EFFECT OF ELEVATED ATMOSPHERIC CO₂ ON NITROGEN DISTRIBUTION AND N UTILIZATION EFFICIENCY IN WINTER RAPE (BRASSICA NAPUS L.)

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7Key word: Oilseed rape (Brassica napus); Elevated CO₂ concentrations; Nitrogen (N) distribution; N loss.

Abstract

We characterized the responses of plant dry biomass, nitrogen (N) distribution and N-utilization efficiency (NUE) to changes in CO₂ concentration through exposure and culture of winter rape under normal (380 μmol mol⁻¹) and elevated CO₂ (760 μmol mol⁻¹) conditions. Brassica napus (Xiangyou 15) was used as an agriculturally important model plant. Plants were cultivated in a greenhouse with sand culture under normal (15 mmol L⁻¹) and limited-N (5 mmol L⁻¹) conditions. NUE increased with elevated CO₂ regardless of whether N was limited. NUE was higher under N limitation than under normal N conditions for both normal- and elevated-CO₂ conditions. N labeling was used to assess the distribution of N from vegetative- to reproductive-organs. N distribution within the plant and during different developmental stages was affected by CO₂ concentration and the level of N application. A higher proportion of N was found in silages at the harvest stage for N-limited plants compared to normal-N plants. The proportion of N absorbed into silages after the stem elongation stage under elevated-CO₂ conditions was significantly higher than under normal CO₂. The proportion of N absorbed at the stem elongation stage from vegetative organs into silages under elevated CO₂ was significantly lower than under normal-CO₂ conditions. However, the proportion of N absorbed at the stem elongation stage and thus lost from the silique under elevated CO₂ was significantly higher than under normal CO₂. In conclusion, limited N or elevated CO₂ generally benefitted plant NUE. N uptake increased during or before stem elongation had the opposite effect.

Key word: Oilseed rape (Brassica napus); Elevated CO₂ concentrations; Nitrogen (N) distribution; N loss.

Introduction

The composition of the atmosphere has changed because of human activities, especially with respect to greenhouse gases, including CO₂, CH₄, and N₂O, which have increased over time (Myers et al., 2014; Bloom et al., 2014). The CO₂ concentration in the atmosphere was 265 μmol mol⁻¹ before the industrial revolution and reached approximately 314 μmol mol⁻¹ by 1958 and 353 μmol mol⁻¹ by 1990 (Loladze et al., 2002). Unfortunately, the atmospheric CO₂ recently reached 380 μmol mol⁻¹ (Wang et al., 2004). If such gases continue to increase at the same rate, atmospheric CO₂ is projected to double by 2050 (Loladze et al., 2002).

Crop growth and development are highly dependent on the interactions of physiological functions and the balance between C and N metabolisms (Reich et al., 2014). Photosynthetic enzymes are synthesized from N metabolism, and N absorption and assimilation require a large pool of C-skeletons for integration. In addition, large amounts of energy and reducing capacity are required during N assimilation (Scheible et al., 1997). The responses of plant growth to CO₂ concentrations can be affected by the C:N balance; plant stem growth can be accelerated by elevated CO₂ but will be inhibited under N-limited conditions (Sun et al., 2002). Although elevated atmospheric CO₂ may accelerate greenhouse effects with possible changes in climate, CO₂ is the raw material for photosynthesis and N assimilation. Thus, crop yields may be positively affected by elevated atmospheric CO₂. Bloom et al. (2014) systematically studied the effects of elevated CO₂ concentrations on crop yields and found that yields increased by 30% when atmospheric CO₂ concentrations doubled. Moreover, regardless of N application levels, seed yields of cotton increased by 56% and 54%, respectively, under elevated-CO₂ concentrations during moist growth conditions (Bloom et al., 2014).

Current studies suggest that CO₂ inhibits nitrate assimilation, as nitrates cannot be assimilated into proteins efficiently under elevated CO₂ (Bloom et al., 2014). Previous studies have shown that the N content in leaves decreases under elevated CO₂ concentrations (Curtis, 1996; Poorter et al., 1997; Cotrufo et al., 1998). The stomatal conductance of leaves decreases during elevated atmospheric CO₂ and leads to decreased N content in plant leaves; this effect is likely due to a decreased absorption of minerals (especially nitrate and potassium) in plant leaves (Morrison & Lawlor, 1999). Other reasons for the decreased N content in leaves may be that the discharge of carbon compounds from roots increases under high CO₂ concentrations (Franzaring et al., 2012) and that more N₂ in the rhizosphere was fixed by microorganism communities, which would result in an N supply that was limiting to plant metabolism (Soussana...
& Hartwig, 1995). Huluka et al. (1994) reported that N content in whole cotton plant tissues decreased under elevated atmospheric CO₂ concentrations. N absorption has also been shown to decrease with elevated CO₂ concentrations in plant tissues of spring wheat, which is more obvious during limited-N application conditions (Li & Kang, 2002).

N redistribution in plant tissues of crops occurs during plant growth stages, for instance, when N in older parts of roots is redistributed to root tips during later growth stages (Zhang et al., 2010). N located in old leaves is redistributed to new leaves, especially after the flowering stage, and most N can be redistributed from vegetative organs to reproductive organs (Martre et al., 2003; Reich et al., 2014). N supply in soils during late growth stages always limits plant growth and crop yield; thus, N redistribution is extremely important for NUE and seed development (Martre et al., 2003; Gallais et al., 2006; Dong et al., 2009). Gallais et al. (2006) showed that the N redistribution rate of oilseed rape averaged 65.1%. NUE has several definitions but is generally defined as an index of production per unit of N taken up (Hirel et al., 1993), which contains 45.0% clay, 46.3% silt and 8.7% sand. N treatments (normal and limited N) were only conducted after transplanting. The average temperature was 25.1°C/10.3°C day/night (controlled by two air conditioners in the greenhouse), the average relative humidity was 75% (controlled by a humidifier in greenhouse), and the average irradiance was 39,000 lux.

Seeds were sown on 28 September 2012 and transplanted on 27 October 2012. One plant was cultured per pot in sand culture (growth matrices were cleared with diluted hydrochloric acid) with complete Hoagland solution used as growth medium. The pot diameter was 20 cm, and the height was 25 cm. The greenhouse length was 15 m and a width of 5 m and a height of 2 m. One greenhouse was used for the normal CO₂ treatments with normal and limited N, and the other greenhouse was used for the elevated CO₂ treatments with normal and limited N. The temperature and humidity of both greenhouses were controlled, and a completely randomized block arrangement was employed for all pots in each greenhouse.

The nutrient solution was composed of 5 mM KNO₃, 1 mM KH₂PO₄, 7 mM MgSO₄, 5 mM Ca(NO₃)₂·4H₂O, 3 mM Fe·EDTA, 46.25 μM B, 6.722 μM Mn, 0.765 μM Zn, 0.316 μM Cu and 0.5 μM Mo. The concentrations of other nutrients in normal- and limited-N treatments were identical, but the concentrations of N [KNO₃ and Ca(NO₃)₂·4H₂O] under the limited-N condition was one-third that of the standard Hoagland solution (Zhang et al., 2012). Nutrient solution was poured onto each plant as follows: 80 ml nutrient solution every day at the seeding stage (15 November 2012–6 February 2013), 150 ml at the stem elongation stage (7 February 2013–2 April 2013), and 100 ml at the harvest stage (3 April 2013–27 April 2013). Nutrient supplementation was ceased on 28 April 2013.

To estimate the distribution of N from vegetative and reproductive organs, ¹⁵N isotope Ca (¹⁵NO₃)₂·4H₂O and K¹⁵NO₃ (Shanghai Chemical Engineering Corporation Research Institute, Shanghai, China; ¹⁵N excess = 20.28%) was used as a labeled N source to follow N distribution within the plant. The culture of ¹⁵N labeled plants was the same as with normal N plants, but these 80 pots were provided 150 ml nutrient solution (containing the ¹⁵N isotope) during the stem elongation stage for 12 days total (7–18 February 2013), which represented the stem elongation stage and without leaching. Forty samples were taken 3 days after the labeling treatment (21 February 2013). The other 40 plants were transplanted into sand culture with no ¹⁵N nutrient and sampled at the harvest stage (6 May 2013) to distinguish between N distribution and absorption. Unlabeled plants (80 total) were sampled at the same growth stages as the ¹⁵N-labeled plants.

Winter oilseed rape (XiangYou15, requiring vernalization), which is commonly cultivated in Hunan Province in southern China, was provided by the Hunan Sub-center of the Improvement Center of the National Oil Crop in China. Experiments were conducted within the Resources and Environment Department, Hunan Agricultural University (N 28°11'00", E 113°04'05''). For the cultivation of seedling transplants, oilseed rape was sown on quaternary red soil, as defined by He et al. (2007), which contains 45.0% clay, 46.3% silt and 8.7% sand. N treatments (normal and limited N) were only conducted after transplanting. The average temperature was 25.1°C/10.3°C day/night (controlled by two air conditioners in the greenhouse), the average relative humidity was 75% (controlled by a humidifier in greenhouse), and the average irradiance was 39,000 lux.

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Materials and Methods

Experimental design: Experiments were conducted at Hunan Agriculture University in two greenhouses (12 m×6 m×2 m) consisting of a steel frame covered by a plastic membrane in which CO₂ cylinders and ceiling fans were installed to ensure high, uniform CO₂ concentrations and airflow. CO₂ was supplied from 8:00 until 18:00 every day. The two levels of CO₂ exposure were as follows: normal (380 μmol·mol⁻¹), which was consistent with natural atmospheric CO₂ concentrations (Bloom et al., 2014), and elevated (760 μmol·mol⁻¹), which was twice that of normal CO₂ concentrations. Two levels for N application were used, including normal (15 mmol·L⁻¹) and limited (5 mmol·L⁻¹). There were four treatments in total, and 10 replicates per sample; 160 total plants were used for N and ¹⁵N measurements at the stem elongation and harvest stages.
Sampling and measuring methods: Samples from different organs were taken from unlabeled and labeled samples at the end of the labeling stage (3 days after labeling treatment, 21 February 2013) and harvest stage (6 May 2013) and were washed, dried in an oven at 105°C for 30 min for the rapid deactivation of enzymes, and then dried at 70°C to a constant weight. Dried samples were collected for biomass calculation (Tables 1 and 2) and were then ground and sieved for N and 15N concentration measurements. Fallen leaves were also collected for measuring N concentration, and roots were the vegetative organs used in calculations. The N contents of plants were measured with a FOSS Kjeldahl apparatus following digestion with concentrated sulfuric acid. Total N was calculated according to biomass and N concentration. The abundance of 15N in different plant tissues was measured using mass spectrometry (Zhang et al., 2010).

Data processing and parameter calculation: Here, we defined NUtE as biomass and grain yield per unit of N in plant tissues, similar to studies in maize (Gallais & Hirel, 2004) and Arabidopsis (Richard-Molard et al., 2008). Experimental data were processed using professional versions of Excel and SPSS (Statistical Product and Service Solutions V17.0, USA) functions for two-way ANOVA (N levels and CO2 levels) and t-tests to compare data for N and CO2 treatments. Physiological parameters were calculated using the following formulas:

\[ TN (mg) = N\% \times \text{biomass (single plant)} \] (Fig. 1)

\[ \text{NUTE based on biomass (g/g)} = \frac{\text{Biomass per plant}}{\text{TN per plant}} \] (Fig. 4A).

\[ \text{NUTE based on grain yield (g/g)} = \frac{\text{Grain yield per plant}}{\text{TN per plant}} \] (Fig. 4B).

Distribution proportion (%) of N in target organ = (TN in target organ / TN per plant) × 100 (Fig. 2).

Distribution proportion (%) of N (absorbed at S stage) in target organs at H stage = (Accumulated amount of 15N in target organs at H stage / accumulated amount of 15N per plant at H stage) × 100 (Fig. 3A).

Distribution proportion (%) of N (absorbed after S stage) in vegetative organs at H stage = [(Accumulated amount of N in vegetative organ at H stage – accumulated amount of N in target organ at S stage) / (TN per plant at H stage – TN per plant at S stage)] × 100 (Fig. 3B)\

Distribution proportion (%) of N (absorbed after S stage) in sili at H stage = [Accumulated amount of N in sili at H stage – (accumulated amount of 15N in sili/T15N per plant at S stage) × TN per plant at S stage] / (TN per plant at H stage – TN per plant at S stage)] × 100 (Fig. 3B)

Transport proportion (%) of N (absorbed at S stage) in sili at H stage = (Accumulated amount of 15N in sili /accumulated amount of 15N per plant at the end of L treatment) × 100 (Table 3).

Transport amount (mg/plant\(^{-1}\)) of N (absorbed at S stage) in sili at H stage = Transport proportion × accumulated amount of N per plant at the end of L treatment (Table 3).

Loss proportion (%) of N (absorbed at S stage) per plant = (T15N per plant at S stage – T15N per plant at H stage) / T15N per plant at S stage × 100 (Table 3).

Lost amount (mg) of N (absorbed at S stage) per plant = N lost proportion × TN per plant at S stage (Table 3).

We hypothesized the transport proportion of 15N to be the transport proportion of N absorbed before stem elongation. Abbreviation note: N concentration = N%, silique = sili, total = T, stem elongation = S, harvest = H, labeling = L.

Results

Effects of elevated-CO2 concentration on plant biomass: At the stem elongation stage under normal- and limited-N conditions, the biomass of roots and stems under the normal-CO2 concentration was significantly lower than that of plants under the elevated-CO2 concentration (Table 1). However, under normal-N conditions at the stem elongation stage, the biomass of leaves under the normal-CO2 concentration was not significantly different from that under the elevated-CO2 concentration (Table 1).

At harvest stage, the biomass of stems and grains under the normal CO2 concentration was significantly lower than that of elevated-CO2 plants under both normal- and limited-N conditions (Table 2). However, at harvest stage, the biomass of roots under normal-CO2 conditions was not significantly different from that of elevated-CO2 concentration plants under either N treatment (Table 2).

Effects of elevated-CO2 concentration on the amount of N absorbed: No significant differences were found for the amount of N absorbed between normal- and elevated-CO2 concentrations under either N application level (Fig. 1). The amount of N absorbed into total plant tissue under normal N was significantly higher than the N absorbed under N limitation for both CO2 concentrations (Fig. 1).

Effects of elevated-CO2 concentration on N distribution in different plant organs: The proportion of N distributed to roots relative to leaves under elevated CO2 was significantly higher than observed in normal-CO2 plants at the stem elongation stage under both N conditions (Fig. 2A). However, the distribution of N to leaves relative to roots under elevated CO2 was significantly lower than that found in normal-CO2 plants at the stem elongation stage under both N treatments.

More N was distributed into leaf tissues under normal N than in limited-N plants; the distribution of N into siliques under normal N was significantly lower compared to that into stems and roots in limited-N plants at harvest. However, no significant differences were found for N distribution in siliques between elevated-and normal-CO2 conditions (Fig. 2).
### Table 1. Effects of elevated-CO$_2$ concentration on biomass of *B. napus* under normal and limited-N at stem elongation stage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Biomass (g/plant)</th>
<th>N concentration (g/kg)</th>
<th>Total N (g/plant)</th>
<th>Biomass (g/plant)</th>
<th>N concentration (g/kg)</th>
<th>Total N (g/plant)</th>
<th>Biomass (g/plant)</th>
<th>N concentration (g/kg)</th>
<th>Total N (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>17.1 ± 1.03ab</td>
<td>15.0 ± 2.36ab</td>
<td>0.24 ± 0.06a</td>
<td>18.2 ± 1.01a</td>
<td>15.5 ± 3.31b</td>
<td>0.27 ± 0.06a</td>
<td>26.8 ± 1.36a</td>
<td>21.5 ± 2.35b</td>
<td>0.57 ± 0.03a</td>
</tr>
<tr>
<td>1/3NC</td>
<td>10.7 ± 1.21b</td>
<td>10.3 ± 1.01c</td>
<td>0.10 ± 0.03b</td>
<td>13.4 ± 2.26b</td>
<td>12.4 ± 1.68b</td>
<td>0.16 ± 0.03b</td>
<td>11.2 ± 1.15b</td>
<td>19.8 ± 2.67b</td>
<td>0.21 ± 0.01b</td>
</tr>
<tr>
<td>N</td>
<td>12.5 ± 1.81b</td>
<td>18.7 ± 3.1a</td>
<td>0.19 ± 0.04a</td>
<td>15.9 ± 0.83b</td>
<td>21.3 ± 2.36a</td>
<td>0.32 ± 0.08a</td>
<td>26.6 ± 2.18a</td>
<td>32.0 ± 3.65a</td>
<td>0.77 ± 0.04a</td>
</tr>
<tr>
<td>1/3N</td>
<td>6.0 ± 0.78c</td>
<td>12.3 ± 1.21bc</td>
<td>0.07 ± 0.02b</td>
<td>9.3 ± 1.07c</td>
<td>13.7 ± 1.21b</td>
<td>0.12 ± 0.06b</td>
<td>9.0 ± 0.89c</td>
<td>24.9 ± 3.14b</td>
<td>0.21 ± 0.02b</td>
</tr>
</tbody>
</table>

Note: Variance analyses (LSD) were conducted using SPSS statistics software. Different letters in the same column denote significant differences (p<0.05). Experimental conditions were as follows: NC indicates normal-N (15 mmol.L$^{-1}$) under elevated-CO$_2$ concentration (760 μmol.mol$^{-1}$); 1/3NC indicates limited-N (5 mmol.L$^{-1}$) under elevated-CO$_2$ concentration; N indicates normal-N under normal-CO$_2$ concentration (380 μmol.mol$^{-1}$); 1/3N indicates limited-N under normal-CO$_2$ concentration.

### Table 2. Effects of elevated-CO$_2$ concentration on biomass of *B. napus* under normal and limited-N at harvest stage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root</th>
<th>Stem</th>
<th>Silique peel</th>
<th>Grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass (g/plant)</td>
<td>N concentration (g/kg)</td>
<td>Total N (g/plant)</td>
<td>Biomass (g/plant)</td>
<td>N concentration (g/kg)</td>
</tr>
<tr>
<td>NC</td>
<td>25.1 ± 2.31a</td>
<td>14.2 ± 1.23a</td>
<td>0.35 ± 0.05a</td>
<td>41.7 ± 2.98a</td>
</tr>
<tr>
<td>1/3NC</td>
<td>15.7 ± 1.85b</td>
<td>9.1 ± 0.87b</td>
<td>0.14 ± 0.02b</td>
<td>30.3 ± 3.31c</td>
</tr>
<tr>
<td>N</td>
<td>26.6 ± 2.79a</td>
<td>15.5 ± 1.65a</td>
<td>0.41 ± 0.03a</td>
<td>37.2 ± 1.23b</td>
</tr>
<tr>
<td>1/3N</td>
<td>13.7 ± 1.48b</td>
<td>9.2 ± 0.79b</td>
<td>0.12 ± 0.01b</td>
<td>19.6 ± 2.16d</td>
</tr>
</tbody>
</table>

Note: Variance analyses (LSD) were conducted using SPSS statistics software. Different letters in the same column denote significant differences (p<0.05). Experimental conditions indicated by NC, 1/3NC, N, and 1/3N are as defined in Table 1. Fallen leaves were also collected for measuring and calculating their N concentration.
Fig. 1. Effects of elevated CO$_2$ concentration on N absorption amount of B. napus at stem elongation stage (Fig. 1A) and harvest stage (Fig. 1B) under normal and limited-N. Note: Variance analyses (LSD) were accomplished using SPSS statistics software. Different letters at the top of histogram bars denote significant differences relative to other data (p<0.05). Experimental conditions were as follows: NC indicates normal-N (15 mmol.L$^{-1}$) under elevated-CO$_2$ concentration (760 μmol·mol$^{-1}$); 1/3NC indicates limited-N (5 mmol.L$^{-1}$) under elevated-CO$_2$ concentration; N indicates normal-N under normal-CO$_2$ concentration (380 μmol·mol$^{-1}$); 1/3N indicates limited-N under normal-CO$_2$ concentration.

![Graph](image1.jpg)

Fig. 2. Effects of elevated CO$_2$ concentration on N distribution in different plant organs at stem elongation stage (Fig. 2A) and harvest stage (Fig. 2B) under normal and limited-N. Different letters at the same rank indicate significant differences (p<0.05). Experimental conditions were as follows: NC indicates normal-N (15 mmol.L$^{-1}$) under elevated-CO$_2$ concentration (760 μmol·mol$^{-1}$); 1/3NC indicates limited-N (5 mmol.L$^{-1}$) under elevated-CO$_2$ concentration; N indicates normal-N under normal-CO$_2$ concentration (380 μmol·mol$^{-1}$); 1/3N indicates limited-N under normal-CO$_2$ concentration.

![Graph](image2.jpg)

Table 3. Effects of elevated CO$_2$ concentration on N (absorbed at stem elongation stage) transport and lost at harvest stage under normal and limited-N.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N (absorbed at stem elongation stage) transport per plant</th>
<th>N (absorbed at stem elongation stage) lost per plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transport proportion (%) of N in silique</td>
<td>Transport amount (mg) of N in silique</td>
</tr>
<tr>
<td>NC</td>
<td>24.1 ± 1.56d</td>
<td>266.1 ± 29.6b</td>
</tr>
<tr>
<td>1/3NC</td>
<td>38.1 ± 3.68b</td>
<td>170.9 ± 11.9d</td>
</tr>
<tr>
<td>N</td>
<td>27.8 ± 1.28c</td>
<td>359.6 ± 31.8a</td>
</tr>
<tr>
<td>1/3N</td>
<td>49.5 ± 5.54a</td>
<td>197.2 ± 13.6c</td>
</tr>
</tbody>
</table>

Note: Different letters in the same column denote significant differences (p<0.05). Experimental conditions indicated by NC, 1/3NC, N, and 1/3N are as defined in Table 1.
Effects of elevated-CO$_2$ concentration on the proportion of N absorbed during or after the stem elongation stage and its distribution to different plant organs at the harvest stage: The proportion of N absorbed during the stem elongation stage that was distributed to roots relative to siliques under normal N supplementation was significantly higher than in plants grown under limited-N conditions (Fig. 3A). No significant difference was found for N distribution into roots absorbed at the stem elongation stage between elevated- and normal-CO$_2$ plants (Fig. 3A).

The proportion of N distributed into the plant root relative to the siliques that was absorbed after the stem elongation stage under elevated CO$_2$ was significantly lower than observed under normal CO$_2$ (Fig. 3B). The proportion of N distributed into the plant stem relative to the siliques that was absorbed after the stem elongation stage under normal-N was significantly higher than observed under limited N (Fig. 3B).

Effects of elevated-CO$_2$ concentration on the harvest-stage transport and loss of N absorbed at the stem elongation stage: The transport proportion and amount of N absorbed at the stem elongation stage from vegetative organs into siliques under elevated CO$_2$ was significantly lower than that observed under normal-CO$_2$ conditions (Table 3). The transport proportion of N absorbed at the stem elongation stage from vegetative organs into siliques
under normal-N conditions was significantly lower than in plants grown with limited N. However, the amount of transport of N absorbed at the stem elongation stage from vegetative organs to siliques under normal-N conditions was significantly higher than that found in limited-N plants due to the significantly higher biomass of normal-N plants compared to limited-N plants (Table 3).

The proportion of N absorbed at the stem elongation stage (and thus lost from plant tissues) under elevated CO₂ was significantly higher than observed under normal CO₂; however, under limited-N conditions, the amount of N absorbed during the stem elongation stage (and thus lost from plant tissues) under elevated CO₂ was significantly higher than in normal-CO₂ plants (Table 3). The proportion and amount of N absorbed at the stem elongation stage (and thus lost from plant tissues) under normal-N conditions was significantly higher than those found limited-N plants (Table 3).

**Effects of elevated-CO₂ concentration on NtUE:** NUtE is defined here as biomass or grain yield per unit N in plant tissues of *B. napus*. The NUtE based on the biomass of elevated-CO₂ plants was significantly higher than that of normal-CO₂ plants under the same N application levels at both the stem elongation or harvest stages (Fig. 4A). The NUtE based on grain yield for the elevated CO₂ concentration was significantly higher than that found in normal-CO₂ treatments for the same levels of N application (Fig. 4B). Whether based on biomass or grain yield, NUtE was significantly lower under normal-N compared to limited-N treatments (Fig. 4).

**Discussion**

Biomass and N absorption increase in wheat when atmospheric CO₂ concentrations are elevated (Li et al., 2003; Yang et al., 2007). Upadhyay et al. (2000) and Hogy et al. (2010) reported that shoot biomass, grain yield per hectare and oil yield of oilseed rape significantly increase under elevated CO₂ concentrations. The biomass of roots and stems at the stem elongation stage and the biomass of stems and grains at the harvest stage under the elevated CO₂ concentration were higher than those of plants grown under the normal CO₂ concentration (Tables 1 and 2); these results agree with previous studies in wheat and oilseed rape (Li et al., 2003; Yang et al., 2007; Franzaring et al., 2012). In addition, there were no significant differences in the amounts of N absorbed per plant under elevated and normal CO₂ concentrations subjected to the same N application level (Fig. 1); however, NUtE was significantly higher in elevated-CO₂ compared to normal-CO₂ plants (Figs 1, and 4). Thus, the results in this study are consistent with the observations of Hulka et al. (1994) in cotton that showed that, although growth under elevated CO₂ supports the same amount of N absorption as does normal CO₂, elevated-CO₂ growth regimes produce higher plant biomass and result in higher NUtE. Zhang & Zhang, (2011) demonstrated that a larger proportion of N was distributed to stems and roots (but not leaves) in *Brassica napus* under elevated-CO₂ growth conditions. In contrast, results during the stem elongation stage showed that compared to normal CO₂ concentrations, a larger proportion of N remained in roots under elevated-CO₂ concentrations, and a smaller proportion of N was transported to leaves under elevated CO₂ (Fig. 2A). Generally, the distribution of N from plant vegetative tissues into reproductive tissues under normal-N conditions tends to be lower than that observed under limited-N conditions and is positively affected by increased CO₂ concentrations (Lobell & Field, 2008; Zhang & Zhang, 2011; Franzaring et al., 2012). The present study produced similar results at the harvest stage, when a smaller proportion of N was distributed to siliques under normal-N supplementation compared to the limited-N regime. However, N distribution proportions in siliques were not affected by CO₂ concentration (Fig. 2B).

Xu et al. (2011) reported that the amount and proportion of N and nutrients distributed from vegetative organs to grains increases under elevated-CO₂ growth in wheat. However, distribution proportions and amounts of N absorbed at a specific growth stage in plant tissues have rarely been reported in previous studies (Loladze, 2002; Xu et al., 2011). The present paper studied the distribution proportion of N absorbed during the stem elongation stage and after the stem elongation stage in different plant tissues. The results showed that a smaller proportion of N was distributed into siliques under normal N supplementation compared to limited-N plants, regardless of whether the N was absorbed during or after the stem elongation stage (Fig. 3). However, the distribution proportions and amounts of N absorbed during the stem elongation stage and after stem elongation in plant tissues differ from those reported in previous studies. For instance, a larger proportion of the N absorbed after stem elongation was redistributed to siliques under elevated-CO₂ conditions than under normal-CO₂ conditions at the harvest stage (Fig. 3B). However, no significant differences were found between elevated-and normal-CO₂ treatments when N was absorbed during the stem elongation stage (Fig. 3A). These results suggest that after stem elongation, elevated CO₂ can accelerate the absorption of N distributed to the main growth organs (siliques) at the harvest stage. NUtE can be enhanced by improving the distribution of N from older plant tissues to vigorously growing plant tissues; therefore, limited N that is distributed to the main growth organs thereby improves the NUtE (Loladze, 2002). This effect occurs because N becomes the limiting substrate in plant tissues under elevated-CO₂ growth conditions. Therefore, a higher proportion of N is distributed to siliques to accommodate the requirements of siliques development and grain yield during later growth stages in rice and wheat (Hou et al., 2006; Yang et al., 2007), results that agree with our study (Fig. 3).

Generally, the proportion and amount of N absorbed at earlier plant growth stages in vegetative organs and transported to reproductive organs increase in wheat when CO₂ concentrations are elevated (Yang et al., 2007). For instance, a larger amount of earlier-absorbed N is distributed to reproductive organs in wheat and *Arabidopsis* under higher CO₂ growth regimes (Yang et al., 2007; Bloom et al., 2014; Tingey et al., 2003). In contrast, our results reported here showed that the transported proportions and amounts of N absorbed
during the stem elongation stage from vegetative organs to siliques under elevated CO₂ were significantly lower than in normal-CO₂ plants (Table 3). It can be concluded that the transport of N (absorbed at earlier growth stages) from vegetative organs to reproductive organs (siliques) at the harvest stage was decreased by the elevated CO₂ concentration, and the proportion of N lost from plant tissues was increased by the elevated CO₂ concentration compared to the normal CO₂ treatment oilseed rape (Table 3). These results support the theory that higher levels of N fertilizer applied at later growth stages under elevated CO₂ will benefit N localization to reproductive organs and reduce the N lost from plant tissues. In addition, a higher proportion of N was distributed from vegetative organs into siliques under limited-N treatments than under normal-N treatments (Table 3), which is similar to results in wheat (Bloom et al., 2014).

NUtE increases under elevated atmospheric CO₂ concentrations in wheat and forest systems (Finzi et al., 2007; Bloom et al., 2014). Zerihun et al. (2000) reported increases of 50% in NUE of Helianthus annuus under elevated CO₂. The current study produced similar results (Fig. 4): The NUE of plants grown in elevated CO₂ was significantly higher than that of plants grown under the normal-CO₂ regime for the same N application level, and the NUE of normal-N plants was significantly lower than that of plants grown under limited-N conditions. Possible reasons for this finding are that more carbon substrate frames were supplied under elevated CO₂ for N assimilation and that a larger proportion of N was distributed from old plant tissues to the vigorously growing plant tissues under limited N supplementation, resulting in a significantly improved NUtE (Loladze, 2002; Bloom et al., 2014).

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