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Effects of temperature and nitrogen application on photosynthetic characteristics and the absorption and distribution of carbon and nitrogen in apple plants

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Abstract

Low temperature has negative effects on apple photosynthesis by inhibiting the accumulations of photosynthates and nitrogen. The interactive effects of low temperature and nitrogen application on photosynthetic parameters and the absorption and distribution of carbon and nitrogen in different organs were assessed to investigate if nitrogen application can relieve the low-temperature stress on gas exchange and the accumulations of carbon and nitrogen inside the apple plants. No matter under normal or low-temperature conditions, nitrogen application both improved the photosynthetic parameters including net photosynthetic rate, intercellular CO_2 concentration, and quantum yield of regulated energy dissipation of PSII as well as the absorption of carbon and nitrogen in roots, stems, and leaves. Thus, we conclude that nitrogen application can relieve the effects of low-temperature stress on photosynthesis and is of benefit for the accumulations of carbon and nitrogen in multiple organs of apple seedlings.

Keywords: chlorophyll fluorescence; gas exchange; *Malus sieversii*; temperature stress; tracing technology.

Introduction

Xinjiang wild apple [*Malus sieversii* (Ledeb.) Roem.] is the ancestor of current cultivated apple species. *M. sieversii* is a precious germplasm resource as it has strong resistance to cold and drought stress, insects, and disease. *M. sieversii* is a primary apple rootstock in China and other production areas due to its strong affinity for grafting with other apple species (Forsline and Aldwinckle 2004, Zhang *et al.* 2009). In recent years, the natural population number and distribution area of wild apple in Xinjiang have decreased sharply due to overpicking,

Highlights

- Nitrogen application alleviated low-temperature-induced inhibition of photosynthesis in wild apple
- Carbon and nitrogen nutrients were preferentially distributed to the roots and stems
- Photosynthesis was closely related to the assimilations of carbon and nitrogen

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Abbreviations: Atom ^{13}C – ^{13}C abundance; Atom ^{15}N – ^{15}N abundance; C_i – intercellular CO_2 concentration; Con ^{13}C – ^{13}C absolute abundance; Con ^{15}N – ^{15}N absolute abundance; DAT – days after temperature and nitrogen application treatment; E – transpiration rate; F_v/F_m – maximum quantum efficiency of PSII photochemistry; g_s – stomatal conductance; P_N – net photosynthetic rate; q_P – photochemical quenching coefficient; Y_{II} – quantum efficiency of PSII; Y_{NPQ} – quantum yield of regulated energy dissipation of PSII; δ ^{13}C – amount of fixed ^{13}C assimilates; δ ^{15}N – amount of fixed ^{15}N assimilates.

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overgrazing, and the influence of insect pests (*Agrilus mali* Matsumura) (Wang *et al*. 2017, Zheng *et al*. 2019). Seedling regeneration is of great value to the breeding of *M. sieversii*, which can promote population evolution and maintain genetic diversity (Kanno and Seiwa 2004, Atici *et al*. 2007). However, *M. sieversii* grows on the steep and fragile hillside of the Tianshan Mountains in Xinjiang, where the range of temperature between day and night is very large. The number of annual seedlings within the canopy of the mother plant is small and they do not survive until autumn each year. At present, it is extremely difficult for *M. sieversii* to establish a new population with seedlings under natural conditions (Liu and Dong 2018). Therefore, it is of great practical significance to develop nitrogen fertilizer and temperature control strategies for *M. sieversii* seedlings under artificial breeding conditions when safeguarding its germplasm resources.

Temperature regulates plants' growth and development. Low temperatures have an inhibitory effect on many physiological processes in plants, such as photosynthesis (Zhang *et al*. 2011). Low-temperature stress has a direct effect on the function of plant leaves. For example, low temperature depresses the CO_2 assimilation, increases the proportion of heat radiation consumption, and severely impairs photosynthesis capabilities (Santos *et al*. 2011, Rihan *et al*. 2017). Furthermore, low-temperature stress inhibits leaf light energy absorption and transformation, and destroys photosynthetic electron transport, resulting in the accumulation of excess excitation energy in the PSII reaction center, which damages the PSII reaction center of the leaves and decreases the leaf photosynthetic capacity (Funk and Cornwell 2013, Ptushenko *et al*. 2014).

Nitrogen is one of the essential elements for plant growth and development, and it is also an important component of protein, chlorophyll, nucleic acid, and some hormones in plants, and participates in plant photosynthesis (Jung 2011, Deng *et al*. 2019). In addition, the demand for nitrogen is greatest during the seedling stage (Kimmins *et al*. 2010). Increasing nitrogen application can improve chlorophyll content and leaf photosynthetic rate (Feng *et al*. 2008, Zhou *et al*. 2020), and an appropriate nitrogen supply is of benefit for promoting the distribution and operation of apple photosynthates (Sha *et al*. 2019). Therefore, it is of great application value to study the protective effects of nitrogen application on photosynthesis of *M. sieversii* under low temperatures.

There is currently a large debate on the effect of nitrogen enrichment on plant photosynthetic carbon fixation (Magill *et al*. 2004, Sparrius *et al*. 2012, Jiang *et al*. 2019, Wang *et al*. 2019). The carbon sequestration capacity of each ecosystem responds differently to nitrogen addition (Liu and Greaver 2010, Chen *et al*. 2020). The distribution of carbon assimilates in plant organs may change after nitrogen application (Silva *et al*. 2019, Zhao *et al*. 2021). Carbon and nitrogen tracing technology is an effective method for studying the distribution of photosynthetic products and nitrogen absorption in plants (Longobardi *et al*. 2017, Weng *et al*. 2020). Thus, this study explores the fixed absorption capacity and distribution direction of carbon and nitrogen nutrients in multiple organs of *M. sieversii* seedlings under different temperature and nitrogen regimes by using ^{13}C and ^{15}N isotope double labeling technology. The objectives of this study were to investigate the effects of temperature and nitrogen application on gas exchange and chlorophyll fluorescence at the apple seedling stage and to explore the relationship between photosynthetic characteristics and carbon and nitrogen absorption.

Materials and methods

Experimental design: This experiment was carried out in the Key Laboratory of Cultivation Physiology and Germplasm Resources Utilization of Featured Fruits and Vegetables of Shihezi University in 2019. The test materials were *M. sieversii* plants. In total, 200 healthy Xinjiang wild apple seeds were soaked in clean water for 1–2 h and disinfected with 0.5% potassium permanganate for 30 min. The seeds were mixed with high temperature sterilized wet sand at a ratio of 1:3 and placed in a germinating box for low-temperature stratification in a refrigerator at 4°C for 90 d. The seeds of *M. sieversii* after stratification treatment were then selected and seeded in the seedling plug-trays (10 cm high, 50 holes, one plant per hole) with the same germination and growth vigor (germ length of approximately 0.5 cm). The substrate ratio was peat soil: vermiculite: apple garden soil = 3:1:0.2 (v:v:v). The seedling plug-trays were placed in the RXZ intelligent artificial climate box [*RXZ-300B*, *Ningbo Jiangnan Instrument Co*., China; 28/25°C day/night temperature, 12/12-h day/night photoperiod, light intensity of 134 μmol(photon) m^{-2} s^{-1}, 75% relative humidity].

The four following treatments were set in the experiment: low temperature, no nitrogen (LTN_0); low temperature, nitrogen (LTN_1); normal temperature, no nitrogen (NTN_0); and normal temperature, nitrogen (NTN_1). A two-factor completely randomized design was employed with three replicates. When the seedlings attained 7–8 true leaves, 48 well-grown plants showing consistent growth were selected. Among them, 24 plants were treated with nitrogen (applying urea at a fertilizer to substrate ratio of 0.43 g kg^{-1}; N_1), and the other 24 seedlings were treated as the control group without fertilization (N_0); all plants were cultured in the artificial climate chamber under room temperature (25°C). After 7 d, seedlings from each treatment were randomly divided into two groups and placed in artificial climate boxes set to LT (5°C) and NT (25°C). The fluorescence parameters were measured 1, 4, and 7 d after temperature and nitrogen application treatment (DAT), respectively. At 7 DAT, photosynthetic parameters, as well as carbon and nitrogen isotope abundances, were measured.

Gas exchange: At 7 DAT, three *M. sieversii* seedlings with the same growth vigor were selected from different treatments. The intercellular CO_2 concentration (C_i), stomatal conductance (g_s), net photosynthetic rate (P_N), and transpiration rate (E) of the third true leaf of each seedling were measured using the portable photosynthesis system (*Li-6400*, *Li-COR Inc*., NE, USA). During measurement, the leaf chamber was equipped with a standard red-blue light source, the light quantum flux density was 500 μmol(photon) m^{-2} s^{-1}, and the CO_2 concentration was 380–390 μmol(CO_2) mol^{-1}.

Chlorophyll fluorescence: At 1, 4, and 7 DAT, *M. sieversii* seedlings were adapted to the dark for 20 min. The maximum photochemical efficiency (F_v/F_m), actual photochemical efficiency (Y_{II}), photochemical quenching coefficient (q_P), and nonphotochemical quenching coefficient (Y_{NPQ}) of PSII in the third mature leaf at the top of the new shoot were measured using the modulated fluorometer (*PAM 100*, *Walz*, Effeltrich, Germany).

^{13}C and ^{15}N: ^{15}N labeling was conducted by applying urea containing ^{15}N on the roots. The root culture medium was then treated with 320 mg of ^{15}N-urea dissolved in water. The ^{13}C pulse was used in a marking chamber made of transparent agricultural plastic film, which was then sealed. After 7 d of nitrogen treatment, 1 mL of HCl solution (1 mol L^{-1}) was aspirated with a syringe and injected into a centrifuge tube containing 0.6 g $Ba^{13}CO_3$ (98% abundance). Following that, HCl solution was injected every 30 min, and the CO_2 concentration in the labeling chamber was maintained at 360 μmol L^{-1}, and the total labeling time was 4 h. After 7 d of ^{13}C labeling, whole plants were sampled destructively, three seedlings from each treatment were selected and divided into three parts: root, stem, and leaf. During the sample separation process, each part was washed with clean water, detergent, 1% hydrochloric acid, and three times with deionized water; then dried at 105°C for 30 min and dried at 80°C to a constant mass. After crushing, the dry sample was filtered through a 0.25-mm sieve, thoroughly mixed, and stored in bags until use. The abundance of ^{13}C and ^{15}N were measured using the *DELTA V* advantage isotope ratio mass spectrometer and analyzed by the China Academy of Forestry Sciences Stable Isotope Laboratory. The formulas used were as follows: $\delta\ ^{13}C$ (or $\delta\ ^{15}N$) = $(R_S/R_{PDB} - 1) \times 1{,}000$; Atom ^{13}C = $(\delta\ ^{13}C + 1{,}000) \times RPDB/[1{,}000 + R_{PDB} \times (\delta\ ^{13}C + 1{,}000)] \times 100$; Atom ^{15}N = $(\delta\ ^{15}N + 1{,}000) \times R_{PDB}/[(\delta\ ^{15}N + 1{,}000) \times R_{PDB} + 1{,}000] \times 100$; Con ^{13}C = Atom $^{13}C \times 0.01 \times C\% \times 0.01 \times 1{,}000$; and Con ^{15}N = Atom $^{15}N \times 0.01 \times N\% \times 0.01 \times 1{,}000$; where $\delta\ ^{13}C$ was the amount of ^{13}C assimilate that was fixed [‰]; $\delta\ ^{15}N$ was the amount of ^{15}N assimilate that was fixed [‰]; R_S was the ratio of ^{13}C to ^{12}C (or the ratio of ^{15}N to ^{14}N); R_{PDB} was the standard ratio of carbon isotopes, *i.e.*, 0.0112372 (or the standard ratio of nitrogen isotopes, *i.e.*, 0.0036765); Atom ^{13}C and Atom ^{15}N were ^{13}C abundance and ^{15}N abundance [%], respectively; Con ^{13}C and Con ^{15}N were ^{13}C absolute abundance and ^{15}N absolute abundance [mg g^{-1}], respectively; and C% and N% were carbon content and nitrogen content, respectively.

Statistical analysis: Analysis of variance (*ANOVA*) was performed using *SPSS 20.0* software. Differences between treatments were considered significant at $P<0.05$ according to *Duncan*'s multiple range tests. The figures were plotted using *SigmaPlot 14.0* software.

Results

Leaf gas-exchange parameters: P_N of the LTN_0 treatment was the lowest among all treatments, and that of the LTN_0 treatment was 25.1% lower than that of the LTN_1 treatment. Compared with the control treatment groups (LTN_0 and NTN_0), the nitrogen treatment groups (LTN_1 and NTN_1) had a promotion effect on P_N. P_N of LTN_1 and NTN_1 treatments were 33.6 and 16.1% higher than these of LTN_0 and NTN_0 treatments, respectively (Fig. 1*A*). C_i could be increased by both normal temperature and

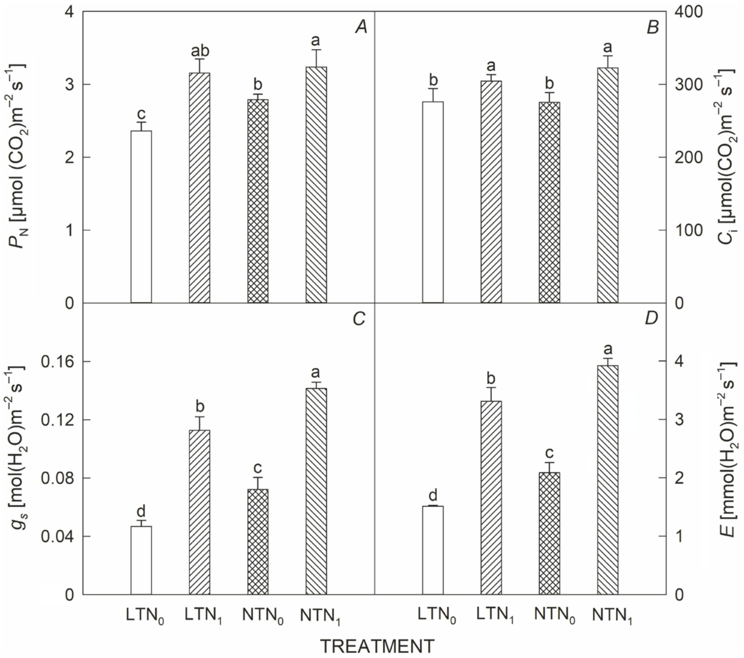

Fig. 1. Photosynthetic characteristics of *Malus sieversii* seedlings after the application of nitrogen (N_1) and without nitrogen application (N_0) under normal temperature (NT) and low temperature (LT) conditions. (*A*) Net photosynthetic rate (P_N), (*B*) intercellular CO_2 concentration (C_i), (*C*) stomatal conductance (g_s), and (*D*) transpiration rate (*E*). Values are means ± SD (n = 3). Values within columns followed by *the same letter* are statistically insignificant at the 0.05 level.

nitrogen application, and C_i of LTN_1 and NTN_1 treatments were 33.6 and 16.1% higher than these of LTN_0 and NTN_0, respectively (Fig. 1*B*). The trend of g_s and E under each treatment was consistent with C_i; g_s, and E of LTN_1 treatment increased by 141.1 and 119.3%, respectively, compared to LTN_0 treatment (Fig. 1*C*,*D*).

Chlorophyll fluorescence: Y_{NPQ} values at 1 DAT did not change compared to the initial values under all four treatments. With the increase of low-temperature treating days, the Y_{NPQ} value of LTN_0 treatment decreased first and then increased. LTN_1 and NTN_0 treatments both showed a downward trend, while NTN_1 treatment showed an upward trend (Fig. 2*A*). With the longer duration of low-temperature treatment, the F_v/F_m of LTN_1, NTN_0, and LTN_0 treatments showed a decreasing trend. However, the F_v/F_m of LTN_1 and NTN_1 treatments decreased slightly at 1 and 4 DAT, and the F_v/F_m of NTN_1 treatment first increased and then decreased. At 7 DAT, the F_v/F_m of all treatments decreased significantly when comparing to 1 DAT, and the F_v/F_m of LTN_0, LTN_1, NTN_0, and NTN_1 treatments decreased by 15.4, 10.4, 16.8, and 3.9%, respectively (Fig. 2*B*). For q_P, with the duration of low-temperature treatment, the q_P of LTN_0 and LTN_1 treatments increased first and then decreased, and the q_P of NTN_0 and NTN_1 treatments decreased first and then increased (Fig. 2*D*).

δ ^{13}C and δ ^{15}N: Under the same temperature treatment, the amounts of fixed ^{13}C assimilate (δ ^{13}C) in roots, stems, and leaves under N_1 were significantly higher than those under N_0 7 DAT (Table 1). Under the same nitrogen treatment, δ ^{13}C in roots, stems, and leaves under NT was higher than those under LT. Furthermore, ^{13}C assimilates were the highest in the stems of nitrogen-treated groups (LTN_1 and NTN_1). The δ value of LTN_1-treated stem was 42.8 and 140.1% higher than those of root and leaf, respectively; the δ value of NTN_1-treated stem was 30.9 and 81.0% higher than those of root and leaf. The amount

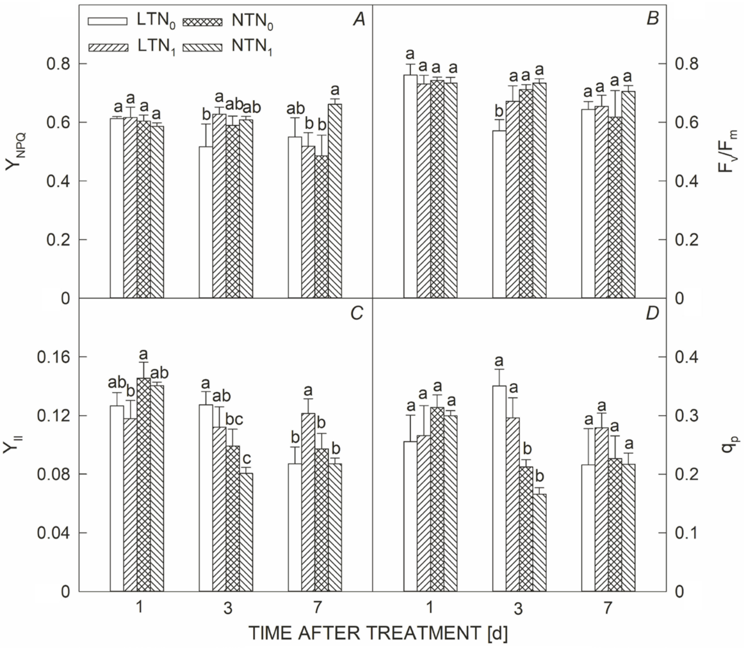

Fig. 2. Chlorophyll fluorescence characteristics of *Malus sieversii* seedlings after the application of nitrogen (N_1) and without nitrogen application (N_0) under normal temperature (NT) and low temperature (LT) conditions. (*A*) Nonphotochemical quenching (Y_{NPQ}), (*B*) quantum efficiency of PSII (Y_{II}), (*C*) initial light energy conversion efficiency of PSII (F_v/F_m), and (*D*) photochemical quenching (q_P). Values are means ± SD ($n = 3$). Values within columns followed by *the same letter* are statistically insignificant at the 0.05 level.

Table 1. The ^{13}C and ^{15}N assimilates (δ ^{13}C and δ ^{15}N) of different organs fixed in *Malus sieversii* seedlings after the application of nitrogen (N_1) and without nitrogen application (N_0) under normal temperature (NT) and low temperature (LT) conditions. Values are means ± SD ($n = 3$). Values within columns followed by *the same letter* are statistically insignificant at the 0.05 level.

Index	Treatment	Root [‰]	Stem [‰]	Leaf [‰]
δ ^{13}C	LTN_0	–36.10 ± 1.05^c	–36.55 ± 0.36^c	–37.75 ± 0.43^c
	LTN_1	21.02 ± 1.79^b	30.02 ± 3.32^b	12.51 ± 1.37^b
	NTN_0	–35.40 ± 0.76^c	–36.28 ± 0.44^c	–36.25 ± 0.68^c
	NTN_1	27.31 ± 1.53^a	35.76 ± 2.72^a	19.76 ± 1.19^a
δ ^{15}N	LTN_0	3.09 ± 1.72^c	3.66 ± 0.51^c	2.46 ± 1.37^b
	LTN_1	6,348.80 ± 1,953.34^b	7,569.71 ± 608.71^b	2,115.19 ± 646.85^b
	NTN_0	5.67 ± 1.10^c	4.72 ± 1.04^c	1.28 ± 0.50^b
	NTN_1	1,2416.28 ± 174.29^a	9,719.44 ± 1,380.31^a	7,508.85 ± 1,936.18^a

of fixed ^{15}N assimilates (δ ^{15}N) in roots, stems, and leaves of NTN_1 were respectively 95.6, 28.4, and 255.0% higher than those of LTN_1; however, there were no significant differences in the δ ^{15}N in roots, stems, and leaves under the LTN_0 and NTN_0 treatments.

Atom ^{13}C and Atom ^{15}N: Under the same temperature treatment, the Atom ^{13}C in roots, stems, and leaves under the N_1 were higher than these under the N_0; the Atom ^{13}C in roots of LTN_1 and NTN_1 were 5.8 and 6.4% higher than those in the roots of LTN_0 and NTN_0, respectively (Fig. 3*A*). Under the same nitrogen treatment, the Atom ^{13}C in roots, stems, and leaves under LT and NT treatments did not differ significantly. Across treatments, the Atom ^{13}C in roots, stems, and leaves were higher under LTN_1 and NTN_1 treatments. Under the same nitrogen treatment, the Atom ^{15}N in roots, stems, and leaves of NTN_1 were significantly higher than these of LTN_1 (Fig. 3*B*). The Atom ^{15}N in roots, stems, and leaves of NTN_1 increased by 78.9, 24.1, and 167.6%, respectively, compared with the LTN_1. However, there was no significant difference between the Atom ^{15}N in roots, stems, and leaves under NTN_0 and LTN_0 treatments. In addition, the organs closer to the soil were supplied with higher levels of N nutrients, which was consistent with the trend shown in Fig. 3*B*, *i.e.*, Atom ^{15}N: roots > stems > leaves.

Con ^{13}C and Con ^{15}N: Under the same nitrogen treatment, the ^{13}C absolute abundance (Con ^{13}C) in roots, stems, and leaves under NT was higher than those under LT. Under the same temperature treatment, the Con ^{13}C in roots, stems, and leaves of N_1 were higher than those of N_0 (Fig. 4*A*). Under the same nitrogen treatment, the Con ^{15}N in the roots, stems, and leaves of NTN_1 were significantly higher than these of LTN_1; the Con ^{15}N in roots, stems, and leaves of NTN_1 increased by 78.9, 64.3, and 193.0% compared with the LTN_1 treatment, respectively (Fig. 4*B*). Under the same temperature treatment, the Con ^{15}N in the roots, stems, and leaves of *M. sieversii* seedlings under N_1 were significantly higher than those under N_0. In the nitrogen treatment groups (LTN_1 and NTN_1), Con ^{15}N in all organs followed the trend roots > stems > leaves.

Correlation analyses: Correlation analyses of photosynthetic parameters and ^{13}C and ^{15}N in the leaves of *M. sieversii* seedlings at 7 DAT showed that the Atom ^{13}C of leaves was positively and significantly correlated with P_N, C_i, g_s, E, Atom ^{15}N, and Con ^{15}N, while Atom ^{15}N and Con ^{15}N were positively and significantly correlated with C_i, g_s, and E (Table 2). These data suggest that maintaining photosynthesis at 7 DAT was a benefit for the efficient accumulation of carbon and nitrogen.

Discussion

Photosynthesis is a complex physiological process. P_N is an important index to reflect photosynthetic capacity, which is defined as the CO_2 assimilation capacity per

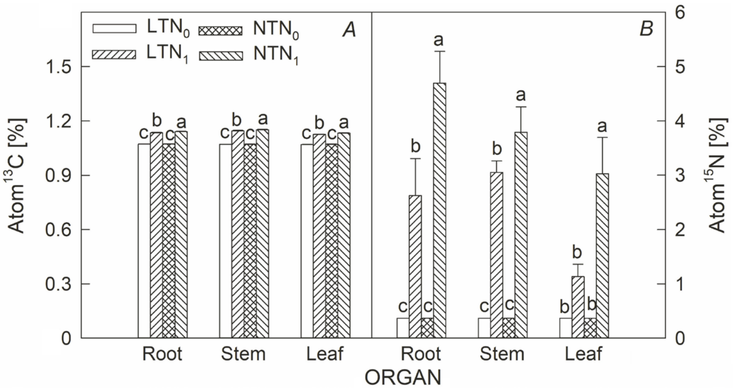

Fig. 3. ^{13}C and ^{15}N abundance in different organs of *Malus sieversii* seedlings after the application of nitrogen (N_1) and without nitrogen application (N_0) under normal temperature (NT) and low temperature (LT) conditions. (*A*) ^{13}C abundance (Atom ^{13}C), (*B*) ^{15}N abundance (Atom ^{15}N). Values are means ± SD (*n* = 3). Values within columns followed by *the same letter* are statistically insignificant at the 0.05 level.

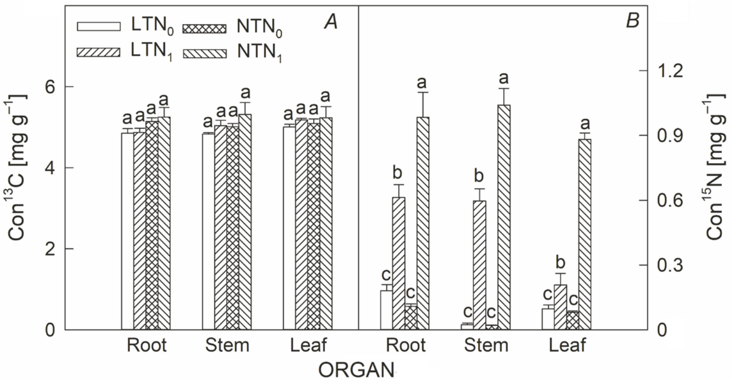

Fig. 4. ^{13}C and ^{15}N absolute abundance in different organs of *Malus sieversii* seedlings after the application of nitrogen (N_1) and without nitrogen application (N_0) under normal temperature (NT) and low temperature (LT) conditions. (*A*) ^{13}C absolute abundance (Con ^{13}C), (*B*) ^{15}N absolute abundance (Con ^{15}N). Values are means ± SD (*n* = 3). Values within columns followed by *the same letter* are statistically insignificant at the 0.05 level.

Table 2. Relationship between the net photosynthesis (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), transpiration rate (E), ^{13}C abundance (Atom ^{13}C), ^{13}C absolute abundance (Con ^{13}C), ^{15}N abundance (Atom ^{15}N), and ^{15}N absolute abundance (Con ^{15}N) in leaves of *Malus sieversii* seedlings. ** – highly significant ($p<0.01$) and * – significant ($0.01<p<0.05$). $n = 12$.

	P_N	C_i	g_s	E	Atom ^{13}C	Con ^{13}C	Atom ^{15}N	Con ^{15}N
P_N	1							
C_i	0.609*	1						
g_s	0.831**	0.863**	1					
E	0.863**	0.828**	0.984**	1				
Atom ^{13}C	0.827**	0.828**	0.936**	0.958**	1			
Con ^{13}C	0.659*	0.159	0.434	0.525	0.473	1		
Atom ^{15}N	0.585*	0.809**	0.848**	0.807**	0.797**	0.212	1	
Con ^{15}N	0.625*	0.749**	0.808**	0.790**	0.754**	0.403	0.942**	1

unit leaf area (Zhu *et al*. 2019). In this study, the low temperature decreased the P_N of *M. sieversii* seedlings. The decreases in P_N can be primarily due to the stomatal and nonstomatal limitations (Yang *et al*. 2008, Zhao *et al*. 2011, Pilon *et al*. 2018). The decrease in P_N caused by stomatal limiting factors was manifested by a decrease in g_s and E and a corresponding decrease in C_i, whereas the decrease in P_N caused by nonstomatal limiting factors was manifested by a decrease in g_s and E and an increase in C_i (Farquhar and Sharkey 1982, Liu *et al*. 2015). Under low-temperature stress, P_N, C_i, g_s, and E of *M. sieversii* seedlings all decreased, indicating that stomatal limitation was the primary cause of P_N decrease in *M. sieversii* seedlings. Previous studies have shown that prolonged low-temperature stress can cause stomatal closure and directly inhibit CO_2 assimilation and that this behavior is regulated by signal transduction, which is ultimately reflected in the decrease of g_s and C_i (Sierla *et al*. 2016, Stewart *et al*. 2016). In this study, nitrogen application significantly improved photosynthetic parameters. The possible reason may be that nitrogen application can indirectly affect photosynthesis by affecting leaf stomatal conductance and mesophyll conductance, thereby increasing the rate of CO_2 diffusion (Tosens *et al*. 2012, Xiong *et al*. 2015). Nitrogen in leaves can also act as a component of the photosynthetic apparatus, influencing photosynthesis (Yamamoto *et al*. 2017). Therefore, nitrogen application could alleviate the inhibitory effect of low-temperature stress on photosynthesis of *M. sieversii* seedlings and improved the photosynthetic capacity of seedling leaves.

Chlorophyll fluorescence parameters can reflect the rapid dynamic changes of photosynthetic rate caused by various environmental factors in plant photosynthesis (Sun *et al*. 2015a, Kalaji *et al*. 2017). q_P and Y_{NPQ} reflect the light energy absorbed by PSII antenna pigment, which is dissipated as heat or electron transport, thus characterizing plant tolerance to stress. It may further represent the degree of damage to the photosynthetic apparatus under stress (Singh *et al*. 2016). F_v/F_m reflects the potential light energy conversion efficiency of the PSII reaction center, and the change in F_v/F_m can be used to determine whether plants were inhibited by photoinhibition (Queiroz *et al*. 2016). The results showed that F_v/F_m and Y_{NPQ} of *M. sieversii* seedling leaves decreased first and then increased under low-temperature stress. This indicated that photoinhibition of *M. sieversii* at low temperature did not cause damage in PSII reaction center. Plants can protect their photosynthetic organs by increasing heat dissipation, which is a protective mechanism of plant adaptation to low temperatures (Li *et al*. 2005, Zhang and Shang 2010). In this study, nitrogen application increased the F_v/F_m and q_P of *M. sieversii* seedlings leaves under low-temperature stress. The results of this study showed that nitrogen application can help *M. sieversii* seedlings maintain strong light-harvesting and transmission ability when subjected to low-temperature stress.

The distribution and transfer of photosynthetically fixed carbon in plants is an important process of the ecosystem carbon cycle that is primarily determined by the physiological and genetic characteristics of plants as well as their growth rhythm (Poorter and Nagel 2000) and is limited by the natural environment of plant growth (such as light, temperature, water, and nutrients) (Lei *et al*. 2020). In this study, ^{13}C isotopes were used to mark the leaves of *M. sieversii* seedlings, and it was found that the abundance of ^{13}C in the roots, stems, and leaves of the seedlings under low-temperature treatment was significantly lower than that of normal temperature treatment. This is because, at low temperatures, the rate of photochemical processes in plants needs to be adjusted to compensate for the decreased metabolic capacity for photosynthate consumption. Thus, the impact of low temperatures on photosynthesis and carbon gain ultimately determines plant growth and distribution (Ball *et al*. 1991, Slot *et al*. 2005, Ensminger *et al*. 2006). In this experiment, the $\delta\ ^{13}C$ and Atom ^{13}C in different parts of the seedlings increased significantly after nitrogen application. This was similar to the findings of Peng *et al*. (2019) on winter jujube. Nitrogen fertilizer is an important factor influencing fruit tree carbon absorption, transport, and distribution (Krapp *et al*. 2005). Increasing the application of nitrogen fertilizer within a certain range can improve the photosynthetic performance of plants and increase their net primary productivity, which in turn increases the total carbon sequestration of plants (Neff *et al*. 2002). The growth center of fruit trees can accumulate photochemical compounds, and the movement and distribution of assimilated products usually change with the change of growth center (Sun *et al*. 2015b). In our study, *M. sieversii* seedlings grew primarily on the assimilates produced by the leaves, and carbon compounds produced by photosynthesis are primarily stored in the stems, indicating that there was no output lag in the photosynthetic products of *M. sieversii* seedlings.

The effect of fertilization on fruit trees is not only related to nutrient uptake, but also its distribution in various organs (Li *et al*. 2011). We used the ^{15}N isotope to label its substrate and found that in the seedling stage of *M. sieversii*, the absorbed nitrogen is mainly transported to the root system, and the nitrogen absorbed by the roots of the *M. sieversii* seedlings could not be quickly absorbed and transferred to the leaves. Higher nitrogen concentrations may have resulted in more nutrients coming into contact with the roots and more nutrients being transported in intercepted forms in the soil (Briat *et al*. 2020). Low-temperature stress can affect nitrogen absorption and assimilation directly or alter patterns of root growth indirectly, thereby reducing nitrogen absorption (Atkin and Cummins 1994, Volder *et al*. 2000). Dong *et al*. (2001) found that increasing soil temperatures from 12 to 20°C increased the total amount and rate of ^{15}N uptake. In this experiment, under nitrogen application treatments, the $\delta\ ^{15}$N, Atom ^{15}N, and Con ^{15}N of different organs of *M. sieversii* seedlings under NT treatments were significantly higher than those under LT treatments. Nitrogen application treatments increased nitrogen uptake by plants at low temperatures, as well as nitrogen concentration in the root environment, resulting in improved recyclable nitrogen effectiveness and transfer nitrogen availability for leaf growth (Atkinson *et al*. 2015).

After 7 DAT, nitrogen application at low temperature increased C_i, g_s, and E by 10.3, 141.1, and 119.3%, respectively. The correlation analysis revealed that C_i, g_s, and E of *M. sieversii* seedling leaves were highly significantly and positively correlated with Atom^{13}C, Atom^{15}N, and Con^{15}N. The decrease in photosynthesis was accompanied by a reduction in carbon and nitrogen metabolism (Wu and Tao 1995, Tian *et al*. 2001, Nghiem *et al*. 2018). Since sufficient nitrogen application at low temperatures can compensate for chlorophyll content, greatly improve light energy-use efficiency, and accelerate the recovery of carbon and nitrogen metabolism in plants (Martin *et al*. 2002, Liu *et al*. 2011, Zhang *et al*. 2017, Shah *et al*. 2021).

Conclusion: In this study, low temperature inhibited the photosynthetic capacity of seedlings and reduced the accumulation of carbon assimilates in roots, stems, and leaves of apple plants. Nitrogen application at low and normal temperatures can promote the absorption and distribution of carbon and nitrogen nutrients in multiple organs of seedlings. In the future, more emphasis could be placed on the relationship between photosynthetic carbon and nitrogen metabolism in *M. sieversii* and its regulation.

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