

# Nitrogen Uptake by Rapeseed Varieties From Organic Matter and Inorganic Fertilizer Sources

CANDACE G CARTER (✉ [carter.candace@gmail.com](mailto:carter.candace@gmail.com))

Colorado State University <https://orcid.org/0000-0003-4993-1810>

Meagan E. Schipanski

Colorado State University

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## Research Article

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# Abstract

## Aims

Improving crop utilization of N from soil organic matter (SOM) has received limited attention despite evidence that half of field crop N is often derived from SOM mineralization. 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  $^{15}\text{N}$  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

While we found limited varietal differences in N uptake dynamics, SOM was an important N source across all varieties 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. High N fertilizer additions increased overall N uptake from SOM by 42% relative to low N fertilizer treatments. In contrast, microbial enzyme activity related to nutrient mineralization was suppressed by 16–58% in high N fertilizer relative to low fertilizer treatments.

## Conclusions

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

## 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 applied is taken up by crops 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 mineral N inputs by improving crop nitrogen use efficiency (NUE), typically defined as the total plant biomass or seed yield divided by the N applied, is an essential part of sustainable agriculture. However, NUE is a complex trait that integrates the efficiency of plant N uptake from the soil environment as well as internal N utilization patterns within

the plant (Kant, Bi, and Rothstein 2011; Stahl, Friedt, et al. 2016; Rathke, Behrens, and Diepenbrock 2006; Perchlik and Tegeder 2017).

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 (Yan et al. 2019; Gardner and Drinkwater 2009; Y. Chen et al. 2014). While SOM is recognized as a source of plant available N in soil testing and fertilizer recommendations, there has been limited research focused on utilizing SOM as a source of N through crop management or crop breeding. 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) these belowground traits have mostly been ignored in traditional breeding programs (Stahl, Friedt, et al. 2016; Dawson, Huggins, and Jones 2008; Jilling et al. 2018).

Root biomass and structure affect soil carbon (C) inputs and root exudates, which can have profound influences on the crop's ability obtain nitrogen from diverse sources (Stahl, Friedt, 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, Conn, and Kaiser 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 (Ju et al. 2015; Bingham et al. 2012; Bowles, Raab, and Jackson 2015; Garnett, Conn, and Kaiser 2009). Belowground traits are recognized as important for N uptake and may be particularly important for improving utilization of N from SOM. However, we have a limited understanding of root traits that increase mineralization of N from SOM.

Indirectly, roots can enhance soil N cycling via their effects on soil microbial communities and activity (Jilling et al. 2018). Plants root exudates can enhance microbial mineralization of C, N, and other essential nutrients, including through increasing the availability of mineral-associated N-containing compounds (Fontaine, Mariotti, and Abbadie 2003; Faucon, Houben, and Lambers 2017; Jilling et al. 2018). Plants can also influence the rate of SOM decomposition by stimulating or inhibiting microbial activity resulting in a positive or negative priming effect (Huo, Luo, and Cheng 2017). The mineralization of soil N requires depolymerization of SOM to monomers by microbially-derived extracellular enzymes (Schimel and Bennett 2004).

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, Friedt, et al. 2016). Generally, rapeseed is considered a high N demanding crop with a low NUE compared to other field crops (Kessel, Schierholt, and Becker 2012; Rathke, Behrens, and Diepenbrock 2006). Thus, high N losses from rapeseed systems are not necessarily due to over-fertilization from the farmers, but instead by narrowed acquisition efficiency of the crops, suggesting that the remainder of the N is supplied by SOM pools (Rathke, Behrens, and Diepenbrock

2006; Stahl, Friedt, et al. 2016). The focus on improving N management has led to more interest in breeding rapeseed varieties with increased NUE capacity (Stahl, Friedt, et al. 2016; Kessel, Schierholt, and Becker 2012). These breeding efforts are primarily focused on one component of NUE, N fertilizer uptake efficiency ( $RE_N$ ), or the percentage of N fertilizer recovered in the aboveground plant biomass during the growing season (Cassman, Dobermann, and Walters 2002). Little attention has been paid to the N supplied by SOM mineralization. Understanding the belowground dynamics of N recovery from SOM, rather than applied N fertilizer, could be an important new direction for improving the sustainability of this globally important crop.

The primary focus of rapeseed NUE studies have been on improving synthetic  $RE_N$  and N translocation within the plant, with yield as the primary response indicator (Stahl, Bissuel-Belaygue, et al. 2016; Stahl, Friedt, et al. 2016). In contrast, this study 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*

To investigate N acquisition in rapeseed (*Brassica napus*), 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, Vail, and Robinson 2017 project, that were selected for development of a germplasm resource to dissect complex traits in *Brassica 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, Vail, and Robinson 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 3.8 Liter pots with a water catch tray. A non-soil mixture of 2/8 sand, 3/8 calcined clay, and 3/8 vermiculite by volume was homogenized. A high organic matter field soil was added to the non-soil mixture to create up to a 6-fold difference in soil organic matter levels across the four treatments. The field soil was a fine loamy Aridic Argiustoll with 6.7% organic matter (3.7% C, 0.38% N) collected from a farm near Fort Collins, Colorado, with a history of organic vegetable production. The field topsoil (0-10cm) was collected and sieved to 8-mm. Soil mixtures were homogenized using a clean cement mixer. 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 to obtain the specified total N additions for the high and

low fertilizer rates as outlined in Table 2 (Damon, Osborne, and Rengel 2007; Balint and Rengel 2008). Fertilizer rates of 50 mg N/pot for low N treatments and 150 mg N/pot for high N treatments were chosen to provide sufficient N through vegetative growth based on the Balint and Rengel 2008 study. The SOM levels were selected by assuming less 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 restricted randomization design with 5 blocks of each of the 4 treatments with each of the 10 varieties for a total of 200 pots. Each of the blocks were divided to have 1 plant of each variety and treatment in a randomly assigned block design in the greenhouse. Each block was planted 1 week apart for 5 weeks.

Four seeds were planted into each pot and at one week they were thinned to one plant per pot. Fertilizer treatments were initiated at 2 weeks after planting when the first true leaves were beginning to emerge. Once per week, 50ml 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. Based on the treatment, supplemental <sup>15</sup>N enriched fertilizer was applied weekly to achieve desired N rates. The first week 100 ml of N-fertilizer solution was applied, and 50 ml was applied in all subsequent weeks for a total of 5 fertilizer applications. 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 pots were watered with a drip emitter irrigation system starting at week three. The irrigation system watered for two minutes each day fertilizer treatments were not applied. A moisture probe was used in the pots twice a week to measure pot moisture and the irrigation amounts were adjusted to equalize moisture levels between 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 when about 75% of the plants in a block were at the elongation stage before flowering, around 6 weeks. Each individual pot in the block was photographed and weighed. 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 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 put in cold storage for further processing. A subsample of the bulk soil from each pot was weighed and dried at 105°C 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 assay (Table 3). Rhizosphere soil samples from each pot were analyzed using the microplate enzyme assay using 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 50mM 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 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 ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) in the soil at the time of harvest using the microplate colorimetric method (Sims, Ellsworth, and Mulvaney 1995).

The Vanadium (III) Chloride ( $\text{VCl}_3$ ) protocol was used to determine soil  $\text{NO}_3^-$ , where 30 $\mu\text{L}$  of the KCl extracted sample was pipetted into microplates with  $\text{VCl}_3$  solution (Doane and Horwath 2003). The salicylate-hypochlorite method was used to determine soil  $\text{NH}_4^+$ , where 70 $\mu\text{L}$  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 are used in 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 uptake. Dried plant samples were ground to 2 mm in a Wiley Mill and then roller ground until the sample was homogenized. All samples were analyzed for total C, total N and  $^{15}\text{N}$  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  $^{15}\text{N}$  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 ( $f_{\text{fertilizer}}$ ) was calculated using the equation:

$$f_{\text{fertilizer}} = (\delta^{15}\text{N}_{\text{sample}} - \delta^{15}\text{N}_{\text{soil}}) / (\delta^{15}\text{N}_{\text{fertilizer}} - \delta^{15}\text{N}_{\text{soil}})$$

Where  $\delta^{15}\text{N}_{\text{sample}}$ ,  $\delta^{15}\text{N}_{\text{soil}}$ , and  $\delta^{15}\text{N}_{\text{fertilizer}}$  represent the atom %  $^{15}\text{N}$  of the total sample, natural abundance of the soil mixture, and fertilizer (8 atom%  $^{15}\text{N}$ ) respectively. The value for  $\delta^{15}\text{N}_{\text{sample}}$  was the sample value output from EA-IRMS analysis. The  $\delta^{15}\text{N}_{\text{soil}}$  was the natural abundance of the soil mixture, 0.3681 atom%  $^{15}\text{N}$  for low SOM and 0.3699 atom%  $^{15}\text{N}$  for High SOM treatments. The contribution of soil

derived N was calculated using the equation  $f_{\text{SOM}} = 1 - f_{\text{fertilizer}}$ . The  $\text{RE}_\text{N}$  was calculated using the equation: Total Plant N/Applied N Fertilizer.

### ***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 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. A subsample of each root sample was analyzed for organic content using the ash correction protocol to obtain an estimated root biomass for each sample (Sparks et al. 1996). Briefly, a subsample of the homogenized sample was weighed in tin weigh boats and placed into a 105°C oven for at 24 hours. The sample was weighed again and then placed into a cold muffle furnace and baked at 450°C for 4 hours. 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, Nadelhoffer, and Blair 1999). The root-shoot ratio was calculated as the ash corrected root biomass estimate divided by the dry shoot weight. Only blocks 1 and 3 were included in the root-shoot ratio estimate.

### ***Data Analysis***

The data were analyzed in R, using a mixed model approach (R Core Team 2017). 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. Block was included as a random variable, while the fixed predictor variables were rapeseed variety and treatment. The response variables were the four different enzyme activities, N uptake, SOM and Fertilizer N uptake, percent N from SOM and fertilizer, total soil inorganic N, soil  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , dry root and shoot biomass, and root-shoot ratio. The data were not normally distributed, so the `lme()` function in the `nlme` package to allow for unequal variances (Pinheiro et al. 2018). The exceptions are  $\text{RE}_\text{N}$  was analyzed with the `lmer()` function, and for  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , the data were transformed by taking the square root and then analyzed using `lmer()` 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. In this case, a constant was added to make all values positive and then were square root transformed. These values were then analyzed with the `lme()` function from the `nlme` package (Pinheiro et al. 2018). A type three analysis of variance `Anova()` from the `with` with Kenward-Roger approximation for degrees of freedom was used from the `car` package (Fox et al. 2018). The `emmeans` function, from the `emmeans` package, was used to make pairwise comparisons of significant predictors (Russell Lenth 2019).

## **Results**

### ***Plant Growth***

The SOM and fertilizer 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 Fertilizer treatment with an average shoot biomass of 4.46 g/plant and plants grown in the Low SOM/Low Fertilizer treatment that had the lowest biomass of 1.62 g/plant (Table 4). Plant biomass production was similar for plants in the High SOM/Low Fertilizer and Low SOM/High Fertilizer treatments. Genotype had no effect on plant biomass and there were no interactions between variety and treatment.

### ***Root Biomass***

Root biomass also differed by soil treatment ( $p < 0.0001$ ). There was no difference between root biomass between the High SOM/High Fertilizer and the High SOM/Low Fertilizer treatments, but there were differences between all other treatments (Table 4). The biomass of the High SOM treatments had root biomass that was 2 to 4-fold higher than the root biomass of the Low SOM treatments (Table 4). The root to shoot ratio differed by treatment ( $p = 0.00091$ ). The highest average root to shoot ratio was the High SOM/Low Fertilizer treatment, followed by the High SOM/High Fertilizer treatment. Variety did not have a significant effect on root biomass ( $p = 0.4197$ ).

### ***Plant N Uptake***

Total plant N uptake differed across all four treatments. Plants in the High SOM/High Fertilizer treatment had the highest total N uptake (195 mg N), and the highest amount of N from SOM (125 mg N) (Fig. 3). Plants in the High SOM/Low Fertilizer treatment had 35% less total plant N a similar amount of N derived from SOM than plants in the High SOM/High Fertilizer treatment (Fig. 3). Conversely, plants in the Low SOM/High Fertilizer had 30% less N uptake from SOM than plants in the Low SOM/Low Fertilizer treatment (Table 4).

The proportion of N acquisition from fertilizer and SOM sources differed between all four treatments (Table 4, 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 Fertilizer and Low SOM/Low Fertilizer both obtained 64% of their total N uptake from SOM (Fig. 3), suggesting a similar balance between N sources at high and low treatment levels. In contrast, High SOM/Low Fertilizer obtained 89% of the total N from SOM, and only 11% from fertilizer, which was more than double the Low SOM/High Fertilizer treatment that obtained 33% of N from SOM (Fig. 3).

The  $RE_N$  was also affected by treatment ( $p = 0.012$ ). However, the  $RE_N$  values had a relatively narrow range from 44% to 49% across treatments (Table 4). The Low SOM/High Fertilizer treatment had the highest fertilizer  $RE_N$ . Pairwise comparisons between treatments show that only the Low SOM/High Fertilizer and the Low SOM/Low Fertilizer 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 ( $p = 0.0022$ ). Although variety was significant in the percentage of total plant N, the interaction between

variety and total plant N was not significant ( $p=.34$ ). Due to the high number of varieties evaluated, no single variety was significantly different from another after adjusting for the multiple comparisons (Table 5). Variety did not influence any other aspects of N uptake (Table 4).

### ***Inorganic nitrogen***

The SOM and fertilizer treatments influenced extractable soil inorganic N ( $\text{NO}_3^-$ -N +  $\text{NH}_4^+$ -N mg/kg soil). The highest to lowest average soil inorganic N by treatment was as follows: High SOM/High Fertilizer, High SOM/Low Fertilizer, Low SOM/High Fertilizer, Low SOM/Low Fertilizer (Table 4). The two extreme treatments differed from one another, but inorganic N availability was similar for the middle treatments (High SOM/Low Fertilizer and Low SOM/High Fertilizer). While total inorganic N differed by treatment, there was no treatment effect on the individual amounts of  $\text{NH}_4^+$  or  $\text{NO}_3^-$  in the soil (Table 4). Total extractable soil inorganic N ( $\text{NO}_3^- + \text{NH}_4^+$ ),  $\text{NO}_3^-$ , or soil  $\text{NH}_4^+$  did not differ by rapeseed variety.

### ***Enzyme Activity***

Across all four enzymes measured we found a decrease in enzyme activity in High Fertilizer treatments relative to Low Fertilizer treatments. In the Low SOM treatments, enzyme activity for all four enzymes was 2.2-2.4 times higher in the Low Fertilizer treatments than in the High Fertilizer Treatments (Fig. 4). We found a similar, but non-significant trend in High SOM treatments, where the Low Fertilizer treatments had activity that was 1.2-1.3 times higher than the High Fertilizer treatments for all four enzymes (Table 4, Fig. 4). Variety was not a significant predictor for any of the measured enzymes (Table 4).

## **Discussion**

Our results highlight the potential importance of organic N sources for rapeseed growth. 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 that an ecosystem-based management 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, instead of focusing solely on fertilizer N management (Drinkwater and Snapp 2007).

Rapeseed studies have primarily focused on enhancing  $\text{RE}_N$  and improving inorganic N fertilizer management in conventional managements systems (Stahl, Bissuel-Belaygue, et al. 2016; Gan et al. 2008; Ma and Herath 2016; Chamorro et al. 2002) and have widely ignored the important role of SOM as an N source. Regardless of total N available, only 44%-49% of the applied N fertilizer was taken up into the plant shoot (Table 4). This low rate of  $\text{RE}_N$  is within the range of  $\text{RE}_N$  from field-based studies of other grain crops worldwide, contributing to the growing problem of N pollution. As N fertilizer losses from agricultural systems continue to have major environmental impacts there has been an increased interest in integrated nutrient management (INM). The goal of INM is to integrate the use of synthetic and

biological plant nutrient sources so that crops can be raised in a productive and sustainable way (Gruhn, Goletti, and Yudelman 2000). Most INM studies have focused on developing countries with degraded soils and on rice, maize and wheat crops (Fan, Zhang, and Jiang 2009). In INM systems with higher SOM, crops show improved yield and field 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 indirect evidence of a positive priming effect on SOM decomposition when plants were under moderate N limitation. 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 (R. Chen et al. 2014). In our study, the Low SOM/Low Fertilizer treatment had significantly less N uptake from SOM than the Low SOM/High Fertilizer treatment (Table 4). The higher degree of N limitation in the Low SOM/Low Fertilizer treatment reduced root growth, likely limiting root foraging and rhizosphere effects on SOM mineralization (Kuzyakov 2002). In contrast, the Low SOM/High Fertilizer treatment had an average plant biomass that was twice of the Low SOM/Low Fertilizer 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 (Kuzyakov 2002; Jilling et al. 2018). This larger root system also likely contributed to the increased  $RE_N$  of 49% in the Low SOM/High Fertilizer treatment relative to 44% in the Low SOM/Low Fertilizer treatment (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 Fertilizer relative to the Low SOM/High Fertilizer (Table 4). Within the high SOM treatments, the High SOM/High Fertilizer treatment also obtained more N from SOM than the High SOM/Low Fertilizer 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 has 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). Microbial biomass is an essential component of SOM parent material. SOM 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 then that enzyme activity was higher in high SOM treatments (Kögel-Knabner 2002).

More surprising, however, was the finding that fertilizer additions suppressed soil enzyme activity across all enzymes that we evaluated. Within SOM treatments, Low Fertilizer treatments had higher enzyme activity than High Fertilizer treatments (Fig. 2). Because all enzymes were suppressed, including those involved in C, N, and P cycling, our findings do not appear to be related to relative N availability. We

considered whether the High Fertilizer treatments may have lowered the pH of the soil thus reducing microbial activity (Geisseler and Scow 2014), but we did not find differences in soil pH by treatment. Another possible explanation is the *copiotrophic* hypothesis, that suggests N additions shift the microbial community composition away from taxa that decompose recalcitrant C and towards those that rely upon more labile C pools, which could influence more than just N cycling enzyme activity (Ramirez, Craine, and Fierer 2012; Fierer et al. 2012). This potential mechanism is more likely; however, our study cannot draw any conclusions as we did not examine microbial community composition.

We did not find a significant effect of variety in our study. All varieties in this study were open pollinated varieties that were chosen based on common spring flowering time and overall genomic diversity. 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 (Karamanos, Goh, and Flaten 2005; Harker et al. 2012; Smith et al. 2010; Brandt et al. 2007). An approach more targeted on diversity of belowground traits could show significance differences in varieties. We have a limited understanding of the role of root traits on NUE because of the challenges of studying root function and architecture in a field setting. Studies support genomic links between belowground traits and N uptake, suggesting that it is possible to incorporate NUE root systems into a breeding program (Coque et al. 2008). We suspect that studies without significant results, which may be more common with studies investigating belowground traits, are not always published due to the bias toward reporting significant differences. This makes belowground traits even harder to study and integrate into belowground targeted breeding approaches because there is not enough literature to help guide what has or has not been examined already.

Although this study did not find significant effects of variety on N uptake patterns, this study provides a new understanding of plant 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.

Our study examined N uptake in the shoots at the plant bolting stage. One study examined the consistency of NUE between vegetative and mature stages in rapeseed. They found that NUE of varieties were not consistent across maturity stages and for breeding purposes may require evaluation at plant maturity (Balint and Rengel 2008). This suggests that further studies should measure N uptake at multiple stages and include assessment at grain maturity. As a greenhouse pot study, our study was limited in scope. 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 have 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

soils mediates the dynamic nature of N uptake as demonstrated by the priming effect in N limited environments. The suppression of enzyme activity in high N environments suggests that N fertilizer has a direct effect on soil microbial dynamics, which warrants further investigation. These findings support an ecosystem-based management system that manages both synthetic and organic N sources for effective nutrient management in rapeseed cropping systems.

## Declarations

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### *Conflicts of interest/competing interests*

Not applicable

### *Availability of data and material*

Data will be placed in a public database at time of publication

### *Code availability*

Not applicable

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## Tables

Table 1

Brassica napus varieties selected for this study chosen from the founder lines of NAM project (Parkin, Vail, and Robinson 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

Table 2

The four treatments evaluated in this study included two levels of organic nitrogen (N) from soil organic matter (SOM) from field soil and two levels of mineral nitrogen fertilizer. Total milligrams (mg) of N from fertilizer and grams (g) of N in SOM per pot by treatment. The percentage (%) of SOM and % by volume of field soil and non-soil mix in each pot for each treatment.

Treatments	Fertilizer (mg N/ pot)	SOM (g N/ pot)	SOM level (%)	Field Soil (%)	Non-Soil Mix (%)
High SOM/ High Fertilizer	150	5.36	3	60	40
High SOM/ Low Fertilizer	30	5.36	3	60	40
Low SOM/ High Fertilizer	150	0.89	0.5	10	90
Low SOM/ Low Fertilizer	30	0.89	0.5	10	90

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

Table 4

Mean plant and soil values for each soil organic matter (SOM) and fertilizer treatment and analysis of variance results for variety and treatment factors. Analysis presented as mean  $\pm$  standard error and associated p-values for variety and treatment. Bolded values indicate that the p-value significant and was below  $\alpha = 0.05$ . Letters denote a significant difference between treatments within the row. There were no significant interactions between treatment and variety. 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).

Measurement	units	High SOM/ High fertilizer	High SOM/ Low Fertilizer	Low SOM/ High Fertilizer	Low SOM/ Low Fertilizer	Variety P-value	Treatment P-Value
Dry shoot biomass	g	4.46 $\pm$ .21 (a)	3.67 $\pm$ .24 (b)	3.24 $\pm$ .21 (b)	1.62 $\pm$ .19 (c)	0.2078	< .0001
Dry root biomass estimate	g	1.71 $\pm$ .15 (a)	1.59 $\pm$ .09(a)	0.61 $\pm$ .05 (b)	.37 $\pm$ .03 (c)	0.4197	< .0001
Root-shoot ratio	g	0.37 $\pm$ .04 (a,b)	0.44 $\pm$ .02 (a)	0.21 $\pm$ .02 (c)	0.28 $\pm$ .01 (b)	NA1	0.0009
Plant N	mg	194.9 $\pm$ 6.03 (a)	127.7 $\pm$ 5.39 (b)	110.1 $\pm$ 4.21 (c)	38.6 $\pm$ 3.73 (d)	0.5678	< .0001
Plant N from Fertilizer	mg	69.9 $\pm$ 2.02	14.0 $\pm$ 0.68	74.1 $\pm$ 1.7	13.1 $\pm$ .48	0.6455	< .0001
Plant N from SOM	mg	125.0 $\pm$ 5.27	113.7 $\pm$ 5.55	36.1 $\pm$ 4.45	25.4 $\pm$ 4.4	0.3323	< .0001
RE <sub>N</sub>	%	46.6 $\pm$ 1.99	46.6 $\pm$ 1.99	49.4 $\pm$ 1.99	43.8 $\pm$ 1.99	0.5476	0.0120
% N from Fertilizer	%	36.0 $\pm$ .96	11.0 $\pm$ .9	67.3 $\pm$ 1.4	35.6 $\pm$ 1.62	0.0022	< .0001
% N from SOM	%	64.0 $\pm$ .93	89.0 $\pm$ .9	32.7 $\pm$ 1.4	64.4 $\pm$ 1.62	0.0022	< .0001
LAP activity	nmol/h/g	65.8 $\pm$ 6.93	84.5 $\pm$ 8.78	12.7 $\pm$ 3.47	30.6 $\pm$ 3.97	0.4151	< .0001
NAG activity	nmol/h/g	3.77 $\pm$ .29	4.68 $\pm$ .44	1.47 $\pm$ .23	3.33 $\pm$ .31	0.1094	0.0261
BG activity	nmol/h/g	45.5 $\pm$ 3.20	54.3 $\pm$ 4.61	11.4 $\pm$ 1.98	25.4 $\pm$ 2.43	0.0912	< .0001

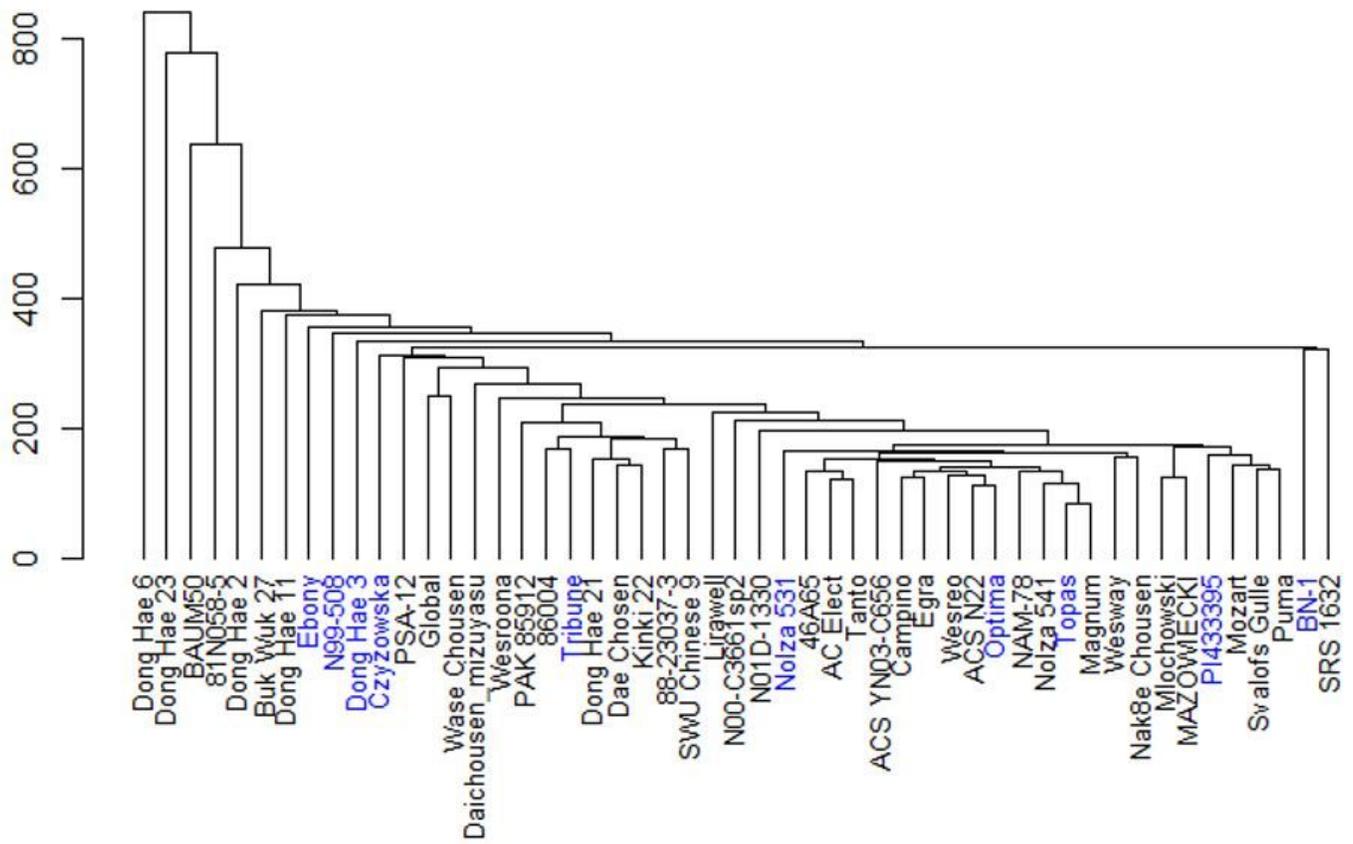
Measurement	units	High SOM/ High fertilizer	High SOM/ Low Fertilizer	Low SOM/ High Fertilizer	Low SOM/ Low Fertilizer	Variety P-value	Treatment P-Value
TAP activity	nmol/h/g	26.45 ± 3.25	33.5 ± 4.08	6.27 ± 1.84	15.15 ± 2.21	0.5084	0.0007
Total Soil inorganic N	mg/kg soil	3.85 (a)	2.34 (b)	1.7 (b,c)	1.13 (c)	0.1713	0.0094
Soil NO <sub>3</sub> <sup>-</sup>	mg/kg soil	3.66 (a)	1.49 (a,b)	1.13 (b)	0.07 (c)	0.1582	0.4651
Soil NH <sub>4</sub> <sup>+</sup>	mg/kg soil	1.02 (a)	1.02 (a)	0.88 (a,c)	1.09 (a,b)	0.9682	0.5445

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

Table 5  
Average Total Plant Nitrogen (N) and Percent (%) of plant N from soil organic matter (SOM) by rapeseed variety.

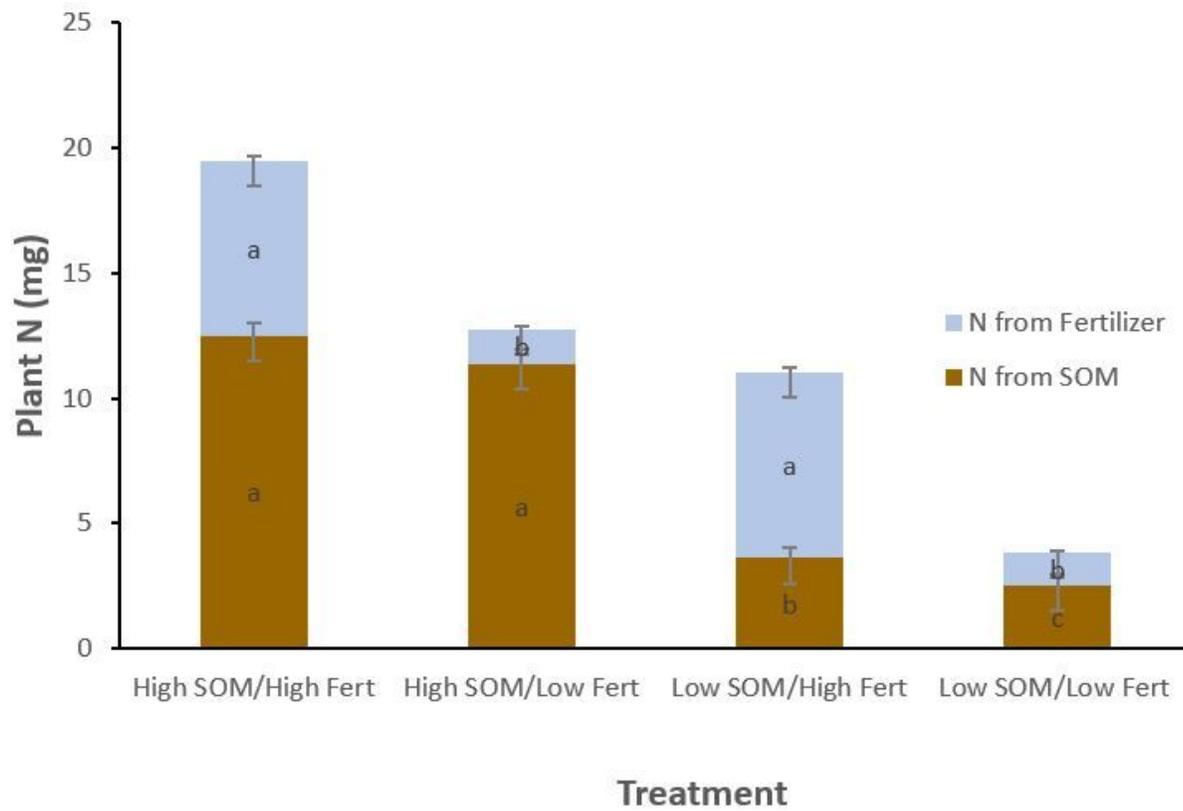
Variety Name	Plant N (mg)	% of N from SOM
BN-1	11.5	63
Czyzowska	11.9	61.5
Dong Hae 3	12.0	62.6
Ebony	11.4	62.9
N99-508	12.4	61.8
Noiza 531	12.2	63.8
Optima	11.4	61.5
US31-2	11.2	62.8
Topas	12.3	62.3
Tribune	11.4	62.9

## Figures



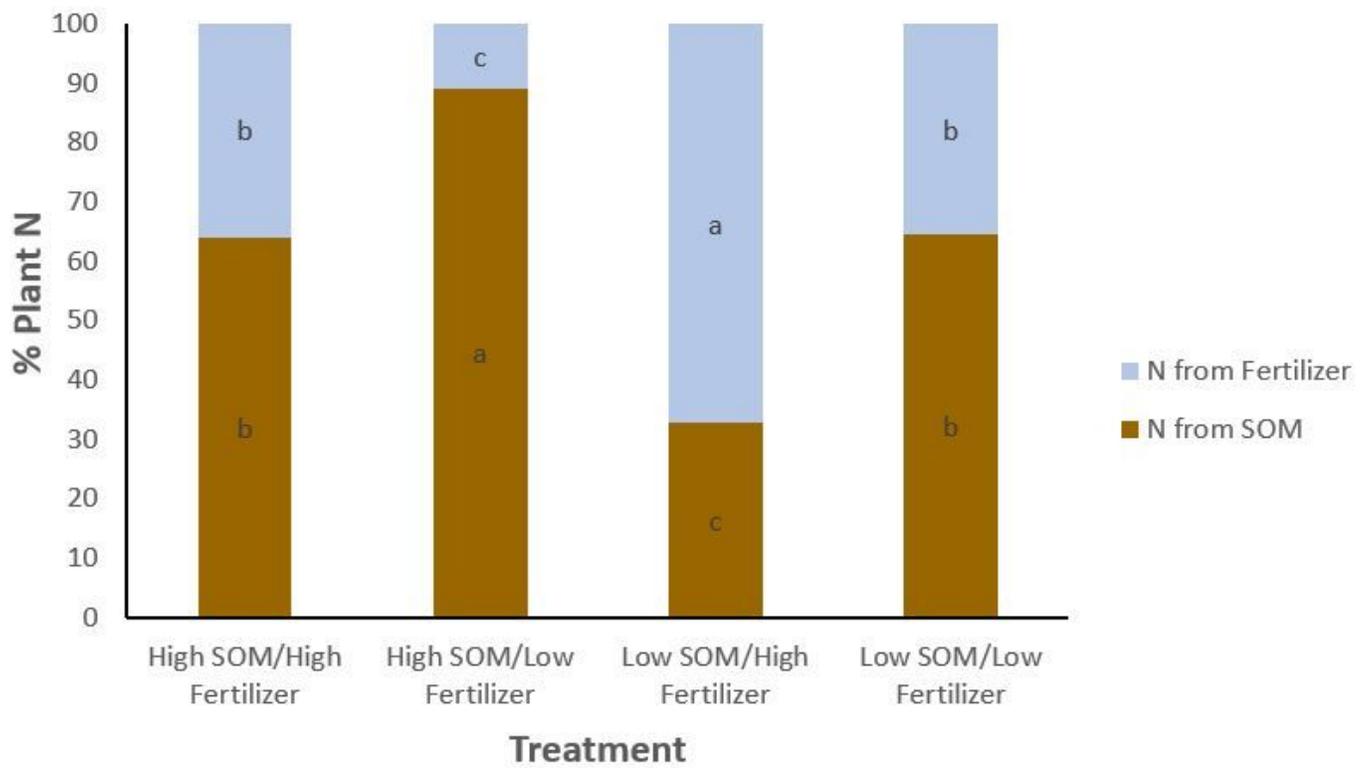
**Figure 1**

Dendrogram of genetic relationships and distances of Brassica napus founder lines of Nested Association Mapping (NAM) project (Parkin, Vail, and Robinson 217). Highlighted lines are varieties selected for this study



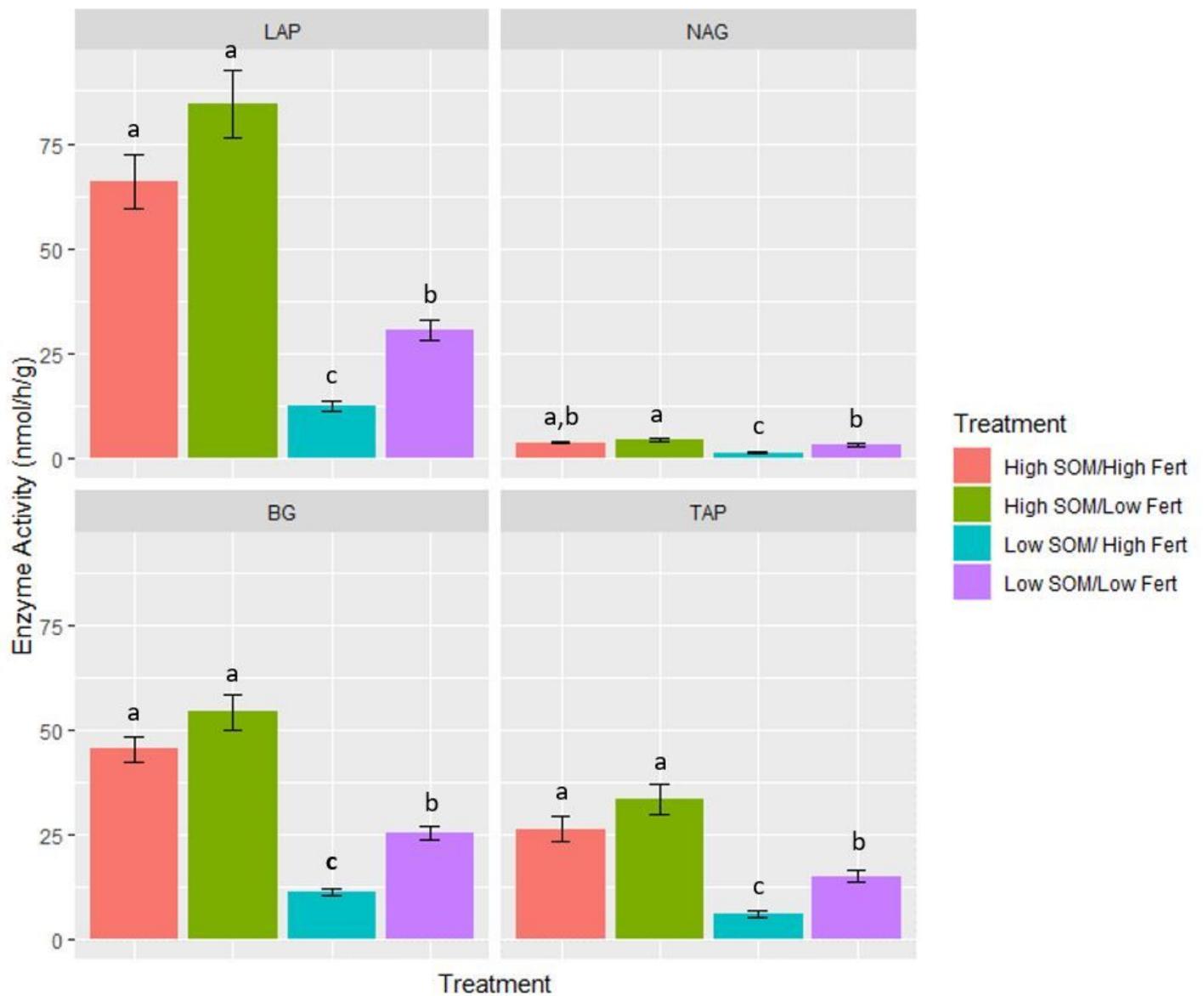
**Figure 2**

Average total plant nitrogen (N) from fertilizer and soil organic matter (SOM) by treatment averaged over variety. Error bars represent standard error (n=4). Letters denote a significant difference between treatments for each N source (SOM or fertilizer) ( $p < 0.05$ ).



**Figure 3**

Percentage of plant nitrogen (N) obtained from fertilizer and soil organic matter (SOM) by treatment. Error bars represent standard error (n=4). Letters denote a significant difference between treatments (p<0.05).



**Figure 4**

Average activity for each soil enzyme measured. Error bars represent standard errors (n=4). 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 ( $p < 0.05$ ).