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Brassica Carinata Growth and Yield Response to Nitrogen and Sulfur Fertilizers and Impacts on Selected Soil Parameters and GHG Fluxes

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BRASSICA CARINATA GROWTH AND YIELD RESPONSE TO NITROGEN AND SULFUR FERTILIZERS AND IMPACTS ON SELECTED SOIL PARAMETERS AND GHG FLUXES

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I would like to dedicate this work to my grandparents, Loknath and Deumaya Bhattarai, parents, Teknath and Lila Devi Bhattarai, lovely wife, Pratiksha KC and sister, Jharana Niroula. This work would have not been possible without your love, care and blessings. I am lucky to have you all in my life.

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BRASSICA CARINATA GROWTH AND YIELD RESPONSE TO NITROGEN AND SULFUR FERTILIZERS AND IMPACTS ON SELECTED SOIL PARAMETERS AND GHG FLUXES

DWARIKA BHATTARAI

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Carinata (*Brassica carinata* A. Braun), a non-food oilseed crop and an alternative bio-jet fuel feedstock, has received attention for its potential as a low-input option for production in the semi-arid regions of the Northern Great Plains of USA. The crop has a lower N fertilizer requirement as compared to the other oilseeds, suggesting less negative impact on soils and GHGs emissions. Carinata is a new crop to South Dakota (SD), thus, the best management practices have yet to be developed. In addition, no sufficient research to address the impact of growing carinata on soils and GHG emissions has been reported. The objectives of the study were to: (i) evaluate the response of seed yield and agronomic traits for carinata to N and S fertilizer rates, and (ii) evaluate the impact of growing carinata with different rates of N and S fertilizers on select soil properties and GHG emissions. Field experiments were conducted in 2017 and 2018 to assess the response of carinata to four N rates (56, 84, 112 and 140 kg N ha^{-1}) and three S rates (0, 22 and 45 kg S ha^{-1}) and) at Brookings, SD under conventional tillage. Increasing N fertilizer rate significantly increased plant height, branching, lodging severity, number of pods plant^{-1} but significantly decreased seed oil concentration. Increasing S fertilizer rate significantly increased plant height, branching, agronomic traits, seed yield, and seed oil concentration. This study showed that the economically optimal N rate was 85 kg N ha^{-1} and the economically optimal S rate was 36 kg S ha^{-1}. Application of N fertilizer had minimal impact on soil parameters; N fertilizer increased soil EC, soil organic carbon (SOC), stable carbon, labile N, soil K, and soil P. Sulfur fertilizer decreased soil EC, SOC, labile N, and soil inorganic N content but increased extractable S content. Results from GHG emissions showed that, in addition to soil temperature and moisture conditions, N fertilizer increased CO_2 and N_2O emissions, whereas, S fertilizer application did not affect emissions. Methane fluxes fluctuated due to the impact of soil temperature and moisture.

Findings from this study suggested that carinata has low nutrient requirements compared to the traditional crops grown in SD, and optimum N and S requirements for this crop were developed. This study also suggested that, in general, carinata has minimal impacts on soils and GHG emissions, however, a long-term monitoring of soils and GHG fluxes under different rotations, soils and environmental conditions can be beneficial in understanding the impacts associated with carinata production.

BRASSICA CARINATA GROWTH AND YIELD RESPONSE TO NITROGEN AND SULFUR FERTILIZERS AND IMPACTS ON SELECTED SOIL PARAMETERS AND GHG FLUXES

INTRODUCTION

Brassica carinata (commonly known as carinata) originated in the Ethiopian Highlands (Warwick, et al., 2009) and is now widely cultivated all over the world (CFIA, 2017). Interest in carinata as a potential crop in the Northern Great Plains (NGP) has increased because of its lower requirements for inputs such as water, and fertilizer compared with other crops. In addition, this crop has high oil content, approximately 45% and high protein content, 43-46%, in the seed depending on the variety and environmental conditions (Agrisoma USA). The oil cannot be used for human consumption due to high erucic acid content (Seepaul et al., 2016); however, oil extract from this crop can be used as biofuel, lubricants and bioplastics (Taylor et al., 2010). The long chain unsaturated fatty acid produced from seed oil of this crop can be used as bio-jet fuel. In addition, the present debate of “food vs fuel” (Ayre, 2007) could be solved by producing biofuel from non-food crops while promoting other oilseed crops such as soybean, sunflower, corn for human consumption. Agrisoma USA reported that roots of carinata penetrate deep into the soil profile improving the soil aggregates as well as helping to improve soil organic matter and carbon sequestering.

Carinata is a new crop to the NGP; hence best management practices have not been developed. Previous research in South Dakota primarily focused on optimum seeding rates and planting dates. While earlier research has also shown optimum N fertilizers rates to be in the range 80-100 kg ha^{-1}, additional research is needed to determine whether N fertilizer interacts with S fertilizer, since carinata has a high S fertilizer requirement.

Soil health, the soil physical, chemical and biological properties, should be least affected due to anthropogenic activities. Application of high rates of fertilizers, and poorly managed tillage operations and cropping systems are responsible for degradation of soil health. Carinata, a low input crop, can help to sustain soil health through low input demand and improvement in soil aggregates and C sequestering. Nitrogen fertilizer can improve soil organic carbon content through increased microbial activity. Application of S fertilizer is crucial for carinata; however, its impact on soil health has not been studied in the NGP.

Greenhouse gas emissions are of global concern. The EPA (2010) showed that the agriculture sector emits 9% of the total GHG emissions in the US. High organic carbon content increases CO_2 and N_2O emissions and soil temperature and moisture suitable for microbial activity increases GHG emission. In the NGP, studies on response of GHG emissions due to long-term application of manure and fertilizers, cropping system, and other management practices have been carried out; however, emissions in response to growing carinata at different N and S fertilizers has not been studied.

Based on the above mentioned research gap and potentiality of carinata to establish in the NGP, the goals of the study were (i) to determine the impact of N and S fertilizers on growth and yield of *B. carinata* (ii) to determine the influence of N and S fertilizers, applied to carinata plots, on selected soil parameters, and (iii) to evaluate the GHG emissions in response to N and S fertilizers applied to carinata plots.

CHAPTER 1: IMPACT OF N AND S FERTILIZERS ON GROWTH AND YIELD OF *BRASSICA CARINATA* IN SOUTH DAKOTA.

LITERATURE REVIEW

Carinata is a new crop in the NGP, thus, there is lack of information about the optimal management practices for the crop. Most crops require N, P and K nutrients for growth and development, while S requirements are higher in *Brassica* species in addition to the major three nutrients (Franzen, 2017). Nitrogen is an important constituent of amino acids, proteins, nucleotides and enzymes (Anjum et al., 2012). It, thus, affects plant height, days to flowering, days to maturity, lodging, pod shatter, number of pods plant^{-1}, number of seeds pod^{-1}, seed weight, seed yield and seed oil concentration. Sulfur plays roles in enzymatic actions, vitamin cofactors, and glutathione formation; it is the major component of amino acids- cysteine and methionine (Ahmad et al., 2005; Hell, 1997) and essential for oil quality and nutritive value (Jamal et al., 2009). Thus, N and S are required for the synthesis of primary and secondary compounds, which play roles in the growth and development of carinata.

Carinata has a determinate growth habit (CFIA, 2017) but has indeterminate flowering (Seepaul et al., 2018) meaning that the plant continue to flower as long as nutrients, light, water and space are available. However, *Brassica* species have higher N nutrient uptake rate from the vegetative stage until flowering stage and then N uptake is reduced after the flowering stage (Wiesler et al., 2001). Seepaul et al. (2016) showed that carinata plant height significantly increased with the increasing level of N fertilizer application. In canola, maximum plant height was obtained at the rate of 150 kg N ha^{-1} (Ma et al., 2015; Öztürk, 2010). However, Seepaul et al. (2016) reported that carinata grows taller (148 cm) than canola (85 cm) at the application rate of 16 mg N L^{-1} Hoagland solution. Although S plays an important role in photosynthesis (Anjum et al., 2012), Seepaul et al. (2017) reported no impact of S fertilizer on carinata height. Sulfur deficiency results in stunted plants (K-State, 2018). There are not many research articles reporting the impact of S fertilizer on carinata plant height.

Addition of N fertilizer significantly increased the yield components of canola (branches $plant^{-1}$, pods $plant^{-1}$ and seeds pod^{-1}) (Ma et al., 2015). There were six more branches $plant^{-1}$ and 150 more pods of rapeseed at the application rate of 180 kg N ha^{-1} compared to 0 kg N ha^{-1} (Khan et al., 2017). Carinata produces a greater number of reproductive branches, nodes and total biomass in response to applied N fertilizer (Seepaul et al., 2016). A review article by Shekhawat et al. (2012) reported an increase in the number of primary branches and number of seeds pod^{-1} of *B. juncea* with increase in N level up to 60 kg N ha^{-1}; however, increasing the N fertilizer rate up to 90 kg N ha^{-1} increased the number of secondary branches and pods $plant^{-1}$. High N fertilizer rates (112-140 kg N ha^{-1}) significantly increase the number of pods $plant^{-1}$ and number of flowering branches (CCC, 2017). Nitrogen deficiency at bolting stage results in less number of branches and poor plant performance (CCC, 2017). Seepaul et al. (2017) reported no significant effect of S fertilizer on the number of branches and pods $plant^{-1}$ in carinata. Similarly, Ma et al. (2015) reported non-significant response of yield components to S fertilizer. Higher rate of S fertilizer application (40 and 80 kg S ha^{-1}) significantly increased the number of pods per plant in rapeseed compared 0 kg S ha^{-1}; however, no impact was seen on the number of branches and seeds per pod (Asare and Scarisbrick, 1995). Significant N and S fertilizers interaction effects on yield components have not been reported under field conditions. Interaction between N and S fertilizers (80, 120 and 160 kg N ha^{-1} and 0, 20, 40 and 60 kg S ha^{-1}) showed significant impact in number of branches plant^{-1} and seeds pod^{-1} in canola; a combination of 160 kg N ha^{-1} and 40 kg S ha^{-1} showed the greatest values compared to other (Ahmad et al., 2011).

Application of N and S fertilizers enhance the overall growth of different crops resulting in early flowering while increasing the flowering period; this effect depends on the crop species (Gan et al., 2007). In the semi-arid regions of the NGP, early flowering for spring carinata can be advantageous to avoid flowering during June-July when high temperatures can result in flower abortion and pod drop. The deficiency of S in *Brassica* species causes delayed and prolonged flowering; reduces life span of petal and subsequently, lessening pollen and attracting fewer pollinators resulting in poor seed filling and low seed yield with poor oil quality (CCC, 2017).

Higher N fertilizer rate results in tall, top-heavy carinata plants that are susceptible to lodging (Pan et al., 2012). When N fertilizer is applied at a rate greater than 100 kg ha^{-1}, lodging may result in yield loss in rapeseed (Wright et al., 1988). Lodging may affect the nutrient relocation into the seed which can cause severe impacts on yield and oil quality (Grant and Bailey, 1993). High rate of N fertilizer promotes increase in plant height thus raising the center of gravity and decreasing lignin and cellulose content resulting in poor stem strength and lodging (Zhang et al., 2014). Increasing N fertilizer rate decreases lodging resistance and N Use Efficiency (NUE) (Khan et al., 2017). Moreover, lodging causes poor light penetration into the canopy providing suitable condition for the development of Sclerotinia stem rot (*Sclerotinia sclerotiorum*) infection (Hanson et al., 2008).

Seed oil concentration has negative correlation with applied N but seed protein content is positively correlated with N fertilizer rate (Johnson et al., 2013; Rana, 2002). Oil concentration increases with applied N fertilizer to a certain point after which it starts decreasing (Brandt et al., 2007; Harker et al., 2012; Malhi et al., 2007; May et al., 2010). Many researchers have provided different reasons for low oil content at high N fertilizer rates; Kutcher et al. (2005) suggested it was the dilution effect of high seed yield with high N fertilizer rates, while Jackson (2000) suggested that N fertilizer prolongs the vegetative growth which results in higher ratio of green seed and poor seed filling. Seed oil content increases with the increased rate of S fertilizer, irrespective of N fertilizer (Mailer, 1989; Malhi and Gill, 2007; Subhani et al., 2003). Sulfur is necessary for the biosynthesis of oil in *Brassica* species; the application of N fertilizer in addition to S fertilizer increased the seed oil content in canola (Jackson, 2000). The amount of applied N and S fertilizers have vital roles on seed oil content of Brassicaceae family. Higher rates of N fertilizer (80 kg N ha^{-1}) resulted in lower oil content (41.6%) compared to 40 kg N ha^{-1} (43.2%) while higher rates of S fertilizer (20 kg S ha^{-1}) led to higher oil content (42.8%) as compared to the plots with no S fertilizer (41.9%) (Ahmad et al., 2007).

Research results on *Brassica* species, including carinata, show that maximum seed yield is obtained at N fertilizer rates of approximately 100 kg N ha^{-1}, with some variations among species (Gan et al., 2007; Johnson et al., 2013; Pan et al., 2012). Khan et al. (2018) reported the greatest rapeseed yield at 270 kg N ha^{-1} compared to 180 and 360 kg N ha^{-1}. Seepaul et al. (2016) reported that the optimum N fertilizer rate of 90 kg N ha^{-1} is required for maximizing carinata yield on a loamy soil. Similar results were shown in a carinata study conducted in semi-arid region of India by Verma et al. (2018). Sulfur fertilizer, in addition to N fertilizer, is important for increasing the yield of oilseed crops. An application rate of 40 kg S ha^{-1} results in the highest yield in canola (Ahmad et al., 2008; Fazili et al., 2010). Similarly, Verma et al. (2018) reported maximum carinata yield (2181 kg ha^{-1})at the application rate of 40 kg S ha^{-1}. There was improvement in seed yield, oil yield, biological yield and harvest index of *B. campestris* with the application of 40 kg S ha^{-1} and 100 kg N ha^{-1} in comparison to 0 kg S ha^{-1} and 100 kg N ha^{-1} (Fazili et al., 2010). Similar interactive effect of N and S was shown in canola; S deficiency at increasing N fertilizer rate resulted in severe yield loss, reduced oil concentration, and low S and N uptake of seed (Malhi and Gill, 2007). Many authors (Ahmad et al., 2008; Fismes et al., 2000) have shown the close link between N and S nutrients in terms of plant uptake; both have synergistic effect at optimum levels and antagonistic effect at higher level of any one (Fismes et al., 2000). Application of 100 kg N ha^{-1} and 40 kg S ha^{-1} resulted in greatest seed yield of rapeseed and mustard (Ahmad et al., 2008), there was no significant difference in the seed yield with the increase in N fertilizer rate beyond 100 kg ha^{-1}. These N and S fertilizers limits were guidelines for setting treatments for the current study.

There is always a limit on the application of nutrients; over-application may lead to luxury consumption. It is important to make wise decisions on the economic fertilizer application rates based on production goals, whether it is maximizing oil content or seed protein. Based on producers’ goal, analysis of economic optimum N fertilizer rate (EONR) and economic optimum S fertilizer rate (EOSR) should be performed. These optimum rates can be used to calculate the economic optimum yield (EOY).

The objectives of this study were to (i) evaluate the response in seed yield, oil yield and other agronomic traits of carinata to four N rates and three S fertilizers rates in SD, ii) determine if N x S interaction occurred, and iii) determine the N and S fertilizers rates for economic optimum yield in SD.

MATERIALS AND METHODS

Site description

The study was conducted at Aurora Agricultural Research Station, Brookings (44º18’35” N 96º40’15.9” W), South Dakota in 2017 and 2018. The study site had slightly acidic medium textured soil. The soil type at the site was Brandt series characterized by fine silty, super active, frigid calcic hapludolls (Malo, 2003; USDA-NRCS, 2017). In both years, the previous crop was winter wheat. Soil properties details for the study site can be found below in Table 1-1.

Treatments and experimental details

The experimental design was a randomized complete block design (RCBD) with 12 treatments replicated four times. Treatments included four different rates of N fertilizer: 56, 84, 112 and 140 kg N ha^{-1} and 3 different rates of S fertilizer: 0, 22 and 45 kg S ha^{-1} arranged in a factorial design. The individual plot size was 1.62 x 9.14 meters (14.86 m^2) with each treatment planted in three adjacent plots. The planting dates in 2017 and 2018 were 24 April and 8 May, respectively. Planting was done using a seven-row Hege 500® (Wintersteiger-Austria). Each plot had seven rows, 22 cm apart with the seeding rate of 11 kg ha^{-1}.

Weeds were managed with pre-emergence application of Prowl H_2O (Pendimethalin, BASF, Research Triangle, NC) herbicide at the rate of 2.8 L ha^{-1} applied 5 cm deep approximately 15 days prior to planting in both years. After crop emergence, Poast (Sethoxydim, BASF, Research Triangle, NC) herbicide was applied at the rate of 2.1 L ha^{-1} 4 weeks after planting to control grassy weeds. Broadleaf weeds were managed by manually removing weeds from within each plot as required.

Nitrogen and S fertilizers were applied in the form of ammonium sulfate (21-0-0-24); additional N fertilizer was applied in the form of urea (46-0-0). Fertilizers were broadcast manually using an automatic hand-held spreader in two equal split rates; first half applied immediately after planting and the next application at bolting stage.

The center plot was harvest for seed yield while the two buffer plots were used for plant samples collection. Plant stands were assessed by counting number of plants in each center plot 4 weeks after planting. The top 3-4 leaves were sampled from 10 random plants within each treatment before bolting stage to determine N and S nutrients content. Days to flowering (50% of flowers open within each plot) and days to maturity (50% of plants with mature pods within each plot) were determined for each plot. At plant maturity, 10 random plants within each treatment were measured from soil line to the top of the plant to determine plant height. Ten random plants were pulled from each plot to determine the number of primary and secondary branches and the number of pods $plant^{-1}$. Out of a 10-plant sample, three plants were randomly selected to measure pod length and to count the number of seeds pod^{-1}; nine pods (three each from top, mid and lower portions of the plant) from each plant were selected. In total, 27 pods $plot^{-1}$ were used to determine average pod length and number of seeds pod^{-1}. Shattering was based on the percent of pods shattered per plot and lodging severity was measured based on a scale of 1 to 9 (where 1 refers to straight standing plants and 9 refers to the plants laid on ground) were recorded before harvest.

After physiological maturity, Roundup® (Glyphosate, Monsanto) was applied at the rate of 2.24 L ha^{-1} to dry down the plants. Center plots were harvested using a Kincaid 8XP® crop research combine (Kincaid Equipment and Manufacturing- Haven, KS) with the assistance of the H2 High Capacity Grain Gage® (Juniper Systems Inc.- Juniper, UT). After harvest, seeds from each plot were cleaned and weighed to determine seed yield. This result was used to calculate total seed yield (kg ha^{-1}). Six samples from the harvested seeds were sent to SGS Mid-West Seed Services, Inc. (Brookings, SD) for oil content analysis using a hexane solvent extraction method. The results of this analysis were used to calibrate the NMR instrument (minispec mq, Bruker-Billerica, MA) for oil content analysis and then the rest of the samples were analyzed using the NMR instrument. Before analyzing the seed for oil content, the seeds were cleaned and then oven dried to a constant weight. At this stage, the seed was considered moisture-free and ready for oil analysis. Oil yield (kg ha^{-1}) was calculated by multiplying the total seed yield by the oil concentration (percent basis).

Statistical analysis and calculation

Data collected from the two years were combined and analyzed together to determine the impact of N and S fertilizers on agronomic traits, seed and oil yield. The statistical model used for analysis was linear mixed for a RCBD in RStudio (Version 1.1.456). The fixed effects in the model were N and S fertilizer rates while year and replication were considered random. Fisher's Least Significant Difference (LSD) was used to compare the differences among treatments at 95% confidence level.

Yield data from both years were used to perform an economic optimum rates (EOR) analysis for N and S fertilizers. EOR is the point at which the last increment of fertilizer results in a yield increase great enough to pay for the additional fertilizer applied (CNRC, 2018). It is calculated using the best-fit polynomial regression equation ($y=a + bx + cx^2$) formed by plotting seed yield (kg ha^{-1}) against N or S fertilizers rate (kg ha^{-1}). The cost of fertilizer N was \$0.84 (using urea- 46-0-0), and cost of fertilizer S was \$1.6 (using ammonium sulfate- 21-0-0-24) (AgFirst Farmers Cooperative). Using these rates and price of carinata, \$ 0.5 kg^{-1} (Elliott et al., 2018) EOR was calculated using the formula shown below. The EOR changes with the change in the price of carinata or the cost of fertilizers.

Equation 1. Economic Optimum Rates (EOR).

$$EOR = \frac{\frac{\$/\,kg}{\$\,/\,kg\ carinata\ seed} - b}{2\ \times c}$$

Where,

\$/kg = Cost of N kg^{-1} or S kg^{-1}

\$/kg carinata seed= Selling price of *B. carinata* seed

b= linear coefficient from the quadratic equation

c= quadratic coefficient from the quadratic equation

The economic optimum yield (EOY) was calculated by substituting the EOR of N and S fertilizers in the respective quadratic equations (St. Luce et al., 2015) and solving the equation.

RESULTS AND DISCUSSION

Weather

The weather data for study location were accessed from the Mesonet at SDSU (Mesonet, 2018).. In addition, 30-year weather data from 1981 to 2010 were used for comparison. The average temperatures in 2017 were similar to the 30-year average. However, the average temperatures in April and July were 1° C and 1.6℃ higher than the 30-year average, respectively. The maximum temperatures during the growing season in 2017 were higher than the 30-year maximum average temperature. In the same year, Brookings received rainfall comparable to the 30-year average in April and May. However, June was significantly drier than the long-term average while late July and August were wetter than long-term average. During the critical crop growth period (June and July), temperature often exceeded 25^{o}C, and one quarter of the total period, exceeded 30^{o}C (Table 1-4). Carinata showed nutrients deficiency symptoms during the early stage of this period (Fig.1-6), with the symptoms disappearing after the rainfall event.

In 2018, the average temperature was extremely low in April when compared to the 30-year average. The freezing temperature and snowfall in April shifted the normal planting time to the 2nd week of May and the harvesting time to September. However, the average daily temperature starting from June was similar to that of 30-year average. The higher average temperature in May, in comparison to long-term average, warmed the soils enough to plant carinata. Unlike 2017, 2018 was a wet year where the total amount of rainfall during the growing period was 43.8 mm more than the long-term average. The critical growing period in 2018 received more than twice the amount of total rainfall received in 2017 during the same period (Table 1-4).

Leaf tissue analysis

Plant available N and S forms are mobile compounds. During nutrient deficit conditions, plants show deficiency symptoms in older leaves because of nutrient translocation to younger leaves (IPNI, 2011). Leaf-tissue nutrient analysis, before bolting and topdressing, showed that application of different N fertilizer rates did not significantly change the leaf N or S concentration but S fertilizer application had significant impact on leaf S concentration (Table 1-5). The rosette stage of a typical canola plant consists of around 5-6 % N (CCC, 2017); these levels are similar to levels observed in the present study. In 2017, carinata leaves from S control plots (0 kg S ha^{-1}) had significantly lower leaf S concentration (0.2 % S) compared to plots with 22 kg S ha^{-1} (0.5% S) and 45 kg S ha^{-1} (0.9% S). Similarly, leaves from S fertilizer control plots in 2018 had significantly low S concentration compared to leaves from leaves from treatment plots (Table 1-5). However, S concentration in 2018 control plots was comparatively higher than in 2017 control plots. Likely, the higher rainfall in 2018 increased the solubility of S and thus more S was available for plant uptake.

Plant stand and plant height

Plant stand was not impacted by N or S fertilizer application (Table 1-6). The mean plant stand four weeks after planting was 76 percent. Depending upon variety, environmental condition and farming practices, canola stand establishment ranges from

40 to 60 % on average (CCC, 2017). The average plant stand from the present study was higher than that for canola. The impact of N fertilizer on plant density was non-significant under rainfed condition in Saskatchewan (Johnson et al., 2013). Hanson et al. (2008) reported variable seedling percent emergence across the NGP depending on precipitation.

Both N (*P*=0.005) and S (*P* <0.001) fertilizers had significant impact on the plant height; however, the interaction was non-significant. Plants were 85 cm tall in the plots with 56 kg N ha^{-1} with this height similar to plants in the 84 kg N ha^{-1} treatment (86 cm) but significantly shorter than plants in the top two N rates (90 cm for 112 and 140 kg N ha^{-1}). Similarly, plants in the S fertilizer control plots were significantly shorter compared to plants in the S fertilizer-applied plots (Table 1-6). The maximum plant height (91 cm) was obtained with the application of 22 kg S ha^{-1}; however, it was statistically similar with plant height at 45 kg S ha^{-1} (89 cm) rate. Similar to our results, Verma et al. (2018) reported significant increase in plant height of carinata with the application of N and S fertilizers in the semi-arid regions of India. This indicates the crucial role of N and S fertilizers on the growth and development of carinata plants.

Branching

Table (1-6) shows that N (*P*<0.001) and S (*P*<0.001) fertilizers significantly influenced the number of primary branches plant^{-1}. Except for the lowest applied N fertilizer rate (5 branches), all other N fertilizer rates produced significantly higher but similar number of primary branches (6 branches). Similarly, the S fertilizer control plants had five primary branches and these were significantly lower that the number of branches for plants in S fertilizer-applied plots (6 branches). A similar impact was observed on the number of secondary branches with lowest N fertilizer rate and S fertilizer control plots having significantly lower number (11-13 branches) of secondary branches compared to higher fertilizer treatment plots (15 branches). Number of secondary branches were greatest (15 branches) at the application rate of 112 and 140 kg N ha^{-1}, and 22 and 45 kg S ha^{-1}. Similar results were reported in many studies in canola. Pan et al. (2011) reported the greatest number of branches (10) in carinata plants receiving 150 kg N ha^{-1} compared to that at the rates of 0, 50, and 100 kg N ha^{-1}; 5, 6 and 7 branches respectively. Similarly, Khan et al. (2002) reported that plots receiving greatest level of N fertilizer (120 kg N ha^{-1}) had maximum number of branches (14), seeds pods^{-1} (28) and greatest yield in canola (2653 kg ha^{-1}). Application of 40 kg S ha^{-1} produced the greatest number of branches (7.8) and remained constant at the application rate of 60 kg S ha^{-1} compared to 0 and 20 kg S ha^{-1} (Ahmad et al., 2011). The same authors also reported that number of branches plant^{-1} increased significantly with the application of 160 kg N ha^{-1} and 40-60 kg S ha^{-1} in canola compared to any other combined application of 80, 120 kg N ha^{-1}, and 0, 20 kg S ha^{-1}.

Lodging

Nitrogen and S fertilizers application significantly affected lodging (Table 1-6). The N x S interaction effects for lodging was also significant (P = .005). Plants lodged more when the N fertilizer rate was equal to or higher than 112 kg N ha^{-1}. Plants in the S fertilizer control treatment lodged significantly less (3.0 score) compared to plants in S fertilizer - applied plots (5.1 score). Table (A1-1) shows that the lodging was greater in 2018 compared to 2017, which might be due to greater amount of rainfall in 2018. Lodging is caused by complex interaction between plants, soil, rain and wind (Yang et al., 2017). Lodging severity was significantly correlated with plant height ($r^2 = 0.97$) meaning taller plants were prone to high lodging severity. In addition, higher fertilizers rates increase stem length, which can induce lodging by increasing twisting force (Wu and Ma, 2016). Seepaul et al. (2016) reported that stem elongation is positively affected by increasing N levels, which might be true in the present study. Integrated agronomic practices including, climate, soil, moisture, fertilization and plant densities influence the phenology and the critical stage when the crop is sensitive to lodging (Gan et al., 2007).

Agronomic traits

Days to flowering for all treatments was between 55 - 58 (±0.85) days after planting and remained unchanged with fertilizer treatments (data not shown). The observed number of days to flowering match with the days reported by Getinet et al. (1996) using a different carinata variety in Canada. Despite the narrow range of days to flowering, both N ($P<0.001$) and S ($P<0.001$) fertilizer rates significantly influenced number of pods plant^{-1} and interaction between the two fertilizers was significant ($P=0.011$) (Table 1-7). Plots receiving 112 kg N ha^{-1} produced the greatest number of pods plant^{-1} (86 pods plant^{-1}), which were similar with number of pods at140 kg N ha^{-1} (80 pods plant^{-1}). Plants receiving 56 and 84 kg N ha^{-1} produced significantly lower number of pods. The three different rates of S fertilizers influenced the number of pods plant^{-1} differently (Table 1-7). Plants in the S fertilizer control plots produced lower number of pods (63 pods plant^{-1}) while the application of 22 and 45 kg S ha^{-1} produced 88 and 72 pods plant^{-1}, respectively, with these values significantly different from each other.

Pod length and number of seeds pod^{-1} were significantly influenced by S fertilizer application (Table 1-7). Although increase in N fertilizer rates showed a trend toward increase in the number of seeds pod^{-1}, the effect was not significant (P=0.670) (Table 1-7). Pods averaged 5 cm-long and contained 12 seeds pod^{-1} in the S fertilizer control plots whereas S fertilizer applied plots produced 6 cm-long pods with an average of 14 seeds in each pod.

Asare and Scarisbrick (1995) reported that application of 240 kg N ha^{-1} increased the number of pods in oilseed rape, compared to 120 kg N ha^{-1}, depending on the location and other environmental factors. A study in canola by Herath et al. (2017) showed that the number of branches were greatest (4) in the plots applied with 150 kg N ha^{-1} compared to 0 kg N ha^{-1}. High variation was seen on the number of pods plant^{-1} (16-39% CV) and seeds plant^{-1} (5-13% CV); however, the numbers were higher in 150 kg N ha^{-1} compared to 0 kg N ha^{-1} in the same study. Another study by Verma et al. (2018) in India reported significant increase in the number of seeds pod^{-1} of carinata from 15 (30 kg N ha^{-1}) to 17 (90 kg N ha^{-1}). Similarly, authors reported significant increase in seeds pod^{-1} with the application of S fertilizer (17 on average) compared to S control plots (16 on average). Moreover, Hossain et al. (2018) indicated that more than average rainfall enhanced plant growth, promoted branching and increased the number of pods plant^{-1}.

Seed yield, oil yield and seed oil concentration

Seed yield was significantly influenced by S fertilizer application (P<0.001); however, N fertilizer effects were not significantly different (P=0.312), neither were their interactions (P=0.702) (Table 1-7). Seed yield increased with increase in S fertilizer rate, the greatest seed yield (1391 kg ha^{-1}) occurred at the 45 kg S ha^{-1} rate; however, this value was similar to the yield obtained at the 22 kg S ha^{-1} rate. The lowest yield (682 kg ha^{-1}) was obtained from the S fertilizer control plots. Sulfur fertilizer application increased yield through increase in number of primary branches, number of pods plant^{-1}, and seeds pod^{-1}. Similar results were reported in other studies in *Brassica* species. Malhi et al. (2007) reported that the seed yield of *Brassica* species is maximized with the application of 30 kg S ha^{-1}, which is similar to our results. Carinata yield and yield components including, primary branches, silique plant^{-1}, seed and dry biomass yield increased with increasing S fertilizer rate up to 40 kg S ha^{-1} in semi-arid region of India (Verma et al., 2018). Sulfur application might have improved the nutritional environment required for carinata by enhancing the enzymatic activities, metabolism and photosynthetic activities, which resulted in optimum growth of carinata and finally, better seed yield.

Table (1-7) shows that the N fertilizer application did not significantly increase seed yield, although, the trend was for increased seed yield from 56 to 112 kg N ha^{-1} with any further increase in N fertilizer rate reducing yield. Scott et al. (1973) suggested that high N fertilizer application extends pod development period and therefore delays maturity resulting in presence of chlorophyll in seeds (green seeds) and hence poor yield and low oil quality. In the present study, plants were taller, thus, lodged more at high N application rate lowering yield. Pan et al. (2012) reported that delayed flowering and lodging because of high N levels might cause yield reduction. Similar results on N fertilizers effects were reported in many other studies (Cheema et al., 2001; Hossain et al., 2018; Öztürk, 2010; Pan et al., 2012). However, the maximum N rate of 200 kg ha^{-1} did not maximize the carinata seed yield in a study conducted by Johnson et al. (2013) in

Canada. Gan et al. (2007) found that different *Brassica* species were responsive to N rates from 0 kg ha^{-1} to 100 kg ha^{-1} in the NGP, but the yield declined at higher N rates. Rapeseed seed yield remained unaffected with the application of S fertilizer (Asare and Scarisbrick, 1995); however, application of 40 kg S ha^{-1} increased carinata seed yield (2130 kg ha^{-1}) compared to 0 kg S ha^{-1} (1592 kg ha^{-1}) in semi-arid region of India (Verma et al., 2018).

We further analyzed the impact of S fertilizer at each N fertilizer rate separately and the results are shown on Figure (1-5). Noticeably, even at higher N fertilizer rates the lack of S significantly reduced seed yield. The lower yield at high N fertilizer rate but lower S fertilizer rate can be due to toxic levels of non-protein N (Anderson and Spencer, 1950). The authors reported that S is required for N nutrient metabolism promoting protein formation within plants. Results from the present study are comparable with Malhi and Gill (2007); they reported seed yield increased in all N fertilizer applied sites only with S fertilizer application. This indicated that S fertilizer was a more limiting nutrient than N fertilizer where no N or S fertilizers were applied. Figure (1-6) shows the S deficiency symptoms in S fertilizer control plots with 112 kg N ha^{-1}. Franzen (2017) reported that 22-32 kg S ha^{-1} can be recommended in canola, regardless of soil test and environmental condition in North Dakota and Agrisoma USA recommended the use of appropriate N: S ratio (approximately 4:1) to maximize carinata yield. Malhi and Gill (2007) suggested applying S fertilizer with N fertilizer in S deficient soils to improve canola seed yield response to N fertilization. Results from the present study agree with these suggestions.

Overall, the seed yield of carinata from the present study was lower compared to other studies (Hossain et al., 2018; Pan et al., 2012; Seepaul et al., 2018; Verma et al., 2018). Cumulative effects of environment, genotype and management might have affected the yield in our study site. In the NGP, heat and drought stress during flowering cause flower abortion in canola (Gan et al., 2016). This might be one of reasons for poor pod setting in the present study. Although this crop can tolerate prolonged heat stress (Taylor et al., 2010), a combination of heat stress and drought can cause severe yield loss because of poor pollination and empty pods (Gan et al., 2004). The average temperature throughout the critical growing period (specifically in June and July) in the present study was very high (Table 1-2) and this could have influenced yields (Table 1-2).

Both N and S fertilizers application (Table 1-7) significantly influenced seed oil concentration. Seed oil concentration at the highest N fertilizer rate of 140 kg N ha^{-1} was significantly lower than in the three other N fertilizer rates. On the other hand, oil concentration in the S fertilizer control plots was significantly lower than in plots with no S fertilizer application. For S fertilizer treatment, the maximum oil concentration (361 g kg^{-1}) was obtained at 22 kg S ha^{-1} while for the N fertilizer treatment the maximum oil concentration (364 g kg^{-1}) was obtained at 84 kg N ha^{-1}. Similarly, N and S fertilizers application significant influenced oil yield (Table 1-7). The lowest (56 kg N ha^{-1}) and the highest (140 kg N ha^{-1}) N fertilizer rates had significantly lower oil yield (378 and 337 kg ha^{-1}, respectively) compared to other two rates and the S fertilizer control plots had significantly lower oil yield (233 kg ha^{-1}) compared to higher S fertilizer rates.

Nitrogen fertilizer application had a negative relationship with seed oil concentration. These results are similar to results from many other studies (Hossain et al., 2018; Pan et al., 2012). The negative relationship of oil content and N fertilizer rate is due to the competition of oil and protein concentration for carbon skeletons during energy metabolism (Rathke et al., 2005); in addition, low carbohydrate availability at high N fertilizer application might be the other reason for decrease in oil content of seeds. In contrast, S fertilizer application has a positive effect on seed oil concentration. Many other studies (Malhi et al., 2007; Malhi and Gill, 2007; Verma et al., 2018) showed positive effect of S fertilizer on oil concentration, supporting the present study. However, oil yield increased with increase in both N and S fertilizer rates, as it is the product of seed yield and oil concentration.

In comparison with other studies (Malhi et al., 2007; Malhi and Gill, 2007; Seepaul et al., 2018; Verma et al., 2018), oil concentration and oil yield, in the present study, were low. Malhi and Gill (2007) reported canola seed-oil concentration ranging from 330 g kg^{-1} to 470 g kg^{-1} depending on different locations. Seepaul et al. (2018) reported carinata oil concentration ranging from 354-370 g kg^{-1} and oil yield ranging from 650-1085 L ha^{-1}. As mentioned earlier, higher temperature (approximately >25^{o}C) during pod filling can result in reduced oil concentration (Deng and Scarth, 1998; Hocking and Stapper, 2001; Öztürk, 2010). Deng and Scarth (1998) reported that plants subjected to more than 30/25^{o}C (day/night) temperature for 40 days produced low amount of polyunsaturated fatty acid. The maximum temperatures during flowering and seed filling (June-July) in the present study were high (32-34^{o}C, Table 1-2), with 48 and 16 days having maximum temperatures higher than 25 and 30^{o}C in 2017 and 39 and 13 days in 2018, respectively (Table 1-2). Seed oil content shows positive response to cooler temperatures ranging from 18-20°C during the reproductive (flowering-maturation) (Roche et al., 2006).

Moreover, Hossain et al. (2018) reported that controlling weeds could improve the oil concentration. This might be true for the 2018 results where weed infestation was severe later in the season. Lodging in 2018 hindered the sunlight from penetrating the canopy resulting in damp seeds during harvest. Lodging reduced oil content and fatty acid composition of winter rapeseed (Khan et al., 2018). Lodged plants lower seed and oil quality by increasing disease attack and making harvest difficult (Baylis and Wright, 1990), which was true in the present study.

Regression analysis results of seed and oil yield on N and S fertilizers are shown in Figures (1-1) to (1-6). The binomial curves for both N and S fertilizer treatments show that seed yield and oil yield increases in curvilinear manner. For N fertilizer, peaks of both curves are in between 80 and 100 kg N ha^{-1} (Fig 1-1 and 1-3). Gan et al. (2007) reported decline in canola yield with the application of N fertilizer greater than 100 kg N ha^{-1} and attributed the response to the decrease in N use efficiency with increasing N fertilizer rate. For S fertilizer, peaks for seed yield and oil yield are in between 30 and 40 kg S ha^{-1} (Fig 1-2 and 1-4). Other studies (Malhi et al., 2007; Malhi and Gill, 2007) in canola and *Brassica* oilseed crops have shown the maximum seed yield at 30 kg S ha^{-1} and reduction in oil yield on further application; however, no regression analysis has been conducted for the oil yield.

The economically optimum N rate (EONR) was 85 kg ha^{-1}, where the cost of N fertilizer was \$0.84 kg^{-1} (AgFirst Farmers Cooperative) and the maximum price of carinata seed was \$0.5 kg^{-1} (the average price was \$0.35 kg^{-1}) (Elliott et al., 2018). This optimum fertilizer rate is lower compared to other crops such as corn, mustard, canola, flax (Gerwing and Gelderman, 2005). At the same carinata price and S fertilizer cost of \$1.6 kg^{-1} (based on purchased rate from local cooperative), the economically optimum S rate (EOSR) was 36 kg ha^{-1}. This S fertilizer rate is similar to that of canola as reported by Malhi et al. (2007). The economic optimum yields of carinata based on the variable costs of N fertilizer in different years and variable price of carinata are presented in Tables 1-8. The price of S fertilizer remained similar (\$1.6 kg^{-1}) from 2017 to 2019 (AgFirst Farmers Cooperative); this resulted in same EOY of 1403 kg ha^{-1} (\$0.5 kg^{-1} carinata seed) and 1397 kg ha^{-1} (\$0.35 kg^{-1} carinata seed) throughout three years.

The individual year regression analysis on seed yield are shown on Appendix Fig. A1-1 and A1-2. Overall, seed yield was greater in 2017 compared to 2018 and the response to N fertilizers was greater in 2017 than in 2018. The heavy rainfall in 2018 might have leached the plant available NO_3^- to deeper levels in the soil profile resulting in low uptake of N (Table 1-5) and thus, lower yield. The 2017 seed yield curve for S fertilizer intersects with the yield curve for 2018 at 10 kg S ha^{-1} (Fig. A1-2). Due to drought in the early growing stages in 2017, S uptake by crop in S control plots was poor (Table 1-5).

CONCLUSIONS

The study demonstrated the impact of N and S fertilizers on growth, yield components and yield of *B. carinata.* Sulfur fertilizer significantly increased most growth traits, agronomic traits, seed yield and seed oil concentration. Similarly, N fertilizer increased plant height, branching, lodging and number of pods plant^{-1} but decreased seed oil concentration and oil yield. However, S fertilizer was the most limiting fertilizer for better seed and oil yield of carinata as indicated by lack of response to N fertilizer in the S control treatment. Based on 2-year data the EONR was 85 kg N ha^{-1} while the EOSR was 36 kg S ha^{-1}. These results show that carinata has low requirement of N fertilizer compared to most crops grown in South Dakota while S fertilizer requirement is similar to other oilseed crops. It must be noted that the impact of N and S fertilizers may vary depending upon soil and climatic conditions. Thus, it benefits growers if they consider all these factors including appropriate variety and agronomic practices.

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Table 1-1. Soil physical and chemical characteristics before planting for both years at Brookings.

Year	Previous Crop	Depth (cm)	Texture Class	pH	Organic Matter (g kg^{-1})	NO_3-N (kg ha^{-1})	Olsen-P (mg kg^{-1})	K (mg kg^{-1})	S (kg ha^{-1})
2017	WW	0-15	Medium	5.6	47	3.4	10.0	141.0	9.0
2018	WW	0-15	Medium	5.7	53	26.5	21.0	220.0	29.0

WW = Winter Wheat

Table 1-2. Monthly temperature data collected throughout the growing period (GP) for 2017 and 2018 at Brookings, South Dakota.

Months	**April**		**May**		**June**		**July**		**August**	
Temp. (°C)	**Max**	**Avg.**	**Max**	**Avg.**	**Max**	**Avg.**	**Max**	**Avg.**	**Max**	**Avg.**
Brookings 2017	25.6	7.7	27.8	13.3	32.2	20.1	34.4	22.7	28.9	18.5
Brookings 2018	27.8	1.7	36.1	17.6	33.3	21.2	32.8	21.7	31.7	20.6
30-year average	12.8	6.7	19.4	13.3	25	18.9	27.8	21.1	26.7	20

30-year average is the mean of the maximum and the average temperatures from 1981-2010.

Table 1-3. Monthly rainfall data collected throughout the growing period (GP) for 2017 and 2018 at Brookings, South Dakota.

Months	**April**	**May**	**June**	**July**	**August**	**Total GP Rainfall**
Rainfall (mm)						
Brookings 2017	45.0	96.8	31.5	118.9	112.0	377.2
Brookings 2018	19.3*	18.8	102.1	216.0	86.9	442.9
30-year average	54.1	74.9	109.0	83.1	78.0	399.1

30-year average is the average rainfall from 1981-2010.
* - Snowfall

Table 1-4. Total days with temperatures above 25 and 30 °C and precipitation during critical growth stages of *B. carinata*, conducted at Brookings, SD in 2017 and 2018.

	Month	**Days**	**Days > 25 ºC**	**Days > 30 ºC**	**Precipitation (mm)**
2017	June	1-10	10	5	0.0
	June	11-20	6	1	30.0
	June	21-30	3	0	1.5
	July	1-10	10	4	0.0
	July	11-20	9	4	48.5
	July	21-31	10	2	70.4
				Total	**150.4**
2018	June	1-10	6	3	13.7
	June	11-20	4	2	47.0
	June	21-30	6	1	41.4
	July	1-10	9	5	32.5
	July	11-20	8	2	182.6
	July	21-31	6	0	0.5
				Total	**317.7**

Table 1-5. Leaf N and leaf S content (in percentages) of carinata at different N and S fertilizers rates, before topdressing, at Brookings in 2017 and 2018.

	2017		**2018**	
	Leaf N	**Leaf S**	**Leaf N**	**Leaf S**
	%	%	%	%
N rates (kg ha^{-1})				
56	5.4	0.6	5.2	0.6
84	5.5	0.5	5.3	0.6
112	5.7	0.6	5.4	0.6
140	5.8	0.6	5.5	0.6
S rates (kg ha^{-1})				
0	5.5	0.2^{b†}	5.3	0.5^{b}
22	5.6	0.5^{a}	5.5	0.7^{a}
45	5.7	0.9^{a}	5.2	0.7^{a}
	Analysis of variance (P>F)			
N rate	0.186	0.667	0.571	0.845
S rate	0.329	<0.001	0.106	<0.001
N x S	0.307	0.413	0.935	0.554

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.

Table 1-6. Plant stand (percentage), plant height (cm), number of primary and secondary branches and lodging (1-9) of carinata at different N and S fertilizers rates at Brookings. Means are average between 2017 and 2018.

	Plant Stand	**Plant Height**	**Number of primary branches**	**Number of secondary branches**	**Lodging**
	%	cm			1-9
N rate (kg ha^{-1})					
56	80.0	$85.0^{b†}$	5.0^{b}	11.0^{b}	3.1^{c}
84	73.0	86.0^{b}	6.0^{a}	13.0^{b}	4.3^{b}
112	76.0	90.0^{a}	6.0^{a}	15.0^{a}	5.1^{a}
140	75.0	90.0^{a}	6.0^{a}	15.0^{a}	5.1^{a}
S rate (kg ha^{-1})					
0	74.0	83.0^{b}	5.0^{b}	11.0^{b}	3.0^{b}
22	77.0	91.0^{a}	6.0^{a}	15.0^{a}	5.1^{a}
45	77.0	89.0^{a}	6.0^{a}	15.0^{a}	5.1^{a}
		Analysis of variance (P>F)			
N rate	0.395	0.005	<0.001	<0.001	<0.001
S rate	0.570	<0.001	<0.001	<0.001	<0.001
N x S	0.836	0.277	0.090	0.058	0.005

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.

Table 1-7. Number of pods per plant, pod length (cm), number of seeds per pod, seed yield (kg ha^{-1}), oil concentration (g kg^{-1}) and oil yield (kg ha^{-1}) of carinata at different N and S fertilizers rates at Brookings. Means are average between 2017 and 2018.

	Number of pods plant^{-1}	**Pod length**	**Number of seeds pod^{-1}**	**Seed yield**	**Oil concentration**	**Oil yield**
		cm		kg ha^{-1}	g kg^{-1}	kg ha^{-1}
N rate (kg ha^{-1})						
56	66.0^{b†}	5.0	13.0	1066.0	352.0^{a}	378.0^{ab}
84	68.0^{b}	5.0	13.0	1110.0	364.0^{a}	406.0^{a}
112	86.0^{a}	6.0	14.0	1184.0	352.0^{a}	422.0^{a}
140	80.0^{a}	5.0	14.0	1063.0	335.0^{b}	337.0^{b}
S rate (kg ha^{-1})						
0	63.0^{c}	5.0^{b}	12.0^{b}	682.0^{b}	337.0^{b}	233.0^{b}
22	88.0^{a}	6.0^{a}	14.0^{a}	1269.0^{a}	361.0^{a}	448.0^{a}
45	72.0^{b}	6.0^{a}	14.0^{a}	1391.0^{a}	357.0^{a}	488.0^{a}
Analysis of variance (P>F)						
N rate	<0.001	0.148	0.670	0.312	0.003	0.030
S rate	<0.001	<0.001	0.017	<0.001	0.001	<0.001
N x S	0.011	0.306	0.004	0.702	0.106	0.328

† Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at P<0.05. No letters are shown where there are no significant differences.

Table 1-8. N fertilizer costs, carinata seed prices, economic optimum N rates (EONR) and economic optimum yield (EOY) based on the costs for 2017 to 2019 and maximum and average carinata prices.

Year	**Cost of N fertilizer**	**Price of carinata seed**	**EONR**	**EOY**
	US\$ kg^{-1}	US\$ kg^{-1}	kg ha^{-1}	kg ha^{-1}
2017	0.85	0.35	77.0	1129
	0.85	0.5	84.0	1144
2018	0.84	0.35	78.0	1130
	0.84	0.5	85.0	1144
2019	0.96	0.35	74.0	1122
	0.96	0.5	82.0	1140

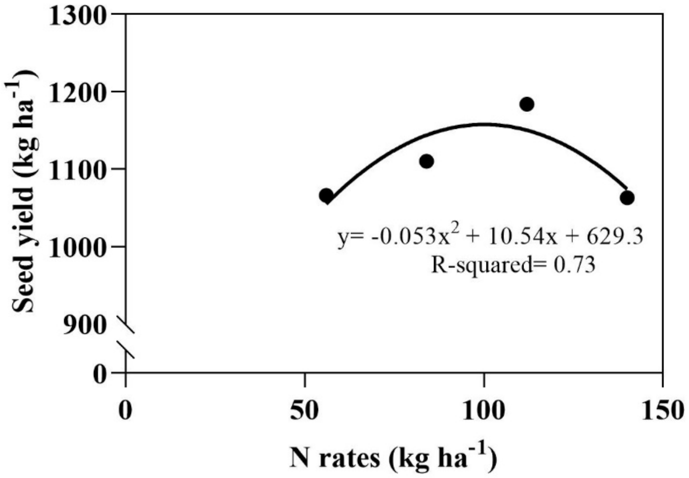

Figure 1-1. Seed yield response to N fertilizer rate for carinata grown at Brookings. Means are averaged over two years (2017 and 2018).

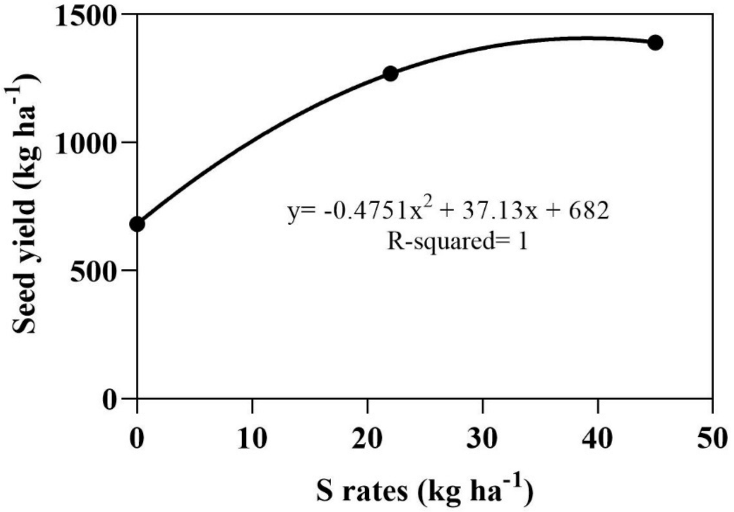

Figure 1-2. Seed yield response to S fertilizer rate for carinata grown at Brookings. Means are averaged over two years (2017 and 2018).

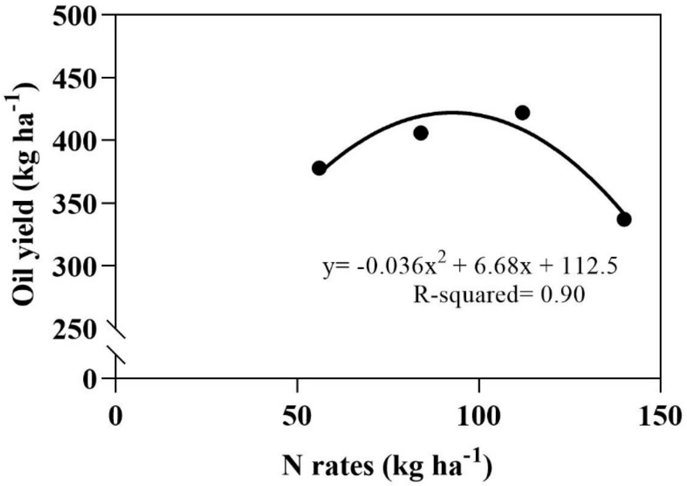

Figure 1-3. Oil yield response to N fertilizer rate for carinata grown at Brookings. Means are averaged over two years (2017 and 2018).

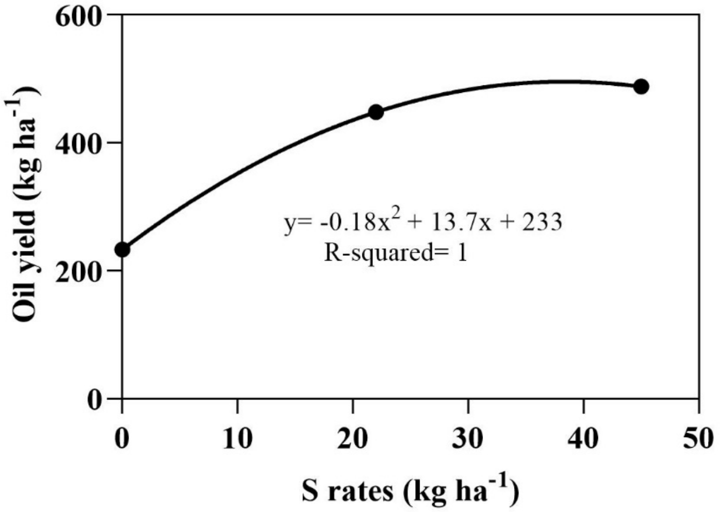

Figure 1-4. Oil yield response to S fertilizer rate for carinata grown at Brookings. Means are averaged over two years (2017 and 2018).

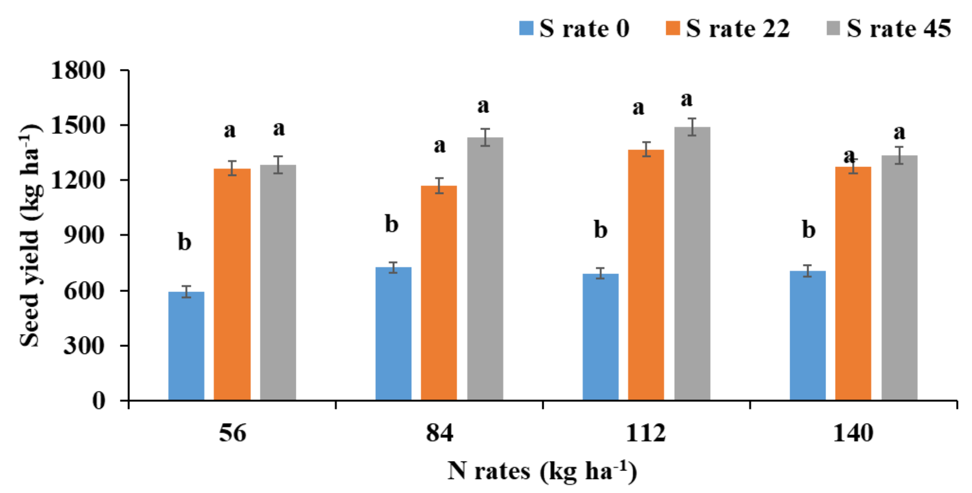

Figure 1-5. Seed yield response to N and S fertilizers rates for carinata grown at Brookings. Means are averaged between two years (2017 and 2018). Letters above bars represent the significant differences due to S fertilizer rates within each N fertilizer rate.

N rate: 112 kg ha^{-1}
S rate: 0 kg ha^{-1}

N rate:112 kg ha^{-1}
S rate: 45 kg ha^{-1}

Figure 1-6. Sulfur deficiency symptoms on S control plots in 2017, Brookings.

CHAPTER 2: IMPACT OF GROWING *BRASSICA CARINATA* ON SOILS AND GREENHOUSE GAS EMISSIONS

LITERATURE REVIEW

Carinata (*Brassica carinata* A. Braun) originated from the Ethiopian Highlands and is commonly known as Ethiopian mustard (Warwick et al., 2009). According to Agrisoma USA, this crop has high oil (45%) and protein (43-46%) content depending on the variety and environmental conditions. The seed has long chain unsaturated fatty acids suitable for the production of bio-jet fuel, lubricants and bioplastics (Taylor et al., 2010). Carinata is heat and drought tolerant (Agrisoma USA), which makes it an ideal crop for production in the semi-arid environments of South Dakota. Moreover, carinata is also an excellent rotational crop for cereal-based rotations enhancing overall crop productivity and also improving the soil health (Wright, 2017). Similar to other crops, oilseed crops require N, P, K and S fertilizers among those N and S fertilizers are crucial for the enhanced yield (Abdallah et al., 2010; CFIA, 2017). A previous study reported that inorganic N fertilizer requirements for carinata in SD is lower compared to the cereal crops (Alberti et al., 2019). This can reduce the negative impact of fertilizers on soil properties and reduce the greenhouse gas (GHG) emissions (Zhong et al., 2016).

Application of inorganic fertilizers in greater amount cause accumulation and concentration of mineral salts that lead to soil compaction and resistance to root penetration (Massah and Azadegan, 2016). However, no impact of inorganic fertilizers (e.g., N, P and K) was found on the soil bulk density in a long-term field study (Blanco-Canqui et al., 2015; Zhou et al., 2017). Similarly, there was non-significant effect on soil bulk density of S fertilizers applied to the mustard field (Prasad et al., 2018). Although inorganic fertilizers may not impact soil properties in short-term, a long-term experiments of fertilizer applications can provide valuable information on the impact of fertilizers on soil properties (Blanco-Canqui et al., 2015).

USDA has defined neutral pH as the pH in the range of 6.6 and 7.3, which is favorable for microbial growth; however, plant nutrients are readily available at the pH range of 6 to 7 for plant growth (USDA-NRCS, 2008). The causes of soil acidity includes younger soil, acidic parent materials, high precipitation, microbial decomposition and nitrification (Plaster, 2013). Nitrification is the process of oxidation of ammonia or ammonium (NH_4^+) to nitrites (NO_2^-) and nitrites oxidize to nitrates (NO_3^-) in the presence of bacteria. During this process, each molecule of ammonium releases two molecules of H^+ ions resulting in low soil pH (IPNI, 2018). The application of urea (46-0-0) fertilizer releases ammonia gas, which converts into ammonium form, i.e. plant available form; excess ammonium oxidizes and releases H^+ ions. Similarly, application of ammonium sulfate (21-0-0-24) undergoes oxidization and produces H^+ ions resulting in increased acidity. Moreover, elemental S can be oxidized to sulfate (SO_4^{-2}) ions and H^+ ions by bacteria producing sulfide oxidase (Konopka et al., 1986).

Electrical conductivity (EC) is a measure of the amount of salt ions in the soil. According to USDA, non-saline soil has EC less than 2 deci Siemens per meter (dS m^{-1}), whereas, strongly saline soil has EC greater than or equal to 16 dS m^{-1}. The EC is an important indicator of soil health, and it helps to determine the status of plant available nutrition through the measurement of resistance developed by the charged ions in the solution. Hence, this measure can direct us for optimum fertilization time and rate (DeBoer, 2015). Salt content in soil solution is directly proportioned to the amount of fertilizer applied; higher rates of fertilizers during planting, especially in semi-arid to arid areas can accumulate salt resulting in stunted plant growth (Evangelou, 1983).

Soil organic carbon (SOC) enters soil through the decomposition of plants and animals residues, soil biota and microorganisms (USDA-NRCS, 2008). Soil organic carbon contains C compounds with varying degree of degradability, easily degradable to recalcitrant carbon. It is the main source of energy for soil microbes. According to the USDA, soil organic matter (SOM) contains approximately 58% C, so 1.72 can be used as the factor to convert SOC to SOM (USDA-NRCS, 2008), however, this fraction varies in differ soils and environmental conditions. The amount of inorganic carbon present in the total organic carbon (TOC) is higher in calcareous soil compared to acidic soil, where inorganic carbon might be negligible. Soil organic carbon can be increased with the application of manure, fertilizers, diverse crop rotations and cover crops. Studies have found that the long-term application of fertilizers has significant effect on SOC (Lugato et al., 2010; Zhou et al., 2013). However, application of inorganic fertilizers alone is ineffective in improving soil aggregation (Zhou et al., 2013). The biomass yield and root growth were improved by the application of inorganic fertilizers, resulting in improved root activity and accelerated organic carbon accumulation (Liu et al., 2013). In contrary, long-term inorganic N fertilizer application reduced the soil microbial activities decreasing SOC, while short-term application of inorganic N fertilizer had limited effects on soil microbial activities (Fauci and Dick, 1994). A research study in a canola field showed that there was indirect increase in organic carbon by increasing crop residue with the application of N fertilizers (Kazemeini et al., 2010). Similarly, another research result showed an increase in biomass yield with the application of N fertilizers but no significant effect on organic carbon (Halvorson et al., 2002). Not many studies studied the impact of S fertilizers on soil C, however, Gupta et al. (1988) reported 2-51% reduction in microbial biomass carbon with the application of elemental S fertilizer.

Total nitrogen (TN) is an important soil nutrient required for plant nutrient uptake. Soil N is organic or inorganic; organic includes amino acids, nucleic acids and urea, whereas, inorganic includes nitrates, nitrites and ammonium, inorganic forms are plant available. Application of N fertilizer is necessary to replenish the depleted N from soil resulting from plant uptake, leaching or evaporation loss. Optimum application of N fertilizers should be considered based on plant nutrient uptake rate and type of soil. Soil inorganic N builds up only when the application rate of N fertilizers is high (200 kg N ha^{-1}) (Bergström and Brink, 1986). These authors found that spring rape (*Brassica napus*) followed by winter wheat (*Triticum aestivum*) takes up higher amounts of soil N preventing possible leaching. The rate of exchangeable ammonium was higher with the increase in N fertilizer level (Broadbent, 1965). Although the application of inorganic N fertilizers is important for plant and soil health, repeated application of high N fertilizers can lead to soil acidity and negative soil health traits (Singh, 2018). A study in canola (*Brassica napus* L.) showed that a field receiving higher rates of N fertilizer significantly increased soil mineral nitrogen (Herath et al., 2017). Another study in canola showed that about 50% of the applied N fertilizer was recovered in seed and the rest remained in plant parts and significant amount returned to soil as mineral N (CCC, 2017).

Greenhouse gas (GHG) emissions have important role in regulating the Earth's surface temperature. General circulation models predict that the Earth's atmospheric temperature increases or decreases in addition to the change in precipitation regime; this may change the GHG exchange (Sillmann et al., 2013). The EPA (2010) has reported that agriculture is responsible for 9% of the total GHG emissions in the US. This agency further mentioned that the total GHG emissions increased by 2% since 1990. Many reports have mentioned that fertilization has mixed effects on GHG emissions. These emissions are sensitive to climate change and land management practices (Rafique et al., 2014). Soil CO_2 fluxes are produced due to the metabolism of plant roots and respiration of soil microbes (Mbonimpa et al., 2015). Soil organic carbon serves as a good substrate to soil microbes that produce CO_2. The application of N fertilizer at higher rates increased the CO_2 emission in a study by Ozlu and Kumar (2018).Higher rates of N fertilizer and manures applied in soil to meet global food demand are responsible for higher N_2O emissions (Kim et al., 2014). The application of N fertilizer, land use change, and climate are the major factors affecting the N_2O

emissions from agricultural soils (Kim et al., 2014). Another major source of N_2O emissions is the microbial process of N transformations, which contribute about 90% of the global N_2O emissions (Li et al., 2013). Soil CH_4 fluxes are mainly produced as a result of microbial processes in SOM (Mbonimpa et al., 2015). Previous studies in South Dakota have reported that CH_4 emissions were not impacted by fertilizer application (Ozlu and Kumar, 2018).

The above studies showed that soil microbial activities as a function of different agricultural activities are responsible for GHG emissions. However, the influence of S fertilizer on GHG emissions have not been reported by any of the above mentioned studies. Gupta et al. (1988) reported that the application S fertilizer reduces microbial biomass. This might result in lower GHG emissions.

Like other crops, *Brassica* crops require major fertilizers (N, P and K) for the enhanced seed and oil production. In addition, higher amount of S fertilizer is required in *Brassica* species compared to the other crops. *B. carinata*, as mentioned earlier, can produce greater yield at less amount of N fertilizer compared to the other crops (like corn and wheat). There are various studies conducted to assess the impacts of N fertilizers on crop production; however, there are insufficient studies that explains the impact of applied inorganic fertilizers including sulfur on soil parameters and GHG emissions. Therefore, the objective of this study was to evaluate the impacts of growing carinata at different rates of N and S fertilizers on select soil parameters, and soil surface GHG fluxes.

MATERIALS AND METHODS

The study was conducted in Aurora Agricultural Experiment Station at near Brookings (44º18’35” N 96º40’15.9” W), South Dakota on the field planted to carinata in 2017 and 2018. The experimental plots were randomized complete block design (RCBD) with 12 treatments replicated three times. Treatments included four different rates of N fertilizer: 56, 84, 112 and 140 kg N ha^{-1} and three different rates of S fertilizer: 0 (control), 22 and 45 kg S ha^{-1} arranged in a factorial design to make 12 treatments within each replication. Urea (46-0-0) and ammonium sulfate (21-0-0-24) were broadcast in two-equal spilt doses: first half immediately after planting and the next 4 weeks after planting, at bolting stage (13 June 2017 and 22 June 2018). There were 36 triplet plots of carinata (var. A120) each year; an individual plot size was 1.62 x 9.14 m (with an area of 14.86 m^2). The planting dates in 2017 and 2018 were 24 April and 8 May, respectively. In 2018, late snow and prolonged frost caused delayed planting of carinata. The weather summary of both years can be found below in Fig. 2-1. Each plot had seven rows, 22 cm apart planted at the seeding rate of 11 kg ha^{-1}.

The study site had slightly acidic and medium textured soil. According to the USDA, the soil type at the study site is Brandt series characterized by fine silty, super active, frigid calcic hapludolls (Malo, 2003; USDA-NRCS, 2017). In both years, the previous crop was winter wheat. The basic soil properties for the study site can be found in Table 2-1.

Soil sampling and analysis

Soil samples were collected using hydraulic probe after crop harvest from four different depths (0-5, 5-15, 15-30 and 30-45 cm) at three different spots within each plot. Intact soil core samples (n = 3 per plot) were also collected from each plot to analyze the soil bulk density for each plot. In addition, samples were air dried, ground and passed through 2 mm sieve to measure soil pH, and EC. Further, soil was passed through 0.5 mm sieve to measure total carbon, total nitrogen and carbon fractions. Soil extractable N, P, K, and S concentration in the soil were also analyzed.

Soil Bulk Density

Soil bulk density was determined for all the four depths by dividing the oven dry weight (g) of soil with the known volume of soil (Grossman and Reinsch, 2002). Soil samples were kept for oven drying at 105℃ for 48 hr.

Soil pH and Electrical Conductivity (EC)

Air-dried soil samples for all four depths were passed through a 2-mm sieve. Soil solution at 1:1 ratio (Miller and Kissel, 2010) was prepared by adding 10 g soil and 10 mL distilled water in centrifuge tube and stirred with vortex for 10 seconds. Fisher’s Scientific® pH meter was used to measure the soil pH. After measuring the soil pH, at the same soil-solution ratio soil EC was measured using the Fisher’s Scientific ® EC meter (Zhang et al., 2005).

Soil Organic Carbon (SOC) and Total Nitrogen (TN)

Total soil carbon and TN were analyzed through dry combustion using Leco® 628 series carbon/hydrogen/nitrogen analyzer (Baron et al., 2006). Soil inorganic carbon (SIC) is the measure of calcium or magnesium carbonate in the soil. Soil with low pH has lower inorganic carbon (Guo et al., 2016), which is negligible in our case. Hence, this study has reported the total soil carbon as the soil organic carbon.

Soil carbon and nitrogen fractions

Soil carbon and nitrogen fractions were analyzed using cold water extraction (for labile carbon and nitrogen) and hot water extraction (for stable carbon and nitrogen) methods (Ghani et al., 2003). For cold water extraction of labile carbon and nitrogen, 3 g soil was placed in 50 mL centrifuge tube with 30 mL of distilled water. Soil suspension was mixed on vortex for 10 seconds and kept in end-to-end shaker at 40 rpm for 30 minutes. The solution was then centrifuged at 3000 rpm for 25 minutes maintaining 4^{o}C, and supernatant was separated from soil by using 0.45 µm pore size syringe filters. Soil remained thereafter was used to determine the stable carbon and nitrogen fractions. Similar to the previous methods, it was mixed on vortex for 10 seconds after adding 30 mL distilled water to the remained soil and kept in end-to-end shaker at the same precisions. The tube was then subjected to hot water bath at 80^{o}C for 12 hr. Thereafter, the solution was centrifuged at 3000 rpm for 25 minutes at 25^{o}C. The supernatant was again filtered using 0.45 µm pore size syringe filters. These total C and N were considered as organic C and organic N in each extract by considering negligible inorganic C in soil as the pH of the soil was less than 6.0. Cold-water, and hot-water C and N were determined for 0-5 and 5-15 cm depths using the TOC-L analyzer (Shimadzu Corporation, model-TNM-L-ROHS).

Soil extractable nitrogen (N), phosphorus (P), potassium (K) and sulfur (S)

Soil from the top two depths (0-5 and 5-15 cm) were sent to Ward Laboratories, Inc., NE for soil extractable N, P, K and S analysis. The Ward Lab uses flow-injection analysis method to analyze NO_3-N (Kanda and Taira, 2003), Mehlich-3 method for P (Mehlich, 1984), ammonium aceate extraction method for K (Brix, 2008) and monocalcium phosphate to extract sulfate (Fox et al., 1964) from soil samples.

GHG monitoring

The GHG emissions were monitored for the whole growing season of carinata. Nine treatments (a factorial combination of 56, 112 and 140 kg N ha^{-1} and 0, 22 and 45 kg S ha^{-1}) in three replications were selected for monitoring the GHG emissions. Polyvinyl chloride (PVC) static chambers (25 cm diameter and 15 cm height) were implanted between crop rows in each plot to monitor soil surface GHG fluxes, according to the guidance of Parkin and Venterea (2010). Gas samples were taken once or twice a week depending on weather conditions from June to August in 2017, and from May to August in 2018. Gas samples at 3-time intervals (0, 20 and 40 minutes) were collected using the 10 mL syringe. At the mean time of gas sampling, soil temperature and moisture, and chamber temperature were recorded. A total of 81 gas samples (9 treatments x 3 replications x 3 point samples) per day were collected and taken through a chamber septum and transferred to a 10 mL, argon-filled vials. Gas Chromatograph [Shimadzu 4B with a CombiPal AOC-500 auto sampler, 2-mL injection loop, a 1/8″stainless-steel Porapack Q (80/100 mesh) column, a Haysep-D 90 column (columns operated at 60°C), and a flame ionization detector and a lepton capture detector each at 260°C)] measured the concentrations of CO_2, N_2O, and CH_4 for each sample. Daily flux (F, mass of gas ha^{-1} day^{-1}) was computed as:

$$F = \frac{\Delta g}{\Delta t} . \frac{V}{A} . k$$

where, $\Delta g/\Delta t$ is the rate of gas change (CH_4, CO_2 or N_2O) concentration inside the chamber (mg CH_4-C, CO_2-C or mg N_2O-N m^{-2} min^{-1}); V is the chamber volume (m^3); A is the surface area circumscribed by the chamber (m^2) and k is the time conversion factor (1440 min day^{-1}). Gas fluxes were calculated from the time vs. concentration data using linear regression or the algorithm (Hutchinson and Mosier, 1981; Ussiri and Lal, 2009), when the time vs. concentration data were curvilinear (Ozlu and Kumar, 2018).

Statistical Analysis

The impacts of N and S fertilizers applied to carinata on select soil parameters in 2017 and 2018 were analyzed using Analysis of Variance (ANOVA) method in the SAS 9.4 (SAS, 2014). Data normality test was done using Kolmogorov-Smirnov method. Statistical comparisons of soil BD, pH, EC, SOC, TN, C-fractions, N-fractions, inorganic N, P, K, and S of selected soil depths among N and S fertilizers rates were obtained using pairwise differences method (adjusted by Tukey) in a mixed model approach using GLIMMIX procedure in SAS 9.4 (SAS, 2014). In this analysis, N and S fertilizers rates were considered as fixed effects and replication as random effect.

The repeated measures analysis for comparing the soil CO_2, CH_4, and N_2O fluxes under different N and S rates was conducted using PROC MIXED in SAS 9.4 (SAS, 2014), with N and S fertilizers as fixed effects, replication as a random effect, and date of gas sampling as a repeated measure variable. Wherever necessary, data transformation was conducted using Box-Cox method (Box and Cox, 1964) using SAS 9.4. All statistical analyses were determined at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Weather data, soil moisture and temperature

The weather data was accessed from the Mesonet at SDSU (Mesonet, 2018).Temperature and rainfall data were recorded throughout the crop growing period at Brookings in 2017 and 2018 and shown in Fig 2-1. The mean maximum temperature during the carinata growing season in 2017 was 34°C in July. In the same year, Brookings received a total rainfall of 377.2 mm over the crop-growing season. Unexpectedly, early days of June did not receive rainfall followed by scattered rainfall at the mid of the month. However, late July and August rainfall compensated the crop for moisture requirements. During the critical crop growth period (June and July), temperature often exceeded 25°C, one quarter of the total period exceeded 30°C. In 2018, the average temperature was extremely low in April, although, it rose at the end of April. The freezing temperature and snowfall in April shifted the normal planting time to the 2nd week of May and the harvesting time to September. Unlike 2017, 2018 was a wet year where the total amount of rainfall during the growing period was 442.9 mm; on a single day, in mid-July, the total rainfall was 150 mm.

Soil moisture (percentage) and soil temperature (°C) recorded during the observed days are presented in Fig. 2-2a and 2-2b. Brookings experienced a period of drought in the mid and late June (Fig. 2-1 and 2-2a) in 2017. Soil moisture rose after the first week of July 2017 and eventually fluctuates until the last observation day; however, the moisture was above 13% on an average. The early observation days in 2018 had lower moisture content (around 10%). The continuous rainfall during the third week of June sharply raised the soil moisture in the field. The 2018 growing season received high amount rainfall, thus, soil moisture for most of the sampling days was greater than 20%. The heavy rainfall after the second week of July increased the soil moisture (Fig 2-1 and 2-2a).

Unlike soil moisture, soil temperature did not rise or fall sharply. In 2017, average soil temperature was 20°C, which decreased to 17°C during the short period of rain in June. During the period of drought, soil temperature gradually increased and reached 23°C. Soil gradually cooled down to 17°C after the second week of July with the onset of rain. Long frost in 2018 resulted in cooler soil temperature until the second week of May. The first sampling day in 2018 had low temperature of 16°C. As there was no rainfall from the second week of May to the first week of June, soil temperature rose sharply to greater than 25°C; after this Brookings received continuous rainfall. During this period, soil temperature decreased to 20°C; however, small fluctuations on soil temperature were observed until the third week of June when the continuous rainfall was observed and the soil temperature decreased to18°C. After the last week of June, most of the days in July had air temperature greater than 25°C (Fig. 2-1) this led to continuous increase in soil temperature during observed days in July. Higher amount of rainfall and gradual decrease in air temperature resulted in lower soil temperature in August.

Average soil temperature was not influenced in response to N treatments (*P*=0.28) in 2017, however, in 2018, plots applied with 56 kg N ha^{-1} had 3.5% and 2.6% higher moisture content compared to plots applied with 112 and 140 kg N ha^{-1}, respectively (*P*=0.005). Average soil moisture was not influenced in response to N treatments in 2017 (*P*=0.41) and 2018 (*P*=0.17). Average soil temperature was not affected by S fertilizer treatments in 2017 (*P*=0.37) and in 2018 (*P*=0.81). In 2017, average soil moisture at 45 kg S ha^{-1} was 8% higher than that at S control plots (*P*<0.001). In 2018, S control plots had 3% higher moisture compared to S fertilizer applied plots (*P*=0.027).

Soil bulk density

Mean of soil bulk density (BD, g cm^{-3}) at different N and S fertilizers treatments were non-significant for both the years; however, BD was significantly different at different depths (Table 2-2a and b). Data for 0-5, 5-15, 15-30 and 30-

45 cm depths for both years are presented in Table A2-1 (a-d). Data showed that neither of the studied treatments significantly influenced soil bulk density in both years. Soil bulk density was greater in 2018 compared to the 2017 because the experimental field was different although the site was the same. In 2017, the lowest soil bulk density was 1.21 g cm^{-3} at 0-5 cm depth, whereas, the highest was the 1.49 g cm^{-3} at lower depths.

Soil BD is the measure of soil compaction and affects the uptake and utilization of rate of nutrient in soil (Jian-jun et al., 2013). Results presented above did not show any significant impact of N and S fertilizers on soil bulk density; however, it increased with the depth. Hati et al. (2006) reported that the application of NPK fertilizers for three years affected the bulk density only at the top depth. Use of tillage equipment, planting and harvesting machines, rainfall event and other activities are responsible for the compaction of soil. The presence of porous soil with high organic matter is the major reason for lower bulk density on the top depth compared to the lower depth (USDA-NRCS, 2018). A short-term study on inorganic fertilizers did not show any significant difference on soil bulk density (Zhang et al., 2017). However, long-term application of manure and fertilizers can significantly improve the soil bulk density (Blanco-Canqui et al., 2015).

Soil pH and EC

The pH data for 0-5, 5-15, 15-30 and 30-45 cm depths for both years are presented in Table A2-1 (a-d). Mean soil pH was acidic, however, was not significantly influenced by different N fertilizer treatments in both years (Table 2-

2a and b). The lowest pH was 4.5 at 0-5 cm depth when higher rates of N fertilizer were applied. It gradually increased up to 6.4 at 30-45 cm depth with no significant difference within each depth. In 2018, N fertilizers influenced soil pH at the top depth only; lower pH (5.2) was obtained at higher N fertilizer rates compared to lower N fertilizer rates. In 2017, S fertilizer treatments significantly increased mean soil pH at 22 kg S ha^{-1}, however, pH were similar at 0 and 45 kg S ha^{-1} treatments (Table 2-2a).

Nitrogen fertilizers can have negative effect on soil parameters through lowering of soil pH due to natural transformations of N in the soil. Data showed the acidic soil in the plots that might be directly related to the application of N fertilizers in the soil. Use of urea and ammonium sulfate produces H^{+} ion during the conversion process of ammonium to nitrate (Gilmour, 2018). Guo et al. (2016) reported that the application of N fertilizers for the long term significantly decreased the soil pH in croplands. The authors further mentioned that plant uptake and removal of base cations are the consequences of high N fertilizer application. High precipitation leaches the base ions such as Ca^{++} and Mg^{++} increasing the concentration of acidic ions such has H^{+} and Al^{+3} on the upper depth of soil (Singh, 2018). This process is responsible for the acidic pH in the soil at upper depth compared to lower depth. In addition, high organic matter on the O-horizon has high microbial activities during which N transformation process is higher in the soil; this releases more H^{+} ions and cause low soil pH (Lamb et al., 2014). A study in winter wheat by Aula et al. (2016) reported that soil pH declines with increasing N fertilization rate. Application of S fertilizer (ammonium sulfate) releases H^+ ions during the conversion of ammonium to nitrate. In addition, elemental S releases H^+ ions during the process of oxidation. Wiedenfeld (2011) reported that increasing rate of S fertilizer application gradually reduces soil pH.

Mean soil EC was influenced by both N and S fertilizers treatments in 2017, although, there was no any interaction between them (Table 2-2a). The result shows that increased N fertilizer rates significantly increase the EC of the soil, whereas, increased S fertilizer rates reduced the EC value. Mean EC at 112 and 140 kg N ha^{-1} are 12.5% and 13% greater than that at 56 kg N ha^{-1}, respectively. The application of 22 and 45 kg S ha^{-1} reduced the EC by 12% and 10%, respectively compared to S control soils. Only S fertilizer application showed significant difference in the mean soil EC in 2018. Application of S fertilizer increased the EC in 2018, which was contrary to that of 2017. Similar to the soil pH, soil EC was different at different depths (Table A2-1 (a-d)). Soil EC decreased with the depth in both years.

Higher rate of N fertilizer releases greater amount of NO_3^- during the process of nitrification, resulting in greater EC at higher N fertilizer rates in 2017. Liu et al. (2014) reported the highest EC at the highest application rate of N fertilizers. Bunt (1988) reported that the application of ammonium sulfate significantly increases the soil EC. Salt stress was reported by Bryla et al. (2010) when ammonium sulfate was applied at the higher rate. Soil EC increases with the application of S fertilizer (SO_4^-) as reported by many authors. For instance, Hashemimajd et al. (2012) reported that the application of elemental S increased the soil EC after it has started converting to SO_4^- ions. Similarly, highest EC was recorded at the highest rate of applied S fertilizer in soil by Turan et al. (2013). A soil quality indicator sheet on soil EC provided by USDA-NRCS (2008) showed that topsoil rich with organic matter improves the water holding capacity of soil and added inorganic fertilizers augment the salt concentration on soil. This might be the reason for higher EC on the upper depth compared to the lower depth in both years. In contrary, decrease in mean soil EC with increasing S fertilizer in 2017 might need some further study to be explained. The larger difference in soil EC between the years might be due to high amount of rainfall in 2018, which might have leached the mineral ions from the soil (USDA-NRCS, 2008).

Soil Organic Carbon

Data on mean soil organic carbon (SOC; g kg^{-1}) at different N and S fertilizer rates for 2017 and 2018 are present in Table 2-2a and b. The data showed that N fertilizer rates influenced the SOC in 2017 but not in 2018. In 2017, the two higher rates of N fertilizer application showed a 5% increase in SOC compared to the lowest N fertilizer rate (56 kg N ha^{-1}). In addition, S fertilizer application showed significant impact only in 2018; S fertilizer applied soils contained 3% lower SOC compared to the S control soils. Table A2-1 (a-d) showed that SOC decreased with increasing depth in both years.

Recous et al. (1995) suggested that N fertilizer application either can increase soil organic matter and promote plant growth or can promote organic matter loss because of microbial transformation and other forms of organic C present in the soil. The first mechanism is widely accepted and true for the data of

2017, whereas, second mechanism might be the reason for non-significant effect of N fertilizer in 2018. Ghimire et al. (2017) showed that the application of inorganic fertilizers including N fertilizer increased the SOC over control. Poffenbarger et al. (2017) stated that application N fertilizer above the optimum rate decreased the SOC storage. Similar to the above study, the SOC content in our study was similar for 112 kg N ha^{-1} and 140 kg N ha^{-1}. Regarding the effect of S fertilizer on SOC, not enough studies have been recorded. However, reduction in the microbial biomass and enzyme activities were reported by Gupta et al. (1988). They reported 2 to 51% decline in microbial biomass carbon content because of S fertilizer application in Canada. Authors mentioned that repeated application of S fertilizer had negative impact on respiration, dehydrogenase, urease, alkaline phosphatase and arylsulfatase activities in soils. High organic matter and microbial activities on the top layer of soil resulted in higher SOC content compared to the lower depth. Similar results were reported by Hao et al. (2017) where the SOC stock was lower in 20-40 cm depth compared to 0-20 cm despite the treatments.

Total Soil Nitrogen

Data on total nitrogen (TN, g kg^{-1}) showed non-significant impact of the treatments in both years (Table 2-2a and b). The average TN in 2017 was 2.70 g kg^{-1} and that in 2018 was 3.10 g kg^{-1}. The highest TN in 2017 was 3.52 g kg^{-1} at the 0-5 cm depth and the lowest was 1.87 g kg^{-1} at 30-45 cm depth. Likewise, in 2018, the highest TN was 3.45 g kg^{-1} at the 0-5 cm depth and the lowest was 2.46 g kg^{-1} at 30-45 cm depth.

Long-term application of manure significantly impacted the soil TN, however, application of inorganic fertilizers did not show significant impact (Ozlu and Kumar, 2018). Liang et al. (2012) reported that the long-term inorganic fertilizer application did not influence TN content. This might be partially due to N loss because of ammonia volatilization, leaching, and denitrification as reported by Ju et al. (2009) in the wheat-maize cropping systems.

Soil carbon and nitrogen fraction

Data on means of labile C and stable C for both years are presented in Table 2-2 (a and b). There was no significant impact of N and S fertilizers on labile and stable C in both years, except for stable C in 2017. Increase in N fertilizer application significantly increased the level of stable C in 2017. The higher concentration of stable C was obtained with 112 kg N ha^{-1}, which was 12.6% greater than the 56 kg N ha^{-1}. Like other parameters, both labile and stable C were significantly different at different depths. Data on the different depths are shown in the Table A2-2. This table showed that stable C for the 0-5 cm depth was influenced by N fertilizer rates only in 2017. The higher concentration of stable C at this depth was obtained in the soil of 112 kg N ha^{-1}, which was 17% greater than that at 56 kg N ha^{-1}. Sulfur fertilizer application at the rate of 22 kg S ha^{-1} significantly reduced the stable C concentration at 5-15 cm depth.

Labile C is the primary C available to the plants and is readily available in soils. Soil organic matter rather than inorganic fertilizers influenced labile C. This is the reason of higher labile C at 0-5 cm depth compared to 5-15 cm. The possible reason for the significantly higher concentration of stable C at higher N fertilizer rates in 2017 might be the activities of soil bacteria under the favorable moisture and temperature. Li et al. (2018) reported that the application of standard rate of mineral fertilizers increased the non-labile soil carbon; however, it was similar to that with the double rate of mineral fertilizers. A study conducted over 41 years on impact of manure and fertilizers on soil properties in India showed that soil carbon increased with the application of fertilizers and manure.

Data on means of labile N and stable N for both years are presented in Table 2-2 (a and b). There was no significant impact of N and S fertilizers on stable N in both years, whereas, N fertilizers influenced labile N in 2017 and S fertilizers affected labile N in 2018. Increase in N application significantly increased the level of labile N in 2017. The greatest concentration of labile N in 2017 was obtained in the soil of 112 kg N ha^{-1}, which was 80% greater than that of 56 kg N ha^{-1}. In 2018, soil in the highest applied S fertilizer rate (45 kg S ha^{-1}) had 17% less concentration of labile N compared to S control soil. Like other parameters, both labile and stable N were significantly different at different depths. Data on the different depths are shown in the Table A2-2. This table showed that stable N for the 5-15 cm depth was influenced by S fertilizer rates in both years. However, S fertilizer affected the labile N for the 0-5 cm depth in 2018 only. Sulfur fertilizer application lowered the concentration of labile N in soil.

Addition of N fertilizer in soil increased the readily available N due to the transformation in soil by bacteria. Urea and ammonium sulfate are converted into nitrate ions which account for the labile soil N. Belay-Tedla et al. (2009) reported the impact of increased temperature on the labile N whereas no influence was seen on the stable N. This might be another reason for the significant increase of labile N in soil due to the interaction between applied N and high microbial activities at optimal temperature. Gupta et al. (1988) reported that microbial activity declines in S applied soil. This can be the reason for the lower concentration of labile N at 45 kg S ha^{-1}.

Soil extractable N, P, K and S

Data on means of soil N, P, K and S for both years are presented in Table 2-2 (a and b). Sulfur fertilizer application showed a negative relationship with soil NO_3-N in 2018. N fertilizer influenced the soil P in both the years. In 2017, soil applied with 56 kg N ha^{-1} showed the lowest soil P concentration, whereas in 2018, soil with 140 kg N ha^{-1} demonstrated the lowest soil P concentration. Soil K was not influenced by any of the treatments in 2017, whereas in 2018, both fertilizers had significant impact on soil K concentration. In 2018, soil with 56 kg N ha^{-1} had the greatest K concentration (250.4 mg kg^{-1}), whereas soil with 140 kg N ha^{-1} had the lowest K concentration (175.7 mg kg^{-1}). Soil applied with 22 kg S ha^{-1} had significantly lower K concentration compared to the S control and soil applied with 45 kg S ha^{-1}. Application of S fertilizer significantly increased the soil S concentration in both years. Soil N, P and K concentration significantly varied with the soil depth, whereas soil S concentration remain unchanged. Data on N, P, K and S on different depths are presented in Table A2-3. This table showed that increase in N fertilizer increased the soil P concentration at 0-5 cm depth in 2017 while it reduced the soil K concentration at 0-5 cm depth in 2018.

In 2017, the greatest rate of S fertilizer significantly increased the soil S concentration at both the depths (0-5 and 5-15 cm), whereas in 2018 the concentration remains unchanged.

Soil bacteria transforms the inorganic N (applied via urea and ammonium sulfate) to nitrate ions, those are plant available. Gupta et al. (1988) reported a decline in microbial activity in soil after the application of S fertilizers. This might be the reason for negative relationship of soil N with applied S fertilizers. Soil P is greatly influenced by microorganisms; they play an important role in supplying P to plant in a sustainable manner (Gyaneshwar et al., 2002). Application of higher rate of N fertilizer, in addition to optimum soil temperature and moisture, might have improved the soil microbial community. This resulted in increase in soil P availability with increasing N rate in 2017. However, higher amount of rainfall in 2018 might have increased nutrient leaching and created unfavorable condition for microbial activity, which resulted in poor soil P availability at 140 kg N ha^{-1}. N and K are available in monovalent ionic forms, NH_4^+ and K^+, in soil. They share similar valance and size properties, and thus compete for same exchangeable and non-exchangeable sites of soil particles (Bar Tal, 2011). The same author mentioned that in short-term, the application of N fertilizer may increase K availability, whereas in long-term, NH_4^+ fertilization depletes the soil K^+ availability.

Daily and seasonal CO_2, N_2O and CH_4 fluxes

Daily average soil surface CO_2 fluxes were higher under 140 kg N ha^{-1} treatment in comparison to the other N fertilizer rates (Fig. 2-3). In 2017, peaks were seen after the application of fertilizers (applied on 13 June) and during the wet soil condition after a long drought (Fig 2-1 and 2-3). The largest difference in fluxes was observed on 29 July, 2017 between 56 kg N ha^{-1} (24.80 kg CO_2 ha^{-1} day^{-1}) and 140 kg N ha^{-1} (30.51 kg CO_2 ha^{-1} day^{-1}). In 2018, peaks were seen after the increase in soil temperature on 8 June 2018. After the application of fertilizers on 22 June 2018, significant difference in CO_2 fluxes was observed among N fertilizer treatments ($P<0.001$) on 24 June 2018. Likewise, peaks were seen on 6 and 15 July after increased soil temperature and soil moisture (Fig. 2-2a) while there was significant influence of N fertilizers on fluxes on those two dates with p-values 0.015 and 0.025, respectively. On the other hand, daily average soil surface CO_2 fluxes were numerically higher under 0 kg S ha^{-1} compared to S fertilizer applied plots; however, no significant difference among the S fertilizer treatments were observed. Similar to the response of the fluxes to N fertilizer rates, fertilizer application and increased soil temperature and moisture resulted higher CO_2 peaks in response to S rates (Fig 2-2b and 2-3). Despite of any treatments, GHG fluxes decreased during the period of continuous precipitation after mid-July of 2018.

Daily average soil surface N_2O fluxes were lower under 56 kg N ha^{-1} compared to higher N rates (Fig 2-4). In 2017, higher peaks and significant differences among the N fertilizer treatments were observed after the application of fertilizers and increased soil temperature (Fig 2-2a and 2-4). Significant difference among different N fertilizer treatments were observed on 16 June 16, 19 and 29 July. In all three days, application of 56 kg N ha^{-1} exhibited 1-6% lower

N_2O fluxes compared to the 140 kg N ha^{-1} (Fig. 2-4). Likewise, fertilizer application, and increase in soil moisture and soil temperature increased peaks in 2018. On 24 June 2018, N_2O fluxes under 56 kg N ha^{-1} (9.51 g N_2O ha^{-1} day^{-1}) was significantly lower (P=0.007) compared to 112 kg N ha^{-1} (15.94 g N_2O ha^{-1} day^{-1}) and 140 kg N ha^{-1} (16.85 g N_2O ha^{-1} day^{-1}). On 14 July 2018, N_2O fluxes under 56 kg N ha^{-1} (4.13 g N_2O ha^{-1} day^{-1}) was significantly lower (P=0.042) compared to 140 kg N ha^{-1} (8.35 g N_2O ha^{-1} day^{-1}). On the other hand, daily average soil surface N_2O fluxes were numerically higher under 0 kg S ha^{-1} compared to S fertilizer applied plots in most of the observed days; however, no significant difference among the S fertilizer treatments were observed. Similar to the response of the fluxes to N fertilizer rates, fertilizer application, and increased soil temperature and moisture resulted higher N_2O peaks in response to S fertilizer rates (Fig. 2-2b and 2-4). Data on daily means of CH_4 fluxes are presented in Fig. 2-5. The graph did not show any specific trend on the CH_4 emission in any of the treatments for both years.

Data on the seasonal means of CO_2, N_2O and CH_4 fluxes as influenced by different N and S fertilizer treatments are presented in the Table 2-3. In 2017, application of N fertilizers significantly influenced CO_2 and N_2O fluxes. CO_2 and N_2O fluxes were comparatively lower in the plots with 56 kg N ha^{-1} compared to higher N rates. Although no significant effect of S fertilizer was observed on the GHGs emissions, S control plots had comparatively greater CO_2 and N_2O fluxes. In 2018, increasing trend of CO_2 and N_2O emissions were observed with increased N fertilizer rates; however, they were not significantly different. Similar to 2017, S fertilizer control plots had greater CO_2 and N_2O fluxes compared to S fertilizer applied plots. Significant interaction of N by S fertilizer was observed on the N_2O fluxes in 2018. Like the other two gases, CH_4 fluxes increased with increase in N fertilizer and decreased with the application of S fertilizer; however, the change was negligible. In both years, GHG fluxes were significantly influenced by the date or sampling days.

Application of N fertilizers, in addition to favorable temperature and moisture, is responsible for higher microbial activity; this increases the CO_2 fluxes. The CO_2 emissions increased during the period of warm temperature and moist soil condition. Data showed that the continuous rainfall in 2018 after July reduced the CO_2 emission; this might be due to the high moisture condition in the soil creating anaerobic condition. Carbon dioxide emissions declined during the long drought period in 2017, which might be because of high temperature and low moisture condition creating unfavorable environment for microbial activity. Sainju et al. (2008) reported a 14% increase in CO_2 fluxes in N applied soils compared to the N control soils in North Dakota. Data from the same study showed that irrigation and tillage operation increased the CO_2 emission. The CO_2 fluxes varied with date of sampling; they peaked after short precipitation and fertilizer application activities. Similar trends were observed in another study by Sainju et al. (2012). In contrary, study by Mbonimpa et al. (2015) reported that the reduction in CO_2 fluxes in N fertilizer applied plots due to the combination of lower SOC, lower porosity and high bulk density. Gupta et al. (1988) reported the reduction in the microbial biomass carbon after the application of S fertilizer in agricultural soil. The reduction in microbial biomass carbon after spraying S fertilizer was true for Li et al. (2016). This could be the primary reason for reduction in CO_2 emissions in S fertilizer applied plots compared to S fertilizer control plots.

The annual N_2O emissions were significantly influenced by N fertilizer application in 2017, whereas no influenced was seen on 2018. The greater amount of precipitation in 2018 might have leached the nitrates, which could be the reason for non-significant impact of N fertilizer in 2018. Similar to CO_2 fluxes, daily N_2O fluxes varied at different observed days; soil temperature and moisture, fertilizer application and amount of precipitation influenced N_2O emissions. According to a review by Snyder et al. (2009), adaptation of best management practices including fertilization, on-site and weather-specific management and appropriate cropping system can minimize the N_2O emissions. N fertilization in soils increased the emissions of N_2O; however, N fertilization with no-tillage and the appropriate legume based crop rotation helps to mitigate the N_2O emissions from agricultural soils (Sainju et al., 2012). Many authors agree on stimulatory response of N_2O emissions to N fertilizer (Dusenbury et al., 2008; Mosier et al., 2006).

Data from the current study did not show any influence of N and S fertilizers on CH_4 emissions. Similar results were shown by Ozlu and Kumar (2018). The application of inorganic fertilizers to the soil might not have increased methane producing microorganisms in soil; these microbes are mostly found in anaerobic conditions. However, the fluctuations in CH_4 fluxes might be due to differentiation of moisture and temperature. In addition, Sejian et al. (2015) reported anaerobic decomposition of manure is the primary factor affecting CH_4 emissions.

CONCLUSIONS

The study demonstrated the impact of different rates of N and S fertilizers applied to carinata, on soil parameters and greenhouse gas emissions. The experiment was conducted in Brookings, South Dakota for 2017 and 2018. The 2018 year was a comparatively wet year with heavy precipitation during the crop-growing season. Data from this study showed mixed impacts of N and S fertilizers on soil parameters. In 2017, N fertilizer rates significantly increased soil EC, SOC, stable carbon, labile N, and soil P, whereas, S fertilizer rates decreased soil pH, EC, while increased soil S concentration. In 2018, N fertilizer rates increased the soil P and K when the rate was 112 kg N ha^{-1} but these parameters decreased with 140 kg N ha^{-1}. Application of S fertilizer increased soil EC and soil S concentration but decreased SOC, labile N and extractable N. Soil properties were impacted by depth for either years, except for soil S concentration in 2018.

The application of N fertilizer at higher rates significantly increased CO_2 and N_2O emissions only in 2017. Although there was no significant influence of S fertilizer application on GHG emissions, yet, a declining trend in GHG fluxes with the increased S fertilizer rates was observed in either years. In general, date of fertilizer application, soil temperature, and soil moisture influenced the GHG emissions. Further, treatments did not influence CH_4 emissions; however, soil temperature and soil moisture were responsible for the observed fluctuation.

From this study, we can conclude that application of N fertilizer helps to improve soils; however, differences were not always significant. Higher N fertilizer rates however, increased the soil surface CO_2 and N_2O fluxes. In contrary to the N fertilizer, the application of S fertilizer decreased the SOC resulting in decreasing trend of GHG fluxes. Further studies focusing on assessing the long-term impacts of N and S fertilization rates applied to carinata on soils and GHG emissions under different crop rotations, soils and environmental condition can be beneficial to explore the sustainable strategies for carinata production.

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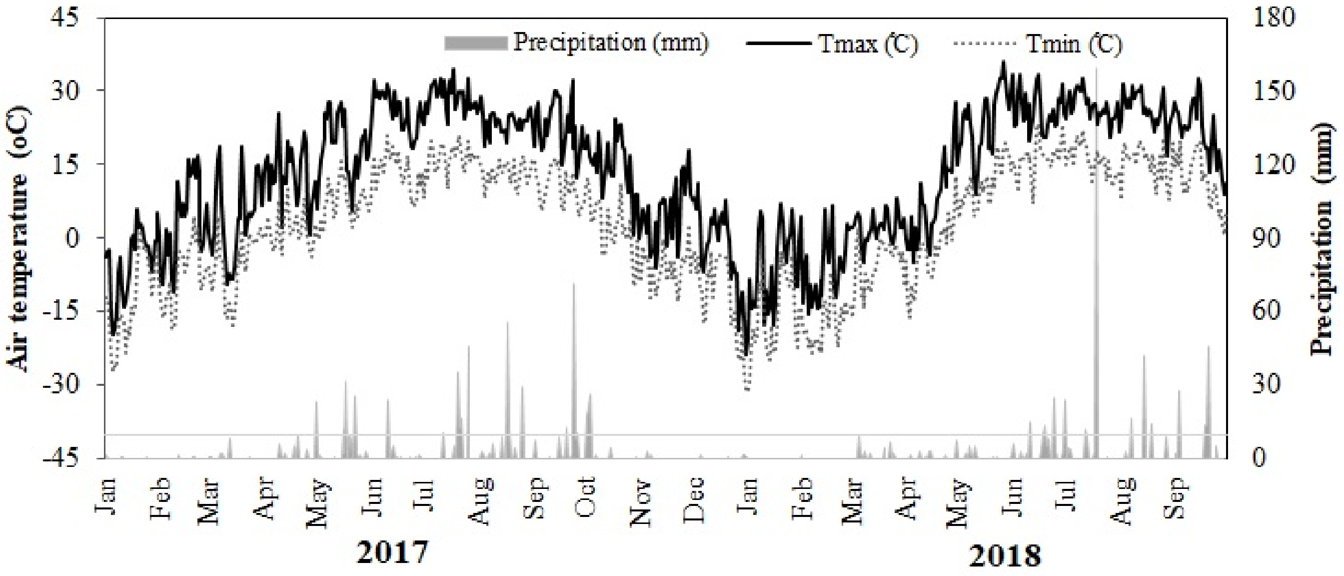


Figure 2-1: Daily maximum and minimum air temperature and precipitation in 2017 and 2018 for Brookings, SD.

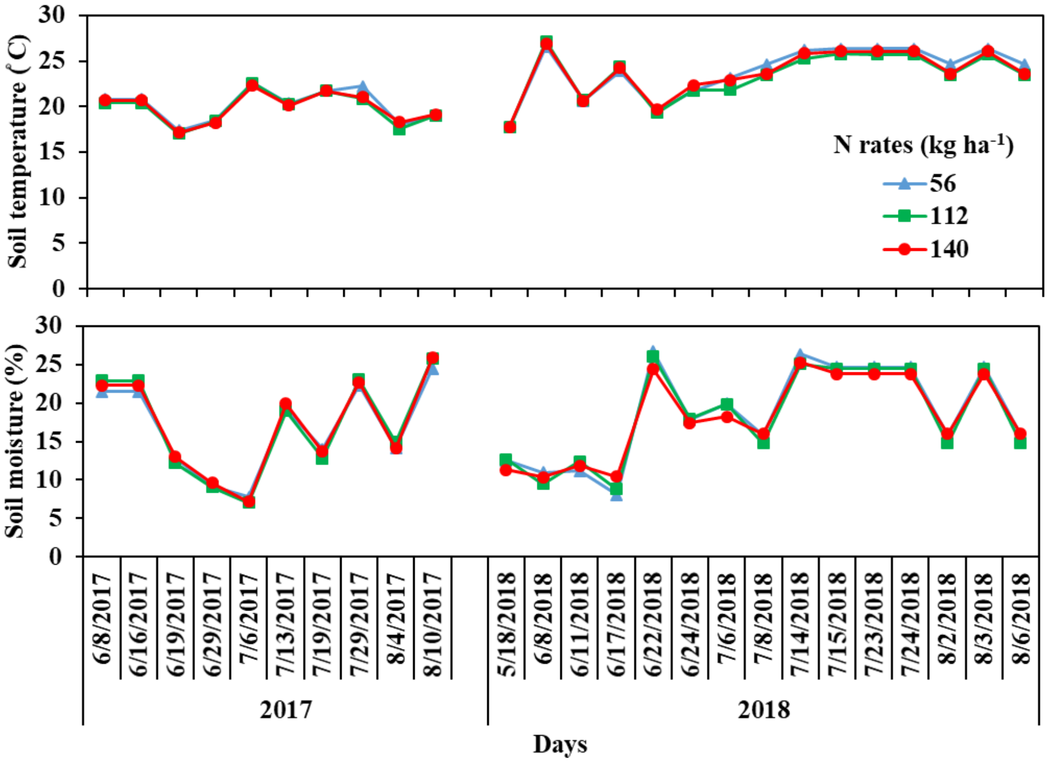


Figure 2-2a: Average soil temperature and moisture of plots applied with different N fertilizer rates over the observed days in 2017 and 2018.

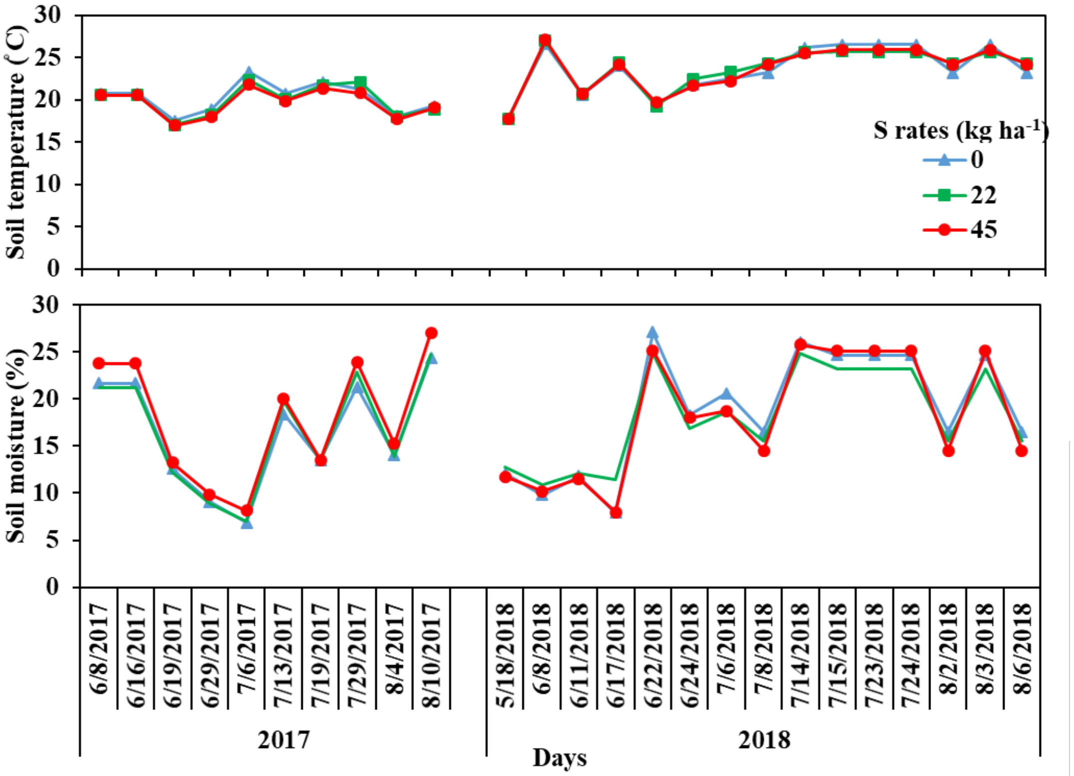


Figure 2-2b: Average soil temperature and soil moisture of plots applied with different S fertilizer rates over the observed days in 2017 and 2018.

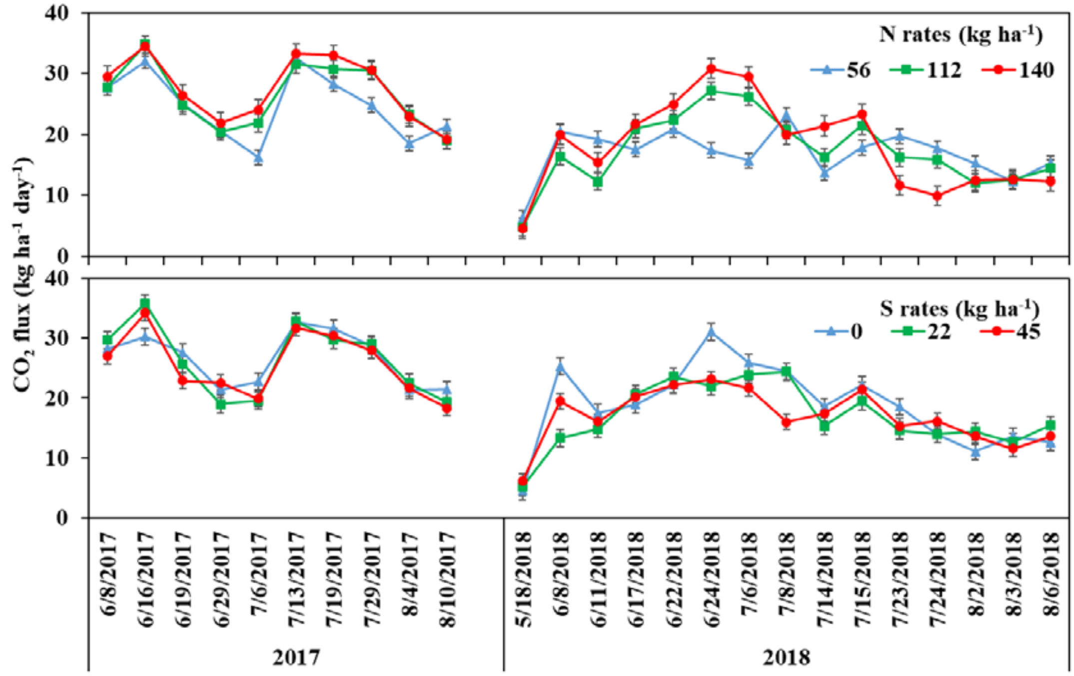


Figure 2-3. Trends of daily mean soil CO$_2$ fluxes from carinata fields under N and S fertilizers rates over the observed days in 2017 and 2018.

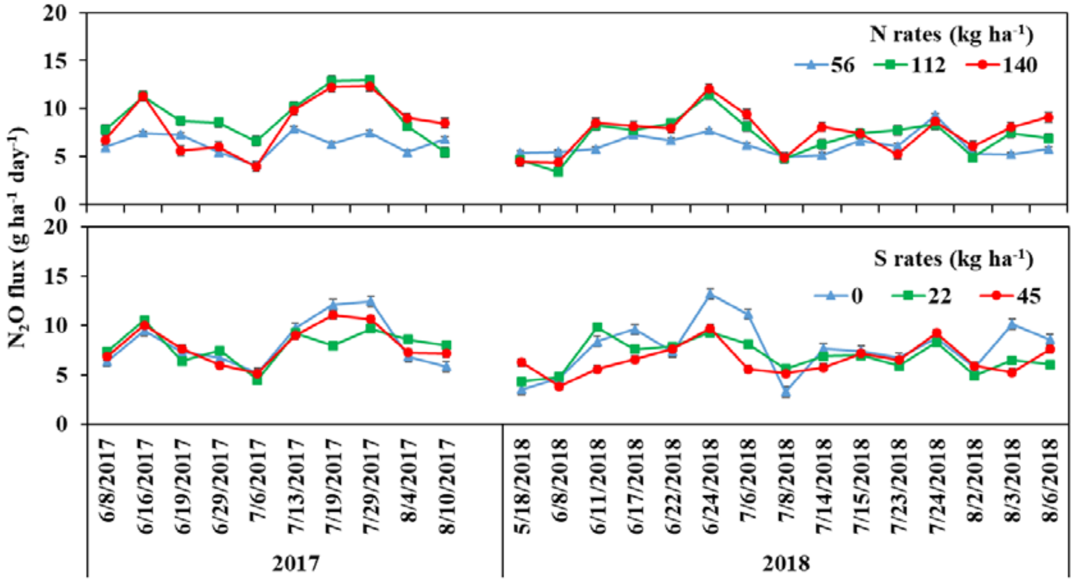


Figure 2-4. Trends of daily mean soil N$_2$O fluxes from carinata fields under N and S fertilizers rates over the observed days in 2017 and 2018.

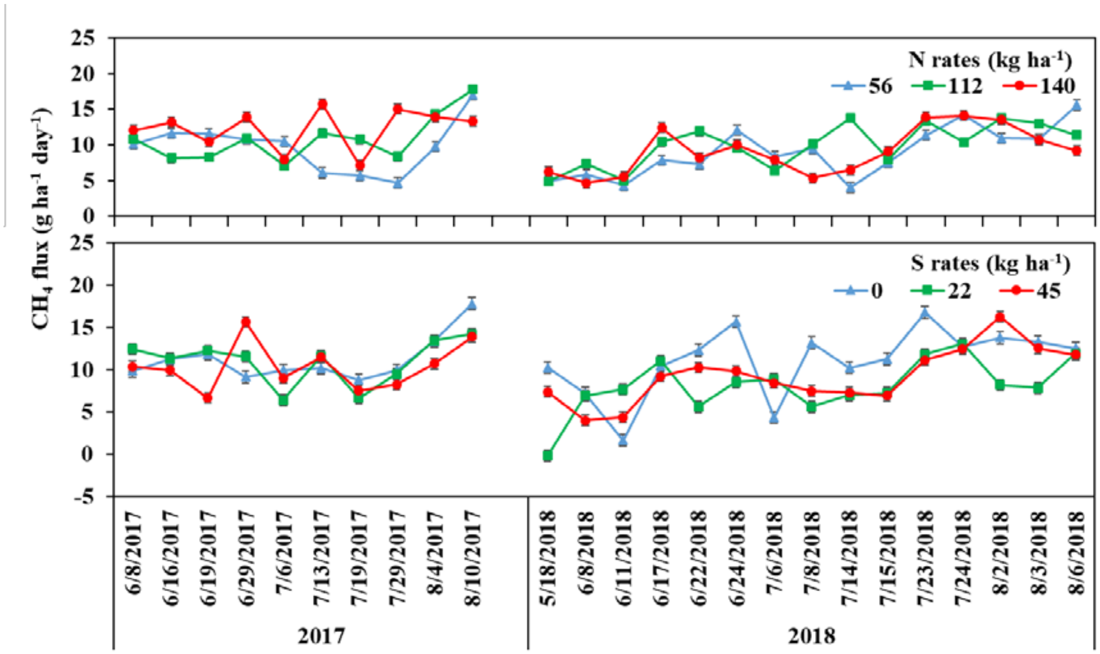


Figure 2-5. Trends of daily mean soil CH_4 fluxes from carinata fields under N and S fertilizers rates over the observed days in 2017 and 2018.

Table 2-1. Soil physical and chemical characteristics (0-15 cm depth) before planting for both years at Brookings.

Year	**Previous Crop**	**Texture Class**	**pH**	**Soluble Salts (mmho cm^{-1})**	**Organic Matter (g kg^{-1})**	**N- NO$_3$ (kg ha^{-1})**	**Olsen-P (mg kg^{-1})**	**K (mg kg^{-1})**	**S (kg ha^{-1})**
2017	WW	Medium	5.6	0.1	47	3.4	10.0	141.0	9.0
2018	WW	Medium	5.7	0.2	53	26.5	21.0	220.0	29.0

WW = Winter wheat

Table 2-2a: Means of soil bulk density (BD), pH, electrical conductivity (EC), organic carbon (SOC), total nitrogen (TN), labile C and N, stable C and N, soil N, P, K, and S as influenced by different N and S fertilizers treatments for the 0-45 cm depth in 2017.

Treatments	**BD**	**pH**	**EC**	**SOC**	**TN**	**Labile C**	**Stable C**	**Labile N**	**Stable N**	**N**	**P**	**K**	**S**
	g cm^{-3}		µS cm^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
N rates (kg ha^{-1})													
56	1.40	5.4	$322.4^{b\dagger}$	24.5^{b}	2.70	288.6	936.3^{b}	50.0^{b}	30.5	68.0	12.8^{b}	229.3	9.7
84	1.40	5.4	319.2^{b}	25.1^{ab}	2.70	307.9	992.5^{ab}	54.4^{b}	33.1	63.8	12.9^{b}	222.5	10.4
112	1.43	5.4	368.6^{a}	26.0^{a}	2.70	311.1	1071.9^{a}	90.0^{a}	35.8	78.7	$18.^{6a}$	240.3	10.4
140	1.39	5.3	370.0^{a}	26.0^{a}	2.90	297.1	1020.4^{a}	67.5^{ab}	34.1	80.8	15.6^{ab}	235.0	9.9
S rates (kg ha^{-1})													
0	1.39	5.3^{b}	369.2^{a}	25.2	2.70	286.1	1016.2	63.1	33.4	77.3	15.9	224.7	8.7^{b}
22	1.41	5.5^{a}	329.9^{b}	25.7	2.70	306.7	967.6	68.5	32.6	67.0	13.9	228.8	9.6^{b}
45	1.42	5.4^{b}	336.1^{b}	25.3	2.80	308.2	1028.2	62.2	33.9	73.2	14.8	240.0	12.0^{a}
							Analysis of Variance (P>F)						
N rates (N)	0.306	0.137	0.009	0.041	0.058	0.573	0.012	0.013	0.114	0.061	0.004	0.629	0.734
S rates (S)	0.363	0.016	0.007	0.634	0.241	0.535	0.103	0.991	0.487	0.117	0.396	0.568	<.001
N x S	0.766	0.687	0.624	0.005	0.107	0.399	0.006	0.346	0.036	0.489	0.939	0.607	0.753
Depth (D)	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
N x D	0.822	0.418	0.891	0.795	0.967	0.319	0.271	0.355	0.551	0.665	0.736	0.781	0.709
S x D	0.854	0.887	0.540	0.751	0.578	0.404	0.157	0.095	0.335	0.746	0.482	0.654	0.260
N x S x D	0.957	0.678	0.535	0.961	0.587	0.351	0.423	0.893	0.717	0.143	0.976	0.322	0.702

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at P<0.05. No letters are shown where there are no significant differences.

Table 2-2b: Means of soil bulk density (BD), pH, electrical conductivity (EC), soil organic carbon (SOC), total nitrogen (TN), labile C and N, stable C and N, soil N, P, K, and S as influenced by different N and S fertilizers treatments for the 0-45 cm depth in 2018.

Treatments	**BD**	**pH**	**EC**	**SOC**	**TN**	**Labile C**	**Stable C**	**Labile N**	**Stable N**	**N**	**P**	**K**	**S**
	g cm^{-3}		µS cm^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
N rates (kg ha^{-1})													
56	1.44	5.6	123.2	28.6	3.10	105.5	420.7	21.9	43.2	8.6	21.6^{ab†}	250.4^{a}	22.6
84	1.45	5.7	120.3	29.0	3.10	110.8	436.2	23.8	46.6	8.7	24.5^{a}	230.0^{a}	20.9
112	1.46	5.6	126.5	28.3	3.00	106.5	418.8	23.3	43.7	8.4	21.1^{ab}	214.9^{ab}	22.9
140	1.47	5.7	125.9	28.4	3.10	100.8	394.6	23.9	41.9	9.2	18.5^{b}	174.7^{b}	20.8
S rates (kg ha^{-1})													
0	1.46	5.6	116.0^{b}	29.0^{a}	3.10	108.6	424.4	24.2^{a}	45.4	9.5^{a}	22.7	232.8^{a}	20.6^{b}
22	1.45	5.7	129.7^{a}	28.1^{b}	3.10	108.9	418.6	25.5^{a}	44.6	9.2^{ab}	20.5	190.0^{b}	21.1^{ab}
45	1.46	5.6	126.6^{a}	28.5^{b}	3.00	100.7	411.6	20.1^{b}	41.9	7.6^{b}	21.6	232.4^{a}	23.3^{a}
Analysis of Variance (P>F)													
N rates (N)	0.804	0.401	0.656	0.266	0.478	0.167	0.180	0.427	0.173	0.845	0.008	0.012	0.216
S rates (S)	0.908	0.281	0.027	0.011	0.254	0.091	0.531	0.001	0.106	0.050	0.161	0.026	0.042
N x S	0.500	0.451	0.183	0.516	0.353	0.329	0.469	0.440	0.477	0.129	0.122	0.079	0.361
Depth (D)	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.149
N x D	0.935	0.295	0.892	0.896	0.722	0.609	0.950	0.972	0.918	0.894	0.881	0.748	0.960
S x D	0.468	0.378	0.780	0.884	0.765	0.956	0.835	0.863	0.790	0.330	0.967	0.781	0.978
N x S x D	0.392	0.942	0.740	0.375	0.451	0.100	0.230	0.786	0.304	0.957	0.783	0.997	0.984

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.

Table 2-3: Means CO_2, N_2O and CH_4 fluxes as influenced by different N and S fertilizers treatments in 2017 and 2018.

	2017			2018		
	CO_2	**N_2O**	**CH_4**	**CO_2**	**N_2O**	**CH_4**
	kg ha^{-1} day^{-1}	g ha^{-1} day^{-1}	g ha^{-1} day^{-1}	kg ha^{-1} day^{-1}	g ha^{-1} day^{-1}	g ha^{-1} day^{-1}
N rates (kg ha^{-1})						
56	24.8 ^{b†}	6.5 ^{b}	9.6	16.8	6.3	8.9
112	26.4 ^{ab}	9.4 ^{a}	10.4	17.3	7.1	9.9
140	27.2 ^{a}	8.5 ^{a}	12.5	18.1	7.6	9.1
S rates (kg ha^{-1})						
0	26.4	8.3	11.3	18.7	7.9	10.9
22	26.3	8.0	11.0	16.9	6.8	8.1
45	25.7	8.0	10.1	16.9	6.5	9.3
			Analysis of variance (P>F)			
N rates (N)	0.015	<0.001	0.118	0.954	0.109	0.623
S rates (S)	0.487	0.893	0.803	0.708	0.054	0.060
NxS	0.376	0.958	0.830	0.723	0.005	0.788
Date (D)	<.001	<.001	0.005	<.001	<.001	<0.001
NxD	0.887	0.112	0.075	0.093	0.830	0.961
SxD	0.975	0.740	0.578	0.448	0.328	0.564
NxSxD	0.935	0.562	0.425	0.421	0.448	0.929

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at P<0.05. No letters are shown where there are no significant differences.

CONCLUSIONS AND RECOMMENDATIONS

Carinata is a non-food oilseed crop with a great potential to fit in the NGP cropping systems because of its low input requirements. The present study had three objectives (i) to determine the impact of N and S fertilizers on growth and yield of carinata (ii) to determine the influence of N and S fertilizers, applied to carinata plots, on selected soil parameters, and (iii) to evaluate the GHG emissions in response to N and S fertilizers applied to carinata plots.

Increasing N fertilizer rate increased plant height, number of primary and secondary branches, number of pods plant^{-1} but significatly decreased seed oil concentration. Sulfur fertilizer application increased plant height, number of primary and secondary branches, seed yield and seed oil concentration. Application of N fertilizer in S deficient plots produced poor seed yield (600-800 kg ha^{-1}) compared to N fertilizer applied to S applied plots (~1300 kg ha^{-1}). The economic optimum N rate for carinata was 85 kg N ha^{-1} whereas the economic optimum S rate was 36 kg S ha^{-1}. It is important to note that these values can change with the changes in the cost of fertilizers and value of carinata seeds.

Nitrogen and S fertilizers showed minimal impact on soil parameters; increase in soil EC, SOC, stable carbon, labile N, soil K, and soil P with higher N fertilizer rates and decrease in pH and SOC with increased S fertilizer rates. The greenhouse gas emission study showed an increase in CO_2 and N_2O fluxes with increase in N fertilizer application. Sulfur fertilizer application showed a numerical decline in emissions of CO_2 and N_2O. Overall, application of fertilizer and increase in soil temperature and mositure increased the emission levels of

CO_2 and N_2O. There was no specific trend for CH_4 emission in response to N or S fertilizer application.

This study confirms that carinata has low N fertilizer requirements compared to other traditional crops grown in SD. This result contributed information for developing best management practices for carinata. The soil parameter results could not provide any conclusive results, a longer term study is needed to fully understand the impacts of growing carinta on soil quality traits. Results from GHG emissions study showed significant increase in CO_2 and N_2O with the application of N fertilizer and numerical decrease in the same gases with S fertilizer application. A long-term study is required to confirm these results and the determine how fertilizer application interacts with temperature and soil moisture to influence GHG emmissions.

APPENDIX

Table A1-1. Mean comparison of plant stand, plant height, number of primary and secondary branches, number of pods plant^{-1}, pod length, number of seeds per pod, lodging severity, seed yield (kg ha^{-1}), oil concentration (g kg^{-1}) and oil yield (kg ha^{-1}) of carinata at Brookings between 2017 and 2018.

	Plant Stand	**Plant Height**	**Number of primary branches**	**Number of secondary branches**	**Number of pods per plant**	**Pod Length**	**Number of seeds per pod**	**Lodging**	**Seed yield**	**Oil concentration**	**Oil yield**
	%	cm				cm		1-9 scale	kg ha^{-1}	g kg^{-1}	kg ha^{-1}
Years											
2017	78.0	78.0^{b†}	6.0^{a}	12.8	57.0^{b}	5.5^{a}	13.0	3.4^{b}	1232.0^{a}	350.0	433.0^{a}
2018	73.0	98.0^{a}	5.0^{b}	14.3	95.0^{a}	5.0^{b}	13.0	5.3^{a}	975.0^{b}	353.0	342.0^{b}
					Analysis of variance (P>F)						
Year	0.061	<0.001	<0.001	0.149	<0.001	0.039	0.901	<0.001	0.006	0.618	0.009

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at P<0.05. No letters are shown where there are no significant differences.

Table A1-2. Mean comparison of plant stand (%), plant height (cm), number of primary and secondary branches, number of pods per plant, pod length (cm), number of seeds per pod, lodging severity (1-9 scale), seed yield (kg ha^{-1}), oil concentration (g kg^{-1}) and oil yield (kg ha^{-1}) of carinata at different N and S fertilizers level at Brookings in 2017.

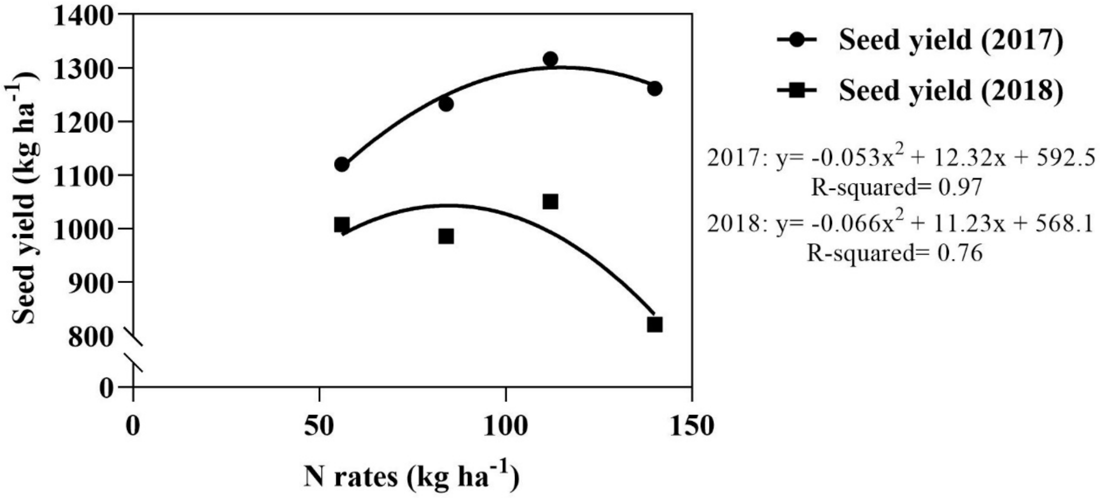
	Plant Height	**Number of primary branches**	**Number of pods per plant**	**Pod Length**	**Lodging**	**Seed yield**	**Oil yield**
	cm			cm	1-9 scale	kg ha^{-1}	kg ha^{-1}
N rates (kg ha^{-1})							
56	76.0	5.8^{c†}	48^{b}	5.3	2.4^{b}	1120.0	398.0
84	75.0	6.3^{b}	51.0^{b}	5.3	3.2^{a}	1233.0	445.0
112	80.0	6.8^{a}	70.0^{a}	5.7	4.1^{a}	1317.0	464.0
140	79.0	6.7^{ab}	60.0^{ab}	5.4	4.1^{a}	1262.0	424.0
S rates (kg ha^{-1})							
0	72.0^{b}	5.9^{b}	45.0^{b}	5.0^{b}	1.9^{b}	611.0^{b}	208.0^{b}
22	80.0^{a}	6.8^{a}	67.0^{a}	5.5^{a}	4.3^{a}	1492.0^{a}	537.0^{a}
45	80.0^{a}	6.6^{a}	60.0^{a}	5.7^{a}	4.3^{a}	1617.0^{a}	569.0^{a}
			Analysis of variance (P>F)				
N	0.362	0.001	0.013	0.320	0.001	0.185	0.222
S	0.010	<0.001	0.001	0.022	<0.001	<0.001	<0.001
Nx S	0.685	0.042	0.066	0.502	0.070	0.476	0.470

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at P<0.05. No letters are shown where there are no significant differences.

Table A1-3. Mean comparison of plant stand (%), plant height (cm), number of primary and secondary branches, number of pods per plant, pod length (cm), number of seeds per pod, lodging severity (1-9 scale), seed yield (kg ha^{-1}), oil concentration (g kg^{-1}) and oil yield (kg ha^{-1}) of carinata at different N and S fertilizers level at Brookings in 2018.

	Plant Height	**Number of primary branches**	**Number of pods per plant**	**Pod Length**	**Lodging**	**Seed yield**	**Oil yield**
	cm			cm	1-9 scale	kg ha^{-1}	kg ha^{-1}
N rates (kg ha^{-1})							
56	93.0^{b}[†]	5.0	85.0	5.2	3.7^{b}	1008.0	356.0^{a}
84	97.0^{ab}	5.0	88.0	5.0	5.5^{a}	986.0	366.0^{a}
112	100.0^{ab}	5.0	107.0	5.2	6.2^{a}	1051.0	380.0^{a}
140	101.0^{a}	5.0	104.0	5.3	6.2^{a}	821.0	252.0^{b}
				S rates (kg ha^{-1})			
0	93.0^{b}	4.0^{b}	88.0	5.0^{b}	4.3^{b}	757.0^{b}	260.0^{b}
22	101.0^{a}	5.0^{a}	105.0	5.3^{a}	5.9^{a}	1031.0^{a}	359.0^{a}
45	99.0^{a}	5.0^{a}	88.0	5.4^{a}	5.8^{a}	1150.0^{a}	407.0^{a}
				Analysis of variance (P>F)			
N	0.020	0.214	0.117	0.243	<0.001	0.206	0.025
S	0.004	0.009	0.115	0.001	0.006	0.001	0.002
Nx S	0.392	0.339	0.447	0.440	0.227	0.165	0.060

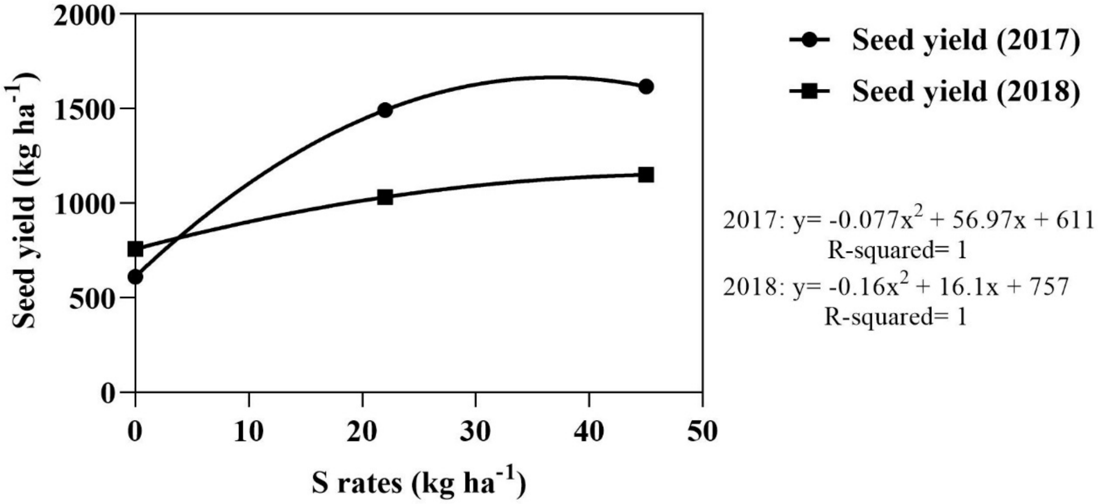
[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.



2017: $y= -0.053x^2 + 12.32x + 592.5$
R-squared= 0.97

2018: $y= -0.066x^2 + 11.23x + 568.1$
R-squared= 0.76

Figure A1-1. Seed yield response to N fertilizer rate for *B. carinata* grown at Brookings in 2017 and 2018.



2017: $y= -0.077x^2 + 56.97x + 611$
R-squared= 1

2018: $y= -0.16x^2 + 16.1x + 757$
R-squared= 1

Figure A1-2. Seed yield response to S fertilizer rate for *B. carinata* grown at Brookings in 2017 and 2018.

Table A2-1a. Means of soil bulk density (BD), pH, electrical conductivity (EC), soil organic carbon (SOC) and total nitrogen (TN) as influenced by different N and S fertilizer treatments for the 0-5 cm depth in 2017 and 2018.

Treatments	**2017**					**2018**				
	BD	**pH**	**EC**	**SOC**	**TN**	**BD**	**pH**	**EC**	**SOC**	**TN**
	g cm^{-3}		µS cm^{-1}	g kg^{-1}	g kg^{-1}	g cm^{-3}		µS cm^{-1}	g kg^{-1}	g kg^{-1}
	0-5 cm									
N rates (kg ha^{-1})										
56	1.25	4.7	508.5	30.3	3.40	1.32	5.3^{ab}[†]	157.4	32.3	3.45
84	1.21	4.6	535.7	31.2	3.38	1.31	5.4^{a}	148.9	32.0	3.39
112	1.26	4.5	625.7	33.4	3.44	1.30	5.2^{b}	155.5	31.6	3.41
140	1.22	4.5	586.9	32.4	3.52	1.33	5.2^{b}	154.8	31.6	3.39
S rates (kg ha^{-1})										
0	1.24	4.6	573.8	32.1	3.41	1.30	5.3	147.4	32.3	3.42
22	1.23	4.6	580.3	32.3	3.44	1.33	5.3	156.6	31.3	3.39
45	1.24	4.6	538.5	31.2	3.46	1.33	5.3	158.5	31.2	3.42
	Analysis of variance (P>F)									
N rates	0.595	0.121	0.468	0.172	0.305	0.899	0.024	0.794	0.699	0.805
S rates	0.937	0.965	0.807	0.659	0.771	0.518	0.954	0.322	0.215	0.887
N x S	0.861	0.850	0.840	0.672	0.499	0.467	0.336	0.975	0.570	0.6312

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at P<0.05. No letters are shown where there are no significant differences.

Table A2-1b. Means of soil bulk density (BD), pH, electrical conductivity (EC), soil organic carbon (SOC) and total nitrogen (TN) as influenced by different N and S fertilizer treatments for the 5-15 cm depth in 2017 and 2018.

Treatments	2017					2018				
	BD	**pH**	**EC**	**SOC**	**TN**	**BD**	**pH**	**EC**	**SOC**	**TN**
	g cm^{-3}		μS cm^{-1}	g kg^{-1}	g kg^{-1}	g cm^{-3}		μS cm^{-1}	g kg^{-1}	g kg^{-1}
	5-15 cm									
N rates (kg ha^{-1})										
56	1.47	4.9	332.1	29.3	3.09	1.45	5.5	124.4	31.3	3.31
84	1.43	5.0	321.9	28.8	3.08	1.47	5.5	115.6	31.3	3.31
112	1.49	4.8	375.8	29.7	3.16	1.47	5.4	118.7	30.7	3.25
140	1.43	4.8	357.2	23.0	3.19	1.49	5.4	116.7	30.6	3.31
S rates (kg ha^{-1})										
0	1.43	4.8	377.5^{a}[†]	29.4	3.18	1.51	5.5	110.0	31.6	3.36
22	1.48	4.9	306.9^{b}	29.7	3.07	1.45	5.4	123.2	30.4	3.27
45	1.46	4.8	355.9^{ab}	29.0	3.14	1.44	5.4	123.3	30.9	3.26
	Analysis of variance ($P>F$)									
N rates	0.530	0.111	0.343	0.651	0.501	0.827	0.760	0.789	0.517	0.655
S rates	0.570	0.152	0.031	0.825	0.412	0.299	0.428	0.152	0.095	0.134
N x S	0.750	0.809	0.859	0.193	0.844	0.869	0.881	0.114	0.658	0.651

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.

Table A2-1c. Means of soil bulk density (BD), pH, electrical conductivity (EC), soil organic carbon (SOC) and total nitrogen (TN) as influenced by different N and S fertilizer treatments for the 15-30 cm depth in 2017 and 2018.

Treatments	2017					2018				
	BD	**pH**	**EC**	**SOC**	**TN**	**BD**	**pH**	**EC**	**SOC**	**TN**
	g cm^{-3}		µS cm^{-1}	g kg^{-1}	g kg^{-1}	g cm^{-3}		µS cm^{-1}	g kg^{-1}	g kg^{-1}
	15-30 cm									
N rates (kg ha^{-1})										
56	1.41	5.8	238.6	23.0	2.47	1.52	5.6	103.7	28.6	3.01
84	1.48	5.8	215.5	23.8	2.55	1.51	5.7	107.9	29.0	3.04
112	1.49	5.7	255.1	23.7	2.51	1.53	5.7	120.4	28.6	3.04
140	1.45	5.8	279.2	24.8	2.68	1.50	5.7	124.2	28.8	3.09
S rates (kg ha^{-1})										
0	1.44	5.7	267.8	29.0	2.48	1.49	5.6	102.2	29.2	3.06
22	1.44	5.9	233.4	23.6	2.56	1.51	5.8	118.4	28.3	3.03
45	1.49	5.7	240.1	23.8	2.62	1.55	5.7	121.5	28.7	3.05
	Analysis of variance (P>F)									
N rates	0.307	0.951	0.124	0.452	0.451	0.848	0.859	0.553	0.912	0.604
S rates	0.443	0.172	0.289	0.867	0.475	0.423	0.079	0.301	0.280	0.824
N x S	0.891	0.284	0.181	0.242	0.374	0.954	0.408	0.418	0.104	0.107

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.

Table A2-1d. Means of soil bulk density (BD), pH, electrical conductivity (EC), soil organic carbon (SOC) and total nitrogen (TN) as influenced by different N and S fertilizer treatments for the 30-45 cm depth in 2017 and 2018.

Treatments	2017					2018				
	BD	**pH**	**EC**	**SOC**	**TN**	**BD**	**pH**	**EC**	**SOC**	**TN**
	g cm^{-3}		µS cm^{-1}	g kg^{-1}	g kg^{-1}	g cm^{-3}		µS cm^{-1}	g kg^{-1}	g kg^{-1}
	30-45 cm									
N rates (kg ha^{-1})										
56	1.46	6.4	210.3	15.9	1.87	1.49	6.1	105.4	22.2	2.46
84	1.45	6.3	203.6	16.6	1.99	1.54	6.2	108.9	23.5	2.56
112	1.47	6.4	217.6	16.6	1.99	1.52	6.1	107.3	22.3	2.45
140	1.49	6.3	256.8	17.6	2.03	1.53	6.3	107.8	22.8	2.56
S rates (kg ha^{-1})										
0	1.47	6.3	257.5[a†]	15.8	1.87	1.53	6.1	104.4	23.2	2.53
22	1.48	6.4	198.8[b]	16.9	2.01	1.50	6.3	117.3	22.6	2.54
45	1.46	6.3	209.9[b]	17.4	2.03	1.52	6.2	103.0	22.3	2.46
	Analysis of variance (P>F)									
N rates	0.918	0.351	0.110	0.565	0.531	0.580	0.300	0.912	0.389	0.302
S rates	0.284	0.298	0.015	0.323	0.224	0.771	0.533	0.272	0.447	0.461
N x S	0.328	0.780	0.364	0.228	0.132	0.103	0.887	0.837	0.560	0.469

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at P<0.05. No letters are shown where there are no significant differences.

Table A2-2a. Means of soil labile carbon (C), stable carbon (C), labile nitrogen (N) and stable nitrogen (N) as influenced by different N and S fertilizer treatments for the 0-5 cm depth in 2017 and 2018.

Treatments	**2017**				**2018**			
	Labile C	**Stable C**	**Labile N**	**Stable N**	**Labile C**	**Stable C**	**Labile N**	**Stable N**
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
	0-5 cm							
N rates (kg ha^{-1})								
56	328.4	1048.2^{b†}	65.3	34.8	117.6	492.9	24.6	51.5
84	356.1	1130.8^{ab}	75.5	37.2	127.0	512.9	26.1	55.4
112	372.5	1269.7^{a}	136.5	43.2	119.0	490.1	25.9	52.4
140	324.4	1127.3^{ab}	98.2	39.1	110.2	453.7	26.7	48.8
S rates (kg ha^{-1})								
0	327.5	1132.7	83.4	37.9	122.2	497.9	26.5^{a}	54.0
22	364.3	1142.1	111.3	39.2	121.0	486.2	28.9^{a}	52.6
45	341.5	1146.2	84.2	38.19	113.1	480.7	22.2^{b}	49.9
	Analysis of variance (P>F)							
N rates	0.495	0.029	0.052	0.112	0.204	0.377	0.829	0.411
S rates	0.657	0.984	0.504	0.933	0.378	0.596	0.035	0.375
N x S	0.403	0.066	0.649	0.088	0.269	0.173	0.984	0.175

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.

Table A2-2b. Means of soil labile carbon (C), stable carbon (C), labile nitrogen (N) and stable nitrogen (N) as influenced by different N and S fertilizer treatments for the 5-15 cm depth in 2017 and 2018.

Treatments	2017				2018			
	Labile C	**Stable C**	**Labile N**	**Stable N**	**Labile C**	**Stable C**	**Labile N**	**Stable N**
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
	5-15 cm							
N rates (kg ha^{-1})								
56	248.8	824.4	34.6	26.2	93.3	348.4	19.2	34.8
84	265.1	868.0	37.5	29.4	94.5	359.4	21.2	37.8
112	249.7	874.0	43.2	28.3	91.9	347.5	20.8	35.1
140	272.8	925.4	40.2	29.7	91.4	335.5	21.0	35.0
S rates (kg ha^{-1})								
0	248.4	910.3^{a}[†]	44.6^{a}	29.3	95.1	350.9	21.7^{a}	36.8
22	254.0	806.5^{b}	32.3^{b}	26.5	95.7	350.9	22.0^{a}	36.6
45	274.8	910.1^{a}	40.2^{ab}	29.7	88.4	342.5	18.1^{b}	33.9
	Analysis of variance (P>F)							
N rates	0.297	0.161	0.326	0.538	0.807	0.635	0.586	0.366
S rates	0.117	0.018	0.042	0.193	0.144	0.788	0.021	0.197
N x S	0.546	0.063	0.224	0.438	0.134	0.786	0.149	0.939

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.

Table A2-3a. Means of inorganic nitrogen (N), phosphorus (P), potassium (K) and sulfur (S) after crop harvest as influenced by different N and S fertilizer treatments for the 0-5 cm depth in 2017 and 2018.

Treatments	2017				2018			
	N	**P**	**K**	**S**	**N**	**P**	**K**	**S**
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
	0-5 cm							
N rates (kg ha^{-1})								
56	90.4	17.1^{b†}	301.0	11.3	12.0	27.4	310^{a}	23.4
84	91.9	18.2^{b}	293.3	11.6	10.4	28.0	277.8^{a}	20.7
112	115.3	26.2^{a}	321.3	12.0	11.2	24.8	199.0^{b}	23.5
140	115.1	21.4^{ab}	320.3	11.2	11.6	22.0	205.9^{b}	21.5
S rates (kg ha^{-1})								
0	106.0	23.0	296.6	9.7^{b}	13.4^{a}	27.4	268.0	20.3
22	99.5	19.2	304.2	11.3^{b}	11.7^{a}	24.5	225.7	21.7
45	101.3	19.8	323.1	13.4^{a}	9.1^{b}	25.6	275.4	23.9
	Analysis of variance (P>F)							
N rates	0.331	0.015	0.639	0.875	0.518	0.099	0.014	0.333
S rates	0.885	0.201	0.586	<.0001	0.004	0.642	0.148	0.073
N x S	0.296	0.986	0.440	0.801	0.261	0.056	0.069	0.884

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.

Table A2-3b. Means of inorganic nitrogen (N), phosphorus (P), potassium (K) and sulfur (S) after crop harvest as influenced by different N and S fertilizer treatments for the 5-15 cm depth in 2017 and 2018.

Treatments	**2017**				**2018**			
	N	**P**	**K**	**S**	**N**	**P**	**K**	**S**
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
	5-15 cm							
N rates (kg ha^{-1})								
56	45.6	8.6	157.7	8.1	6.9	17.6	206.3	21.8
84	35.6	7.6	151.6	8.2	7.4	21.0	187.4	20.6
112	46.6	10.9	159.3	8.7	6.6	18.1	198.3	22.2
140	46.4	9.7	149.7	8.5	7.2	15.7	143.3	20.0
S rates (kg ha^{-1})								
0	48.6	8.8	152.7	7.6[b†]	6.9	19.2	203.8	20.0
22	37.0	8.6	153.3	7.9[b]	7.3	17.1	155.7	20.6
45	45.1	9.8	156.9	9.6[a]	6.8	18.3	191.6	22.6
	Analysis of variance (P>F)							
N rates	0.143	0.128	0.159	0.765	0.998	0.141	0.153	0.540
S rates	0.059	0.766	0.695	0.001	0.931	0.445	0.133	0.247
N x S	0.787	0.883	0.294	0.526	0.290	0.569	0.454	0.460

[†] Mean values followed by different lower letters between each treatment within the column represent significant differences due to the treatments at $P<0.05$. No letters are shown where there are no significant differences.