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EFFECT OF ELEVATED ATMOSPHERIC CO_2ON NITROGEN DISTRIBUTION AND N UTILIZATION EFFICIENCY IN WINTER RAPE (*BRASSICA NAPUS* L.)

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Abstract

We characterized the responses of plant dry biomass, nitrogen (N) distribution and N-utilization efficiency (NUtE) to changes in CO_2 concentration through exposure and culture of winter rape under normal-(380 μmol·mol^{-1}) and elevated-CO_2 (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. NUtE increased with elevated CO_2 regardless of whether N was limited. NUtE was higher under N limitation than under normal N conditions for both normal- and elevated-CO_2 conditions. ^{15}N labeling was used to assess the distribution of N from vegetative- to reproductive-organs.Ndistribution within the plant and during different developmental stages was affected by CO_2 concentration and the level of N application. A higher proportion of N was found in siliques at the harvest stage for N-limited plants compared to normal-N plants. The proportion of N absorbed into siliques after the stem elongation stage under elevated-CO_2 conditions was significantly higher than under normal CO_2. The proportion of N transport, as well as the total amount of N, absorbed at the stem elongation stage from vegetative organs into siliques under elevated CO_2 was significantly lower than under normal-CO_2 conditions. However, the proportion of N absorbed at the stem elongation stage and thus lost from the silique under elevated CO_2 was significantly higher than under normal CO_2. In conclusion, limited N or elevated CO_2 generally benefitted plant NUtE. In addition, after the stem elongation stage, elevated CO_2 promoted the redistribution of N from plant vegetative tissues to reproductive organs; however, elevated CO_2 during or before stem elongation had the opposite effect.

Key word: Oilseed rape (*Brassica napus*); Elevated CO_2 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 CO_2, CH_4 and N_2O, whichhave increased over time (Myers *et al*., 2014; Bloom*et al*., 2014). The CO_2 concentration in the atmosphere was 265μmol·mol^{-1} before the industrial revolution and reached approximately 314μmol·mol^{-1} by1958 and 353 μmol·mol^{-1} by 1990 (Loladze *et al*., 2002). Unfortunately, the atmospheric CO_2 recently reached 380 μmol·mol^{-1} (Wang *et al*., 2004). If such gases continue to increase at the same rate, atmospheric CO_2 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 CO_2 concentrations can be affected by the C-N balance; plant stem growth can be accelerated by elevated CO_2 but will be inhibited under N-limited conditions (Sun *et al*., 2002). Although elevated atmospheric CO_2 may accelerate greenhouse effects with possible changes in climate, CO_2 is the raw material for photosynthesis and N assimilation. Thus, crop yieldsmay be positively affected by elevated atmospheric CO_2. Bloom *et al*. (2014)systematically studied the effects of elevated CO_2 concentrations on crop yields and found that yields increased by 30% when atmospheric CO_2 concentrations doubled. Moreover, regardless of N application levels, seed yields of cotton increased by 56% and 54%, respectively, under elevated-CO_2 concentrations during moist growth conditions (Bloom *et al*., 2014).

Current studies suggest that CO_2 inhibits nitrate assimilation, as nitrates cannot be assimilated into proteins efficiently under elevated CO_2 (Bloom *et al*., 2014). Previous studies have shown that the N content in leaves decreases under elevated CO_2 concentrations (Curtis,1996; Poorter *et al*., 1997; Cotrufom *et al*., 1998). The stomatal conductance of leaves decreases during elevated atmospheric CO_2 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 (Morison & Lawlor, 1999). Other reasons for the decreased N content in leaves may be that the discharge of carbon compounds from roots increases under high CO_2 concentrations (Franzaring *et al*., 2012) and that more N_2 in the rhizosphere was fixed by microorganism communities, which would result in an N supply that was limiting to plant metabolism (Soussana

& Hartwing, 1995). Huluka *et al*. (1994) reported that N content in whole cotton plant tissues decreased under elevated atmospheric CO_2 concentrations. N absorption has also been shown to decrease with elevated CO_2 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 NUtE 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%. NUtE has several definitions but is generally defined as an index of production per unit of N taken up (Hirel *et al*., 2001; Good *et al*., 2004). However, studies of the effects of elevated CO_2 concentrations on the distribution of nutrients in plant tissues have primarily focused on root/shoot nutrient ratios (Rogers *et al*., 1996), and few studies have explored N distribution in different plant tissues during growth stages under elevated CO_2 conditions (Reinert and Ho, 1995; Retuerto & Woodward, 1993) or the trade-offs between the NUtE of different nutrients, which are strongly influenced by environment-, plant- and nutrient-specific variables (Reich *et al*., 2014). Sand-cultured oilseed rape was used to study the responses of N-absorption, N-distribution and NUtE to elevated CO_2 concentrations under normal- and limited-N application levels. We hypothesized that elevated CO_2 would have different effects on N distribution in plant tissues for earlier and later plant growth stages and would critically affect NUtE and lost N. The results of this study will contribute to a scientific theory for evaluating the effects of elevated CO_2 concentrations on plant growth in general and will help guide the reasonable application of N fertilizer as atmospheric CO_2 concentrations rise.

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 CO_2 cylinders and ceiling fans were installed to ensure high, uniform CO_2 concentrations and airflow. CO_2 was supplied from 8:00 until 18:00 every day. The two levels of CO_2 exposure were as follows: normal (380 μmol·mol^{-1}), which was consistent with natural atmospheric CO_2 concentrations (Bloom *et al*., 2014), and elevated (760 μmol·mol^{-1}), which was twice that of normal CO_2 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 ^{15}N measurements at the stem elongation and harvest stages.

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.

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 dilute 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 with a width of 5 m and height of 2 m. One greenhouse was used for the normal CO_2 treatments with normal and limited N, and the other greenhouse was used for the elevated CO_2 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 KNO_3, 1 mM KH_2PO_4, 7 mM MgSO4, 5 mM Ca $(NO_3)_2{\cdot}4H_2O$, 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 [KNO_3 and $Ca(NO_3)_2{\cdot}4H_2O$] 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 seedling 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, ^{15}N isotope Ca $(^{15}NO_3)_2{\cdot}4H_2O$ and $K^{15}NO_3$(Shanghai Chemical Engineering Corporation Research Institute, Shanghai, China; ^{15}N excess = 20.28%) was used as a labeled N source to follow N distribution within the plant.The culture of ^{15}N labeled plants was the same as with normal N plants, but these 80 pots were provided 150 ml nutrient solution (containing the ^{15}N 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 ^{15}N 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 ^{15}N-labeled plants.

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 ^{15}N concentration measurements. Fallen leaves were also collected for measuring N concentration, and roots were the vegetative organs used in calculations.TheN 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 ^{15}Nin 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 CO_2 levels) and t-tests to compare data for N and CO_2 treatments. Physiological parameters were calculated using the following formulas:

TN (mg) = N% × biomass (single plant) (Fig. 1)

NUtE based on biomass (g/g) = Biomass per plant / TN per plant (Fig. 4A).

NUtE based on grain yield (g/g) = Grain yield per plant / 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 ^{15}N in target organs at H stage / accumulated amount of ^{15}N 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 ^{15}N in sili/$T^{15}N$ 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^{15}Nin sili /accumulated amount of^{15}Nper 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 = ($T^{15}N$ per plant at S stage – $T^{15}N$ per plant at H stage) / $T^{15}N$ 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 ^{15}N 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-CO_2 concentration on plant biomass: At the stem elongation stage under normal- and limited-N conditions, the biomass of roots and stems under the normal-CO_2 concentration was significantly lower than those of plants under the elevated-CO_2 concentration (Table 1). However, under normal-N conditions at the stem elongation stage, the biomass of leaves under the normal-CO_2 concentration was not significantly different from that under the elevated-CO_2 concentration (Table 1).

At harvest stage, the biomass of stems and grains under the normal CO_2 concentration was significantly lower than that of elevated-CO_2 plants under both normal- and limited-N conditions (Table 2). However, at harvest stage, the biomass of roots under normal-CO_2 conditions was not significantly different from that of elevated-CO_2 concentration plants under either N treatment (Table 2).

Effects of elevated-CO_2 concentration on the amount of N absorbed: No significant differences were found for the amount of N absorbed between normal- and elevated-CO_2 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 CO_2 concentrations (Fig. 1).

Effects of elevated-CO_2 concentration on N distribution in different plant organs: The proportion of N distributed to roots relative to leaves under elevated CO_2 was significantly higher than observed in normal-CO_2 plants at the stem elongation stage under both N conditions (Fig. 2A). However, the distribution of N to leaves relative to roots under elevated CO_2 was significantly lower than that found in normal-CO_2 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-CO_2 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.

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

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 $\mu mol.mol^{-1}$); 1/3NC indicates limited-N (5 $mmol.L^{-1}$) under elevated-CO_2 concentration; N indicates normal-N under normal-CO_2 concentration (380 $\mu mol.mol^{-1}$); 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.

Treatment	Root			Stem			Silique peel			Grains		
	Biomass (g/plant)	N concentration (g/kg)	Total N (g/plant)	Biomass (g/plant)	N concentration (g/kg)	Total N (g/plant)	Biomass (g/plant)	N concentration (g/kg)	Total N (g/plant)	Biomass (g/plant)	N concentration (g/kg)	Total N (g/plant)
NC	25.1 ± 2.31a	14.2 ± 1.23a	0.35 ± 0.05a	41.7 ± 2.98a	9.7 ± 2.78b	0.40 ± 0.03a	15.4 ± 0.63b	9.3 ± 0.73b	0.14 ± 0.02b	14.9 ± 1.12a	31.2 ± 2.15a	0.46 ± 0.05a
1/3NC	15.7 ± 1.85b	9.1 ± 0.87b	0.14 ± 0.02b	30.3 ± 3.31c	5.0 ± 0.43c	0.14 ± 0.02b	14.4 ± 1.38b	7.9 ± 0.49c	0.11 ± 0.01bc	11.0 ± 1.31b	30.0 ± 3.06a	0.33 ± 0.03b
N	26.6 ± 2.79a	15.5 ± 1.65a	0.41 ± 0.03a	37.2 ± 1.23b	13.0 ± 1.12a	0.48 ± 0.04a	17.0 ± 0.79a	11.9 ± 1.32a	0.20 ± 0.02a	11.4 ± 1.35b	34.1 ± 2.87a	0.39 ± 0.04a
1/3N	13.7 ± 1.48b	9.2 ± 0.79b	0.12 ± 0.01b	19.6 ± 2.16d	6.4 ± 1.65bc	0.11 ± 0.02b	10.5 ± 1.43c	9.4 ± 0.69b	0.10 ± 0.01c	8.6 ± 0.98c	34.0 ± 3.69a	0.29 ± 0.04b

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

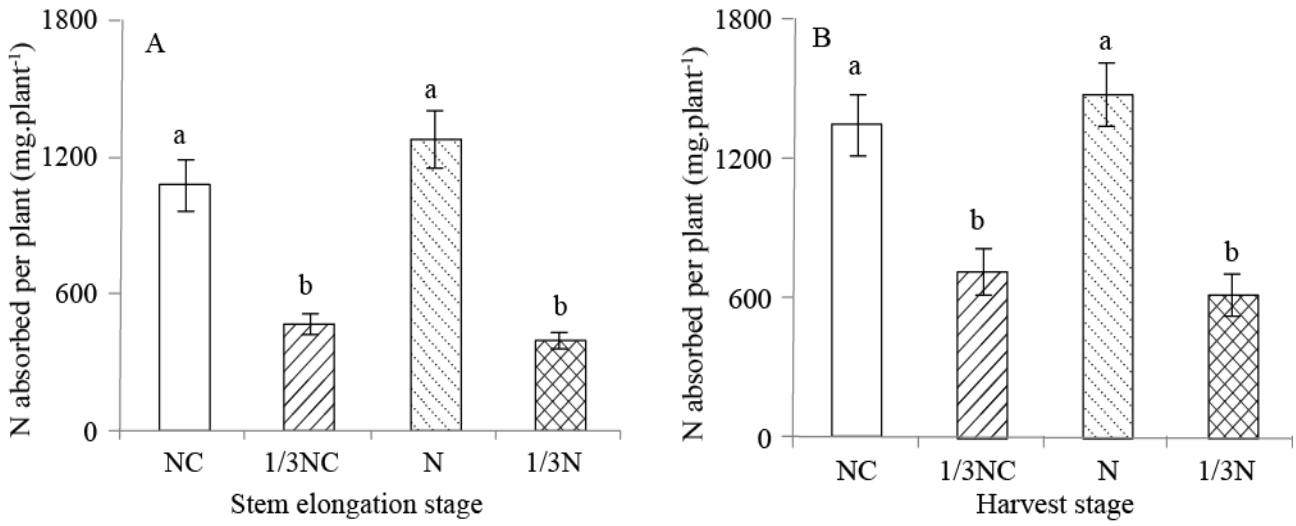

Fig. 1. Effects of elevated-CO_2 concentration on N absorption amount of *B. napus*at stem elongation stage (Fig. 1A) and harvest stage (Fig. 1B)under normal and limited-N. Note: Variance analyses (LSD) were accomplished using SPSS statistics software. Different letters at the top of histogram bars denote 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.

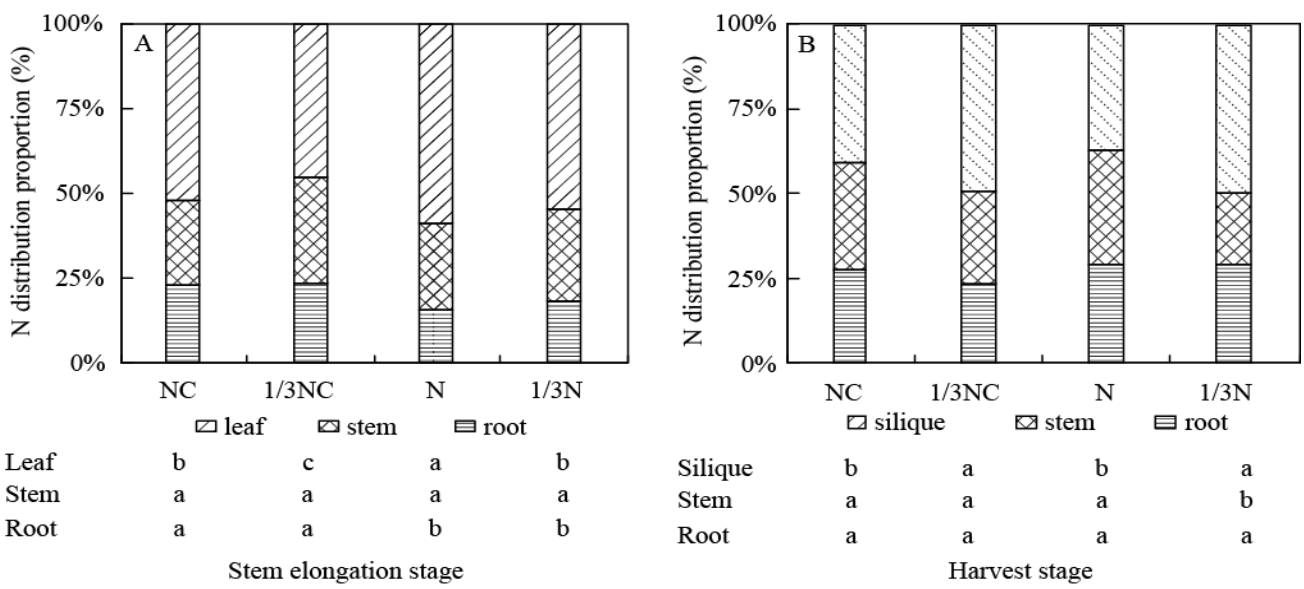

Fig. 2. Effects of elevated-CO_2 concentration on N distribution in different plant organs at stem elongation stage (Fig. 2A) and harvest stage (Fig. 2B) under normal and limited-N. Different letters at the same rank indicate significant differences ($p<0.05$). Experimental conditions were as follows: 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.

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.

Treatment	N (absorbed at stem elongation stage) transport per plant		N (absorbed at stem elongation stage) lost per plant	
	Transport proportion (%) of N in silique	Transport amount (mg) of N in silique	Loss proportion (%)	Loss amount (mg)
NC	24.1 ± 1.56d	266.1 ± 29.6b	42.8 ± 2.67a	472.2 ± 45.5a
1/3NC	38.1 ± 3.68b	170.9 ± 11.9d	29.0 ± 2.46c	143.5 ± 18.6b
N	27.8 ± 1.28c	359.6 ± 31.8a	37.7 ± 2.19b	489.0 ± 39.8a
1/3N	49.5 ± 5.54a	197.2 ± 13.6c	18.0 ± 1.85d	74.5 ± 9.4c

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

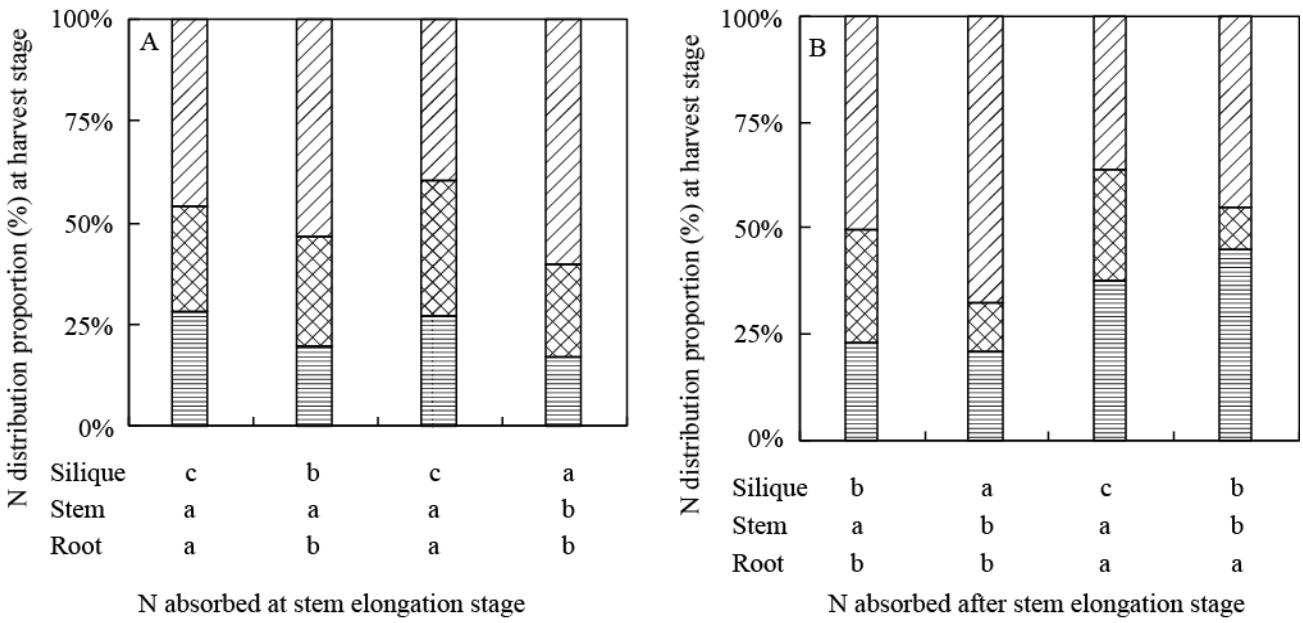

Fig. 3. Effects of elevated-CO_2 concentration on distribution proportion of N absorbed at the stem elongation stage (Fig. 3A) and after the stem elongation stage (Fig. 3B) in different plant organs at harvest under normal- and limited-N. Different letters at the same rank indicate significant differences ($p<0.05$). Experimental conditions indicated by NC, 1/3NC, N, and 1/3N are as defined in Fig. 2.

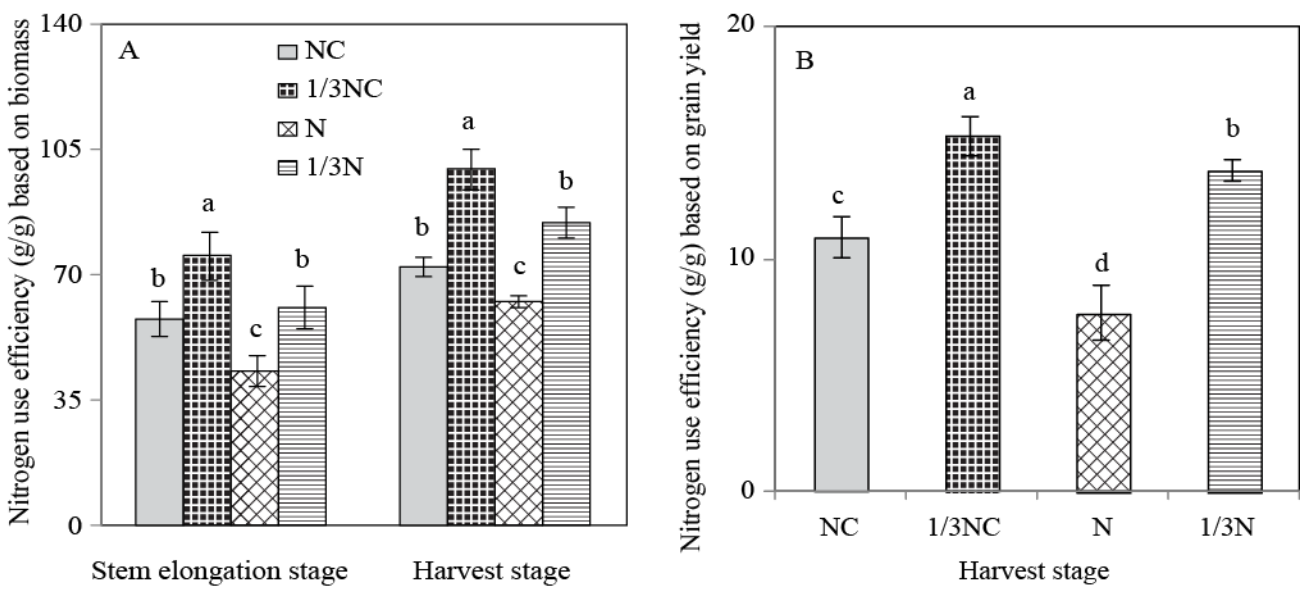

Fig. 4. Effects of elevated-CO_2 concentration on N utilization efficiency (NUtE) of *B. napus* under normal- and limited-N. Different letters above histogram bars indicate significant differences ($p<0.05$).Fig. 4A: NUtE based on biomass (biomass/total N); Fig. 4B: NUtE based on grain yield (grain yield/total N). Experimental conditions indicated by NC, 1/3NC, N, and 1/3N are as defined in Fig. 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 silique 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 silique 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 CO_2 was significantly higher than observed under normal CO_2; however, under limited-N conditions, the amount of N absorbed during the stem elongation stage (and thus lost from plant tissues) under elevated CO_2 was significantly higher than in normal-CO_2 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-CO_2 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-CO_2 plantswas significantly higher than that of normal-CO_2 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 CO_2 concentration was significantly higher than that found in normal-CO_2 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 CO_2 concentrations are elevated (Li *et al*., 2003; Yang *et al*., 2007). Upretyet *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 CO_2 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 CO_2 concentration were higher than those of plants grown under the normal CO_2 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 CO_2 concentrations subjected to the same N application level (Fig. 1); however, NUtE was significantly higher in elevated-CO_2 compared to normal-CO_2 plants (Figs 1, and 4). Thus, the results in this study are consistent with the observations of Huluka *et al*. (1994) in cotton that showed that, although growth under elevated CO_2 supports the same amount of N absorption as does normal CO_2, elevated-CO_2 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-CO_2 growth conditions. In contrast, results during the stem elongation stage showed that compared to normal CO_2 concentrations, a larger proportion of N remained in roots under elevated-CO_2 concentrations, and a smaller proportion of N was transported to leaves under elevated CO_2 (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 CO_2 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 CO_2 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-CO_2 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-CO_2 conditionsthan under normal-CO_2 conditionsat the harvest stage (Fig. 3B). However, no significant differences were found between elevated-and normal-CO_2 treatmentswhen N was absorbed during the stem elongation stage (Fig. 3A). These results suggest that after stem elongation, elevated CO_2 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-CO_2 growth conditions. Therefore, a higher proportion of N is distributed to siliques to accommodate the requirements of silique 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 CO_2 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 CO_2 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 CO_2 were significantly lower than in normal-CO_2 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 CO_2 concentration, and the proportion of N lost from plant tissues was increased by the elevated CO_2 concentration compared to the normal CO_2 treatmentin oilseed rape (Table 3). These results support the theory that higher levels of N fertilizer applied at later growth stages under elevated CO_2, 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 CO_2 concentrations in wheat and forest systems (Finzi *et al*., 2007; Bloom*et al*., 2014). Zerihun *et al*. (2000) reported increases of 50% in NUtE of *Helianthus annuus* under elevated CO_2. The current study produced similar results (Fig. 4): The NUtE of plants grown in elevated CO_2 was significantly higher than that of plants grown under the normal-CO_2 regime for the same N application levels, and the NUtE 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 CO_2 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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