboveground\ N) / (Applied\ N\ Fertilizer) \tag{3}$$

Root biomass

The root biomass was obtained by washing the growth media away from the bulk and rhizosphere samples of blocks 1, 2 and 3. The washed roots were dried for a minimum of 5 days at 55 °C and weighed for dry biomass. The samples were a mixture of roots, vermiculite, and particulate organic matter. The root samples were homogenized in a ball grinder. Each root sample was analyzed for organic content using the ash correction protocol to obtain an estimated root biomass for each sample (Nelson and Sommers 1996). Briefly, the ground sample was weighed in tin weigh boats and placed into a 105 °C oven for 24 h. The sample was weighed again and then placed into a cold muffle

Table 5 Average aboveground biomass and the percent (%) of aboveground plant N from soil organic matter (SOM) ± standard error by rapeseed variety harvested approximately 6 weeks after planting at the onset of reproduction across all soil treatments (n = 16). ANOVA p-values are presented from mixed model analysis including block as a random factor and fixed factors of Variety, Soil Treatment, and their interaction. Differences between varieties were analyzed using a *post-hoc* Tukey's LSD multiple means comparison test. This multiple means approach did not detect specific varietal differences as indicated by the same letters following each variety

Variety Name	Plant biomass	N from SOM
	(g)	(%)
BN-1	3.14 ± 0.31 (a)	63.0 ± 5.3 (a)
Czyzowska	3.36 ± 0.35 (a)	61.5 ± 5.6 (a)
Dong Hae 3	3.61 ± 0.38 (a)	62.6 ± 5.4 (a)
Ebony	2.95 ± 0.28 (a)	62.9 ± 5.4 (a)
N99–508	3.56 ± 0.39 (a)	61.8 ± 5.3 (a)
Noiza 531	3.37 ± 0.32 (a)	63.8 ± 5.3 (a)
Optima	3.00 ± 0.29 (a)	61.5 ± 5.4 (a)
PI433395	2.95 ± 0.28 (a)	62.8 ± 5.2 (a)
Topas	3.39 ± 0.37 (a)	62.3 ± 5.5 (a)
Tribune	3.16 ± 0.33 (a)	62.9 ± 5.3 (a)
ANOVA test p-values		
Variety	**0.0441**	**0.0022**
Soil Treatment	**<0.0001**	**<0.0001**
Variety* Soil Treatment	0.856	0.337

furnace and heated to 450 °C for 4 h. Once samples were cooled to at least 200 °C, they were weighed again. The difference in the sample weights were used to correct root weights for inorganic compounds and get an ash corrected estimated root biomass for each sample (Harmon et al. 1999). The root-shoot ratio was calculated as the ash corrected root biomass estimate divided by the dry shoot weight.

Data analysis

The data were analyzed in R, using a mixed model approach (R Core Team 2019). Due to a data loss of block 2 aboveground dry plant weights, only four of the five blocks were used in analyses that relied on plant biomass. Due to the loss of root biomass data, only blocks 1 and 3 were included in the root-shoot ratio estimates. Block was included as a random variable, while the fixed predictor variables were

rapeseed variety and treatment. The response variables included the four different enzyme activities, plant N content, SOM and Fertilizer N uptake, percent N from SOM and fertilizer, total soil inorganic N (NH_4^+-N + NO_3^--N), soil NH_4^+, soil NO_3^-, dry root and shoot biomass, and root-shoot ratio. We used the lme() function in the nlme package to allow for unequal variances (Pinheiro et al. 2018) with a few exceptions. The RE_N response variable was analyzed with the lemr() function. For NH_4^+ and NO_3^-, the data were transformed by taking the square root and soil inorganic N was log-transformed and then analyzed using lemr() function of the Lme4 package (Bates et al. 2015). Due to near zero nitrate levels in some samples, some samples had negative values after subtracting sample blanks. Therefore, a constant was added to make all values positive before applying the square root transformation. These values were then analyzed with the lme() function from the nlme package (Pinheiro et al. 2018). A type three analysis of variance Anova() with the Kenward-Roger approximation for degrees of freedom was used from the car package (Fox et al. 2019). The emmeans function, from the emmeans package, was used to make pairwise comparisons of significant predictors (Lenth et al. 2018). P values less than 0.05 were considered significant.

Results

Plant growth and development

The SOM and Fert N treatments resulted in a range of N available for plant uptake and biomass production. There was almost a 3-fold difference in biomass ranging from High SOM/High Fert treatment with an average shoot biomass of 4.46 ± 0.24 g plant^{-1} and plants grown in the Low SOM/Low Fert treatment that had the lowest biomass of 1.62 ± 0.19 g plant^{-1} (Table 4). Plant biomass production was similar for plants in the High SOM/Low Fert and Low SOM/High Fert treatments (Table 4). Variety had a modest effect on plant biomass (p = 0.04), but post-hoc tests found no differences between any two specific varieties and there were no interactions between variety and treatment (Table 5).

Plant development was impacted by soil treatment, block and variety. There were small differences in phenological stage (pre-bud, bud, early flowering) by block and variety, but no soil treatment effects (data not shown). There were, however, differences in leaf number by soil treatment, block, and variety. The Low SOM/Low Fert treatment had lower mean leaf number than all of the other soil treatments at the time of harvest (Supplemental Table 1). Root biomass also differed by soil treatment ($p < 0.0001$, Table 4). There was

Table 4 Least squares means ± standard error for plant and soil variables for each soil organic matter (SOM) and fertilizer (Fert) treatment and associated model p values estimated using mixed model ANOVA with block as a random factor and variety and treatment as fixed fa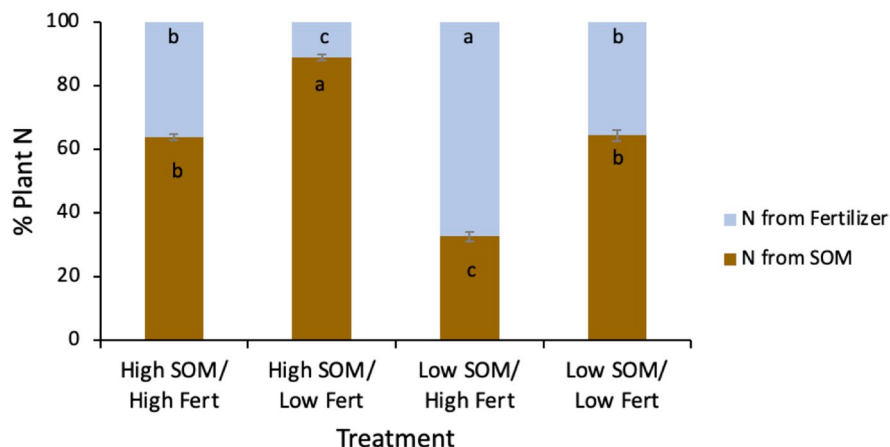ctors. Bolded values indicate that the p value was less than 0.05. Different letters in parentheses denote a significant difference between treatments within the row. There were no significant interactions between treatment and variety

Measurement	units	High SOM/ High Fert	High SOM/ Low Fert	Low SOM/ High Fert	Low SOM/ Low Fert	Variety P value	Treatment P Value
Shoot biomass	g	4.46 ± 0.21 (a)	3.67 ± 0.24 (b)	3.24 ± 0.21 (b)	1.62 ± 0.19 (c)	0.2078	**<0.0001**
Root biomass	g	1.71 ± 0.15 (a)	1.59 ± 0.09 (a)	0.61 ± 0.05 (b)	0.37 ± 0.03 (c)	0.4197	**<0.0001**
Root-shoot ratio	g	0.37 ± 0.04 (a,b)	0.44 ± 0.02 (a)	0.21 ± 0.02 (c)	0.28 ± 0.01 (b)	NA[a]	**0.0009**
Shoot N	mg	194.9 ± 6.03 (a)	127.7 ± 5.39 (b)	110.1 ± 4.21 (c)	38.6 ± 3.73 (d)	0.5678	**<0.0001**
RE_N	%	46.6 ± 1.99 (a,b)	46.6 ± 1.99 (a,b)	49.4 ± 1.99 (a)	43.8 ± 1.99 (b)	0.5476	**0.0120**
Soil inorganic N	mg kg soil^{-1}	4.04 ± 0.55 (a)	2.35 ± 0.35 (b)	2.19 ± 0.32 (b,c)	1.24 ± 0.19 (c)	0.1713	**0.0094**
Soil NO3$^-$	mg kg soil^{-1}	2.96 ± 0.20	1.22 ± 0.09	1.24 ± 0.10	0.04 ± 0.05	0.4651	**0.1582**
Soil NH4$^+$	mg kg soil^{-1}	1.09 ± 0.16	1.23 ± 0.16	0.95 ± 0.16	1.20 ± 0.16	0.9682	**0.5445**

[a]Not available: Sample size not large enough to support analysis

no difference in root biomass between the High SOM/High Fert and the High SOM/Low Fert treatments, but there were differences among all other treatments (Table 4). The root biomass of the High SOM treatments was 2 to 4-fold higher than the Low SOM treatments (Table 4). The root to shoot ratio also differed by treatment (p = 0.0009, Table 4). The highest average root to shoot ratio was the High SOM/Low Fert treatment, followed by the High SOM/High Fert treatment. Variety did not have a significant effect on root biomass (p = 0.42).
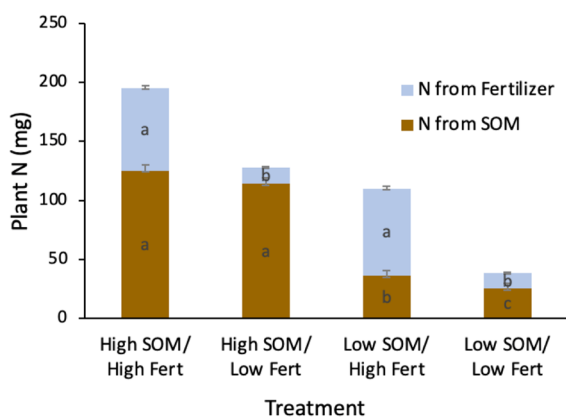
Fig. 2 Least squares means of total plant nitrogen (N) from fertilizer (Fert) and soil organic matter (SOM) across 10 rapeseed varieties by treatment from mixed model ANOVA with block as a random factor and soil treatment as a fixed factor. Error bars represent standard error (n = 10). Letters denote a significant difference between treatments for each N source (SOM or fertilizer) (p < 0.05)

Plant N content and source

Total plant N content differed across all four treatments. Plants in the High SOM/High Fert treatment had the highest total N (195 ± 6 mg N), and the highest amount of N from SOM (125 ± 5 mg N) (Fig. 2). Plants in the High SOM/Low Fert treatment had 35% less total plant N content as plants in the High SOM/High Fert treatment but accessed a similar amount of N from SOM across both High SOM treatments (Fig. 2). Conversely, plants in the Low SOM/High Fert had 30% more N uptake from SOM than plants in the Low SOM/Low Fert treatment (Fig. 2). Variety did not have a significant effect on total plant N uptake, the amount of N taken up from Fert, or N from SOM (p = 0.57, p = 0.65, p = 0.33, respectively).

The proportion of N acquisition from fertilizer and SOM sources differed among all four treatments (Fig. 3). SOM was an important source of N for plants in all four treatments, ranging from 33% to 89% of total plant N. The extreme treatments High SOM/High Fert and Low SOM/Low Fert both obtained 64% of their total N uptake from SOM (Fig. 3), indicating a similar balance in N availability between fertilizer and SOM sources at the high and low end of the N gradient created across the 4 treatments. In contrast, High SOM/Low Fert obtained 89% of the total N from SOM, and only 11% from fertilizer, which was more than double the Low SOM/High Fert treatment that obtained 33% of N from SOM (Fig. 3).

The RE_N was also affected by treatment (p = 0.012, Table 4). However, the RE_N values had a relatively narrow range from 44% to 49% across

Fig. 3 Percentage of plant nitrogen (N) obtained from fertilizer (Fert) and soil organic matter (SOM) across 10 rapeseed varieties from mixed model ANOVA with block as a random factor and soil treatment as a fixed factor. Error bars represent standard error of % N from SOM (n = 10). Letters denote a significant difference between treatments for each N source (p < 0.05)

treatments (Table 4). The Low SOM/High Fert treatment had the highest fertilizer RE_N. Pairwise comparisons between treatments show that only the Low SOM/High Fert and the Low SOM/Low Fert were significantly different from each other. Variety did not have a significant effect on RE_N (Table 4).

Variety had a significant effect on the percentage of total plant N that came from fertilizer and SOM ($p = 0.0022$, Table 5). Although variety was significant in the percentage of total plant N, the interaction between variety and total plant N was not significant ($p = 0.34$). Similar to the plant biomass results and likely due to the high number of varieties evaluated, no single variety was significantly different from another in *post-hoc* analyses that adjusted for the multiple comparisons (Table 5). Variety did not influence any other aspects of N content or source ($p > 0.05$).

Inorganic nitrogen

The SOM and fertilizer treatments influenced extractable soil inorganic N. The highest to lowest average soil inorganic N by treatment was as follows: High SOM/High Fert, High SOM/Low Fert, Low SOM/High Fert, Low SOM/ Low Fert (Table 4). The two extreme treatments differed from one another, but inorganic N availability was similar for the middle treatments (High SOM/Low Fert and Low

SOM/High Fert). While total inorganic N was significantly affected by treatment, there was no treatment effect on the individual amounts of soil extractable NH_4^+ or NO_3^- (Table 4). Total extractable soil inorganic N, NO_3^-, or NH_4^+ did not differ by rapeseed variety (Table 4).

Enzyme activity

Enzyme activity was consistently higher in High SOM soils relative to Low SOM soils (Fig. 4). Variety was not a significant predictor for any of the measured enzymes ($p > 0.05$). Due to the strong effect of SOM level on background soil enzyme activity, we analyzed correlations between soil enzyme activity and plant N uptake from SOM within each SOM level and found weak positive associations between soil enzyme activity and plant N uptake from SOM only within the low SOM treatment ($R^2 = 0.04, 0.08, 0.09, 0.07$; $p = 0.06, 0.01, 0.01, 0.01$ for NAG, LAP, BG, and TAP enzymes respectively). There were no significant correlations between soil enzyme activity and plant N from SOM within the high SOM treatments ($p > 0.05$).

Discussion

Our results highlight the potential importance of organic N sources for rapeseed growth but did not

Fig. 4 Least squares means of soil enzyme activity (nmol per gram of dried soil per hour) across 10 rapeseed varieties from mixed model ANOVA with block as a random factor and soil treatment as a fixed factor. Error bars represent standard errors (n = 10). Enzyme types are represented as Leucine amino peptidase (LAP), B-1, 4-n-acetyl-glycosaminidase (NAG), B-1, 4-glucosidase (BG), and tobacco acid pyrophosphatase (TAP). Letters indicate the significant differences between treatments for each enzyme (*p* < 0.05)
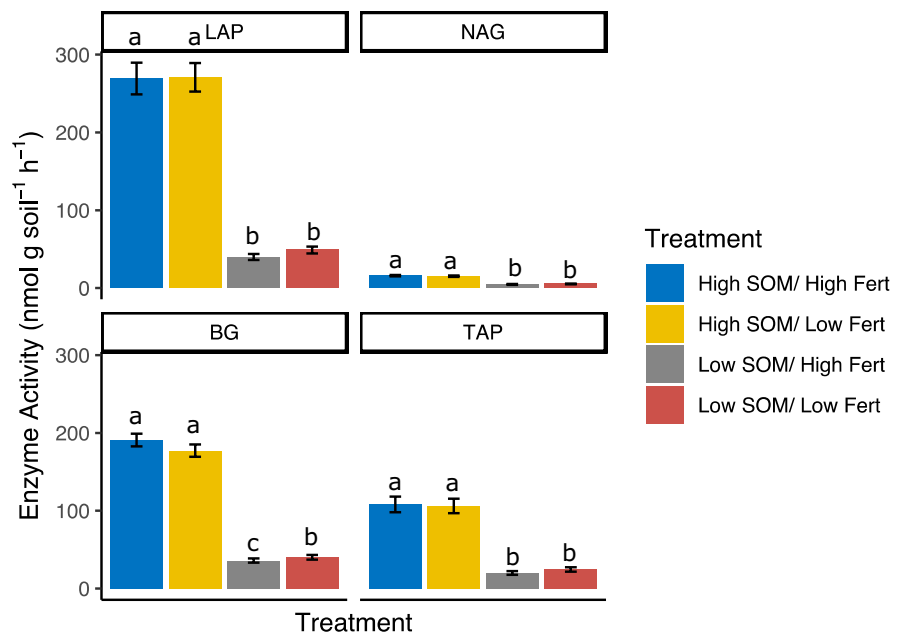
identify specific genotypic traits to enhance N uptake from SOM. Across all treatments, rapeseed plants effectively accessed N from SOM for crop growth and the effect was additive and not a substitute for fertilizer. The enhanced productivity when plants were supplied with both SOM and fertilizer N sources suggests than an integrated nutrient management (INM) system is likely to be an effective approach for improving N uptake and reducing N losses in rapeseed cropping systems. This approach actively manages organic and inorganic N pools and strategically uses all available nutrient sources (Drinkwater and Snapp 2007).

Rapeseed studies have primarily focused on enhancing RE_N and improving inorganic N fertilizer management in conventional management systems (Chamorro et al. 2002; Gan et al. 2008; Ma and Herath 2016; Stahl et al. 2016) and have rarely directly measured N derived from SOM. Regardless of total N available, only 44%–49% of the applied N fertilizer was taken up into plant shoots (Table 4). This low rate of RE_N is within the range of RE_N from field-based studies of other grain crops worldwide (Yan et al. 2019). As N fertilizer losses from agricultural systems continue to have major environmental impacts there has been an increased interest in INM. The goal of INM is to integrate the use of synthetic and biological plant nutrient sources to optimize crop production and resource conservation (Gruhn et al. 2000). Most INM studies have focused on developing countries with degraded soils and on rice, maize and wheat crops (Zhang et al. 2011). In INM systems with higher SOM, crops show improved performance, while reducing the need for inorganic N additions (Zhou et al. 2019). While oilseed rape is a major worldwide crop, fewer INM studies to this point have focused on rapeseed. Studies looking at INM in rapeseed systems have focused on organic fertilizer additions and agronomic practices, and have not focused on the importance of SOM as an N source.

There was an interactive effect between N acquisition from SOM and fertilizer that suggested that any plant-mediated effects on SOM decomposition likely shift as plants become more N limited. The rhizosphere priming effect is dependent on the relative availability of mineral N and labile C (Kuzyakov 2002). Previous studies have found a net positive priming effect with N fertilizer additions, but generally only when there is also an addition of labile C such as from root exudates (Chen et al. 2014. In our study, plants

growing in the Low SOM/Low Fert treatment had significantly less N derived from SOM than in the Low SOM/High Fert treatment (Fig. 2). The higher degree of N limitation in the Low SOM/Low Fert treatment reduced plant development and root growth, likely limiting root foraging and rhizosphere effects on SOM mineralization (Kuzyakov 2002). In contrast, the Low SOM/High Fert treatment had an average plant biomass that was twice that of the Low SOM/Low Fert treatment and a root biomass that was about 70% greater (Table 4). Larger root systems can contribute more labile C, indirectly stimulating N mineralization via microbial processes, or directly enhancing N availability by destabilizing mineral-associated organic compounds (Jilling et al. 2018; Kuzyakov 2002). This larger root system also likely contributed to the modest increase in RE_N from 44% to 49% of plants in the Low SOM/Low Fert and Low SOM/High Fert treatments, respectively (Table 4).

However, plants increased the relative allocation of resources to support belowground biomass under greater N limitation. We found greater root-shoot ratio under the Low SOM/Low Fert relative to the Low SOM/High Fert (Table 4). Within the high SOM treatments, the High SOM/High Fert treatment also obtained more N from SOM than the High SOM/Low Fert treatment and had slightly lower root-shoot ratio, but the differences were not significant (Table 4). This suggests that plants in the High SOM treatments were not as N limited and the added N fertilizer had less of an effect on belowground C dynamics.

We found that High SOM treatments had higher enzyme activity, in all four measured enzymes, than the Low SOM treatments (Fig. 4). Soil organic matter provides both habitat and a food source that can support larger and more active microbial communities. To access C and other nutrients in SOM, soil microorganisms produce exoenzymes to catalyze SOM decomposition and nutrient mineralization. Thus, it is not surprising that enzyme activity was higher in high SOM treatments as the high SOM treatments likely started with higher microbial biomass as well as potentially higher residual exoenzymes in the substrate (Kögel-Knabner 2002). In addition, soil N cycling enzymes (NAG and LAP) did not show stronger relationships with plant N content relative to the C and P cycling enzymes (BG and TAP). Thus, the soil environment had a large impact on soil microbial activity, but we were unable to link plant N acquisition to specific microbial N cycling enzyme activity.

We did not find differences between specific varieties in our study for any response variable. Rather, we found a relatively narrow range in RE_N across all of the varieties included. All varieties in this study were open pollinated varieties chosen based on common spring flowering time and overall genomic diversity. We had limited knowledge of the genetic variation in belowground traits of the varieties included in this study. Some studies suggest that high yielding herbicide tolerant hybrid rapeseed varieties have different N requirements and uptake patterns than open pollinated varieties. Hybrid varieties could have been included in this study to examine differences in uptake patterns (Brandt et al. 2007; Harker et al. 2012; Karamanos et al. 2007; Smith et al. 2010). While we did not find varietal differences in this study, there is evidence of genomic links between belowground traits and N uptake from other studies that suggest it is possible to incorporate NUE of root systems into a breeding program (Coque et al. 2008; Kelly et al. 2022; Mwafulirwa et al. 2021).

Although this study did not find significant effects of variety on N content and source, this study provides a new understanding of rapeseed response to different N environments and the importance of SOM as a N source and could be valuable for future studies focused on belowground trait genetics. In particular, the additive effect of these different N sources suggests that there may be distinct traits that foster uptake of these different N pools. Our study examined N content in the shoots at the plant bolting stage. After this stage, internal N translocation dynamics can influence measurements of NUE at seed maturity (Balint and Rengel 2008). However, N uptake does continue as reproduction and internal translocation processes proceed, and future studies should consider N content at multiple stages and include assessment at grain maturity. While greenhouse pot studies are valuable for more controlled mechanistic studies, further examination of N source uptake in a field setting will be needed to further guide integrated nutrient management recommendations.

Conclusion

Much of sustainable agriculture research has focused on how to decrease the need for surplus N

fertilizer additions by increasing fertilizer use efficiency (RE_N) of crops. While many studies have focused on N fertilizer uptake and use, we focused on the belowground mechanisms in relation to N uptake from SOM. Our results indicate the importance of SOM as an N source to support crop growth, even when an abundance of mineral N is available. Our results also suggest that the interaction between plants and soil environments mediates the dynamic nature of N uptake as demonstrated by reduced fertilizer N uptake efficiency as plants became more N limited. These findings support an integrated nutrient management system that manages both synthetic and organic N sources for effective nutrient management in rapeseed cropping systems.

Availability of data and material Data will be placed in a public database at time of publication.

Code availability Not applicable.

Declarations

Conflict of interest Not applicable.

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