Ammonium Transporter (BcAMT1.2) Mediates the Interaction of Ammonium and Nitrate in Brassica campestris

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The provision of ammonium (NH4+) and nitrate (NO3−) mixture increases the total nitrogen (N) than the supply of sole NH4+ or NO3− with the same concentration of total N; thus, the mixture contributes to better growth in Brassica campestris. However, the underlying mechanisms remain unknown. In this study, we analyzed NH4+ and NO3− fluxes using a scanning ion-selective electrode technique to detect under different N forms and levels in B. campestris roots. We observed that the total N in fluxes with NH4+ and NO3− mixture were 1.25- and 3.53-fold higher than those with either sole NH4+ or NO3−. Furthermore, NH4+ and NO3− might interact with each other under coexistence. NO3− had a positive effect on net NH4+ in flux, whereas NH4+ had a negative influence on net NO3− in flux. The ammonium transporter (AMT) played a key role in NH4+ absorption and transport. Based on expression analysis, BcAMT1.2 differed from other BcAMT1s in being upregulated by NH4+ or NO3−. According to sequence analysis and functional complementation in yeast mutant 31019b, AMT1.2 from B. campestris may be a functional AMT. According to the expression pattern of BcAMT1.2, β-glucuronidase activity, and the cellular location of its promoter, BcAMT1.2 may be responsible for NH4+ transport. Following the overexpression of BcAMT1.2 in Arabidopsis, BcAMT1.2-overexpressing lines grew better than wildtype lines at low NH4+ concentration. In the mixture of NH4+ and NO3−, NH4+ influxes and NO3− effluxes were induced in BcAMT1.2-overexpressing lines. Furthermore, transcripts of N assimilation genes (AtGLN1.2, AtGLN2, and AtGLT1) were significantly upregulated, in particular, AtGLN1.2 and AtGLT1 were increased by 2.85–8.88 times in roots, and AtGLN1.2 and AtGLN2 were increased by 2.67–4.61 times in leaves. Collectively, these results indicated that BcAMT1.2 may mediate in NH4+ fluxes under the coexistence of NH4+ and NO3− in B. campestris.

Keywords: AMT1.2, Brassica campestris, interaction, NH4+ flux, NO3− flux

Frontiers in Plant Science | www.frontiersin.org February 2020 | Volume 10 | Article 1776

ORIGINAL RESEARCH
published: 04 February 2020
doi: 10.3389/fpls.2019.01776

Edited by:
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Specialty section:
This article was submitted to Plant Nutrition, a section of the journal Frontiers in Plant Science

Received: 20 September 2019
Accepted: 19 December 2019
Published: 04 February 2020

Citation:
Zhu Y, Huang X, Hao Y, Su W, Liu H, Sun G, Chen R and Song S (2020) Ammonium Transporter (BcAMT1.2) Mediates the Interaction of Ammonium and Nitrate in Brassica campestris. Front. Plant Sci. 10:1776. doi: 10.3389/fpls.2019.01776
INTRODUCTION

The efficiency and availability of nitrogen (N) have decisive influences on plant growth and crop productivity (Hachiya and Sakakibara, 2017). For most plants, nitrate (NO$_3^-$) and ammonium (NH$_4^+$) are major sources of inorganic N. In C$_3$ plants, NO$_3^-$ reduction is inhibited by elevated carbon dioxide (CO$_2$), whereas NH$_4^+$ assimilation is affected little (Bloom et al., 2010). NH$_4^+$ is believed to be a preferable N source for the future when global levels of CO$_2$ are predicted to increase (Hachiya and Sakakibara, 2017). However, NH$_4^+$ at millimolar concentrations in the soil solution or hydroponic culture causes growth suppression and chlorosis (ammonium toxicity) in plants, unlike NO$_3^-$ at the same concentration (Miller and Cramer, 2004).

Extensive studies suggest that a mixture of NO$_3^-$ and NH$_4^+$ nutrition stimulates plant growth beyond that observed with NO$_3^-$ or NH$_4^+$ alone (Britto and Kronzucker, 2001). The use of the mixture enhances N-use efficiency and improves crop productivity (Wang and Shen, 2011; Hachiya et al., 2012). The mixture greatly improves plant growth and population productivity in maize, especially in high planting density (Wang et al., 2019). When NO$_3^-$ and NH$_4^+$ co-exist, NH$_4^+$ responses are altered by NO$_3^-$ and vice versa (Hachiya and Sakakibara, 2017). Previous researchers have investigated the interaction between NH$_4^+$ and NO$_3^-$ fluxes. Compared with the influx with sole NH$_4^+$, net NH$_4^+$ influx has been shown to increase with a mixture of NH$_4^+$ and NO$_3^-$ in rice using an N labeling technique (Kronzucker et al., 1999); and a similar effect has been observed in Brassica napus (Babourina et al., 2007), Populus popularis (Luo et al., 2013), and Triticum aestivum (Zhong et al., 2015) using the microelectrode technique, whereas a negative effect has been observed in tea (Ruan et al., 2016). Similarly, NH$_4^+$ affects NO$_3^-$ fluxes (Kronzucker et al., 1999; Zhong et al., 2015; Ruan et al., 2016). Therefore, the interaction between NH$_4^+$ and NO$_3^-$ may depend on plant species or N conditions.

Under natural conditions, plant growth and development are typically limited by N availability; thus, plants have evolved different transport and signaling mechanisms to adapt to different N sources (Kiba and Krapp, 2016). NH$_4^+$ and NO$_3^-$ fluxes are mediated by specific genes for ammonium transporters (AMTs) and nitrate transporters (NRTs), respectively (Nacry et al., 2013). In Arabidopsis, NRTs include 72 members belonging to four families: nitrate transporter 1/peptide transporter family (NRT1/PTR), NRT2, chloride channels (CLC), and slow anion channel-associated 1/slow anion channel homologs (SLAC1/SLAH) (Krapp et al., 2014). Some of these genes are related to NO$_3^-$ uptake, xylem loading, and efflux systems (Krapp et al., 2014). AMTs generally contain AMT1 and AMT2 subfamilies (Loque and von Wirén, 2004; McDonald and Ward, 2016). In Arabidopsis, AMT1.1, AMT1.2, AMT1.3, and AMT1.5 are expressed in roots (Yuan et al., 2007), and play different roles during NH$_4^+$ assimilation (Yuan et al., 2007). AMT1.1, AMT1.3 and AMT1.5 contribute to NH$_4^+$ absorption from the soil, whereas AMT1.2 mediates NH$_4^+$ uptake via the apoplastic transport route (Yuan et al., 2007), and exclusively regulates NH$_4^+$ flux into the vasculature (Straub et al., 2017). Furthermore, plant cells eliminate the activity of AMT1.1 (Lanquar et al., 2009) or AMT1.3 (Wang et al., 2013) to avoid excessive NH$_4^+$ accumulation.

AMTs transcript levels are affected by the N status of plants. N deficiency strongly induces AMT1.1, AMT1.3, and AMT1.5 transcription (Yuan et al., 2007; Camañes et al., 2009), whereas that of AMT1.2 is not affected to a large extent (Pearson et al., 2002). When NH$_4^+$ is resupplied to N-deficient plants, AMT1.1, AMT1.3, and AMT1.5 genes are downregulated (Yuan et al., 2007); whereas AMT1.2 is upregulated (Pearson et al., 2002; Yuan et al., 2007). Furthermore, AMTs transcript levels are subjected to control by NO$_3^-$ (Camañes et al., 2009). However, AMT homologs in different species are often not similarly regulated, which may reflect the different nutritional needs of particular species (Loque and von Wirén, 2004).

Flowering Chinese cabbage (Brassica campestris L. ssp. chinensis var. utilis Tsen et Lee) is a prominent vegetable in South China due to the taste and nutrient content of its flower stalk, and it has the largest growing area and yield in South China (Song et al., 2012). In our previous study, we showed that NH$_4^+$ and NO$_3^-$ mixtures were more beneficial to B. campestris qualities than sole N source, and they improved N-use efficiency (Song et al., 2012). However, there is no information regarding the interactions between NH$_4^+$ and NO$_3^-$ and how this affects N uptake at physiological, morphological, and molecular levels. In this study, we examined the characteristics of NH$_4^+$ and NO$_3^-$ fluxes and their interactions in B. campestris using the scanning ion-selective electrode technique (SIET). Regarding the analysis of AMT1s transcripts, we observed that the expression pattern of BcAMT1.2 differed from those of other BcAMTs in B. campestris. Furthermore, the GUS activity of BcAMT1.2pro–GUS and used reverse genetic approaches in Arabidopsis suggested to elucidate the physiological roles of BcAMT1.2 in response to the coexistence of NH$_4^+$ and NO$_3^-$.

Altogether, these results indicated that BcAMT1.2 participated in the interaction between NH$_4^+$ and NO$_3^-$ in B. campestris.

Abbreviations: AMT, ammonium transporter; CBL, calcineurin B-like protein; CIPK, CBL-interacting serine/threonine-protein kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GDH, glutamate dehydrogenase; GOGAT, glutamate dehydrogenase and NADH-dependent glutamate synthase; GS, glutamine synthetase; GUS, β-glucuronidase; HATS, high-affinity transport system; KD, kilo-dalton; MES, 2-(N-morpholino)ethanesulfonic acid hydrate buffer; LATS, low-affinity transport system; N, nitrogen; NH$_4^+$, ammonium; NO$_3^-$, nitrate; NRT, nitrate transporter; ORF, open reading frame; qPCR, quantitative real-time polymerase chain reaction; SIET, scanning ion-selective electrode technique; TM, transport membrane.

MATERIALS AND METHODS

Plant Materials and Culture Conditions

The flowering Chinese cabbage variety “Youlv80”, which was provided by the Guangzhou Academy of Agriculture Sciences (Guangdong Province, China), was used in this study. Experiments were carried out in a controlled-environment...
growth chamber programmed for 16 h light/8 h dark and a 25/23°C day/night cycle, relative humidity of 70%, and light intensity of 150 μmol m⁻² s⁻¹. Seeds were sterilized in 2.5% (w/v) NaClO for 10 min, washed five times with sterile distilled water, and cultured on vertical 0.7% agar plates (17.5 cm long × 16 cm wide × 3 cm high). The agar medium contained 1/2 no-N basal modified MS salt (pH 5.8), supplemented with 4 mmol L⁻¹ NaNO₃ as the N source. On the 6th day of germination, the seedlings were hydroponically cultured in 1/2 MS as an N-deficient treatment for 7 d. The nutrient solution was replaced every 2 days and continually aerated by air pumps. After N starvation, the seedlings were harvested to measure ion fluxes or other treatments.

**Measurement of NH₄⁺ and NO₃⁻ Ion Fluxes on the Surface of *B. campestris* Roots**

To monitor net fluxes of NH₄⁺ and NO₃⁻ in *B. campestris* roots in response to different N treatments, the primary roots were selected and immersed in measuring solutions with different treatment [A. 0.25 mmol L⁻¹ NH₄⁺: 0.1 mmol L⁻¹ CaCl₂, 0.3 mmol L⁻¹ 2-(N-morpholino) ethanesulfonic acid hydrate buffer (MES) (pH 5.8, same as below), and 0.25 mmol L⁻¹ NH₄Cl; B. 1.0 mmol L⁻¹ NH₄⁺: 0.1 mmol L⁻¹ CaCl₂, 0.3 mmol L⁻¹ MES, and 1.0 mmol L⁻¹ NH₄Cl; C. 0.25 mmol L⁻¹ NO₃⁻: 0.1 mmol L⁻¹ CaCl₂, 0.3 mmol L⁻¹ MES, and 0.25 mmol L⁻¹ NaNO₃; D. 1.0 mmol L⁻¹ NO₃⁻: 0.1 mmol L⁻¹ CaCl₂, 0.3 mmol L⁻¹ MES, and 1.0 mmol L⁻¹ NaNO₃; E. NH₄⁺: 0.1 mmol L⁻¹ NH₄Cl, 0.3 mmol L⁻¹ MES, 0.25 mmol L⁻¹ NH₄Cl, and 0.75 mmol L⁻¹ NaNO₃]. Prior to analysis, *B. campestris* roots were transferred to Petri dishes containing 10 mL of measuring solution and equilibrated for 10 min. The equilibrated roots were moved to another Petri dish containing fresh measuring solution to measure NH₄⁺ or NO₃⁻ flux. Ion flux was measured using SIET (MA01002 system; Younger USA Science and Technology Limited Liability Company, Amherst, MA, USA), which was conducted on-site at Xuyue Science and Technology Company Limited (Beijing, China). The SIET system and its application process for ion flux detection have been previously described in detail (Zhong et al., 2015; Ruan et al., 2016).

To determine the regions along the root where the maximal ion influxes of NH₄⁺ or NO₃⁻ occurred, a preliminary experiment was conducted, in which an initial measurement was performed at different points from the root tip (1, 2, 4, 10, 15, 20, 25, 30, and 35 mm). Based on this experiment, we selected 20 and 30 mm from the root apex as the measurement site of NH₄⁺ and NO₃⁻ influxes (Supplementary Figure S2). The recording rate of ion flux was one reading every 6 s and this lasted for 10 min in each root. Six similar seedlings per treatment were measured.

To evaluate the interaction of NH₄⁺ and NO₃⁻ fluxes, the roots of *B. campestris* were soaked in measurement solutions. The effect of NO₃⁻ on NH₄⁺ flux [F (with NO₃⁻): 0.1 mmol L⁻¹ CaCl₂, 0.3 mmol L⁻¹ MES, 0.1 mmol L⁻¹ NH₄Cl, and 1 mmol L⁻¹ NaNO₃; G (without NO₃⁻): 0.1 mmol L⁻¹ CaCl₂, 0.3 mmol L⁻¹ MES, 0.1 mmol L⁻¹ NH₄Cl]. The NH₄⁺ flux was measured using SIET for 3 min after equilibration in measuring solution for 10 min. Thereafter, 1.0 mmol L⁻¹ NH₄Cl was added to the measuring solution, which was mixed thoroughly by expelling and drawing it into a pipette during the first 1–2 min. NO₃⁻ flux was measured using SIET for 17 min. The effect of NH₄⁺ on NO₃⁻ flux [H (with NH₄⁺): 0.1 mmol L⁻¹ CaCl₂, 0.3 mmol L⁻¹ MES, 0.1 mmol L⁻¹ NaNO₃, with 1 mmol L⁻¹ NH₄Cl; I (without NH₄⁺): 0.1 mmol L⁻¹ CaCl₂, 0.3 mmol L⁻¹ MES, 0.1 mmol L⁻¹ NaNO₃]. NO₃⁻ flux was measured utilizing SIET for 3 min after equilibrated in measurement solution for 10 min. Thereafter, 1.0 mmol L⁻¹ NaNO₃ was added to the measuring solution. The test process was the same as that described above. Six biological replicates were used for each measurement.

**Analysis of AMTs and NRTs Transcripts in Roots**

*B. campestris* seedlings that had been N-starved for 7 d were subjected to different N treatments. The treatments were as follows: (1) exposure to different N levels: 0, 0.25, and 1.0 mmol L⁻¹ NaNO₃/NH₄Cl were added, then roots were harvested after 20 min during the N-resupply treatments; (2) effect of NH₄⁺ on NO₃⁻: 1 mmol L⁻¹ NH₄Cl was added into the solution with or without NaNO₃, then roots were harvested at 0, 10, and 20 min after adding NH₄Cl; (3) effect of NO₃⁻ on NH₄⁺: 1 mmol L⁻¹ NaNO₃ was added into the solution with or without NH₄Cl, then roots were harvested at 0, 10, and 20 min after adding NaNO₃. All samples were immediately frozen in liquid nitrogen and stored at –80°C for quantitative real-time polymerase chain reaction (qPCR).

**qPCR**

Total RNA was extracted from samples using an Eastep® Super Total RNA Extraction Kit (Promega, Beijing, China) and was reverse transcribed using a PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa Bio, Dalian, China). The qPCR was performed in a LightCycler 480 Real-Time PCR System (Roche, Basel, Switzerland), using SYBR® Premix Ex Taq™ (TaKaRa Bio). The primer pairs used are listed in Supplementary Table S1. GAPDH was used as an internal control. Three biological replicates were used to calculate relative gene expression levels.

**BcAMT1.2 Cloning and Sequence Analysis**

Based on the AMT1.2 sequence of *Brassica rapa* (retrieved from GenBank, accession no. XM_009113156.2), primers (Supplementary Table S1) were designed to amplify the full-length of *BcAMT1.2* by PCR using the cDNA of *B. campestris* as the template. The PCR product was cloned into binary vector pCAMBIA3301 (Dingguo Biotechnology, Beijing, China) that carried two CaMV 35S promoters (35Sписыва) and phosphinothricin resistance marker genes and was sequenced. Based on the deduced amino acid sequence, transmembrane motifs, subcellular localization, and signature motifs were predicted using Protter (http://wlab.ethz.ch/protter/), Softberry (http://www.softberry.com), and Weblogo (http://weblogo.berkeley.edu/logo.cgi), respectively. The multiple sequence alignment of 32 AMT proteins from plants was performed using the ClustalW method and a phylogenetic tree was constructed using MEGA 6.0 based on the neighbor-joining algorithm. Bootstrap analysis was carried out with 1000 replicates. The
accession numbers of the amino acid sequences of the AMTs are listed in Supplementary Table S2.

**Heterologous Expression of BcAMT1.2 in Yeast**

The open reading frame (ORF) of BcAMT1.2 was amplified by PCR using the primers (Supplementary Table S1) and constructed into pYES2 vector (Waryong Biotechnology, Beijing, China). As described by Yuan et al. (2007), pYES2 and pYES2-BcAMT1.2 plasmids were transformed into yeast mutant cells 31019b (\(\Delta mep1, \Delta mep2, \Delta mep3,\) and \(\text{ura}3\)). Growth complementation assays were performed on a solid yeast N base medium at pH 5.8 and were supplemented with 2% galactose and 2 mmol L\(^{-1}\) arginine or NH\(_4\)Cl as the sole N source. Yeast cells were incubated at 30°C for 3 days.

**BcAMT1.2::GUS Constructs Used for Arabidopsis Transformation and β-Glucuronidase (GUS) Assays**

The BcAMT1.2::GUS construct, containing 1519 bp of BcAMT1.2 promoter cloned by our lab, was amplified by PCR from the DNA of B. campestris using special primers (Supplementary Table S1). They were ligated into the pCAMBIA1391 vector which harbored GUS, without a promoter (Dingguo Biotechnology), yielding a pCAMBIA1391-BcAMT1.2pro::GUS construct. Via Agrobacterium tumefaciens-mediated transformation, BcAMT1.2pro::GUS transgenic plants were generated in a wildtype (Col-0) background. Second generation (T\(_2\)) seeds were germinated on a medium containing 1/2 modified MS, 4 mmol L\(^{-1}\) NaNO\(_3\) and 0.7% agar for 14 d (growth conditions as described above). Some seedlings were subjected to N-free MS treatment for 4 d, and transferred to either the nutrition of N-free MS or the one of N-free MS containing 0.25 mmol L\(^{-1}\) NH\(_4\)Cl, and 0.75 mmol L\(^{-1}\) NaNO\(_3\), and NH\(_4\)\(^+\) and NH\(_4\)\(^+\) fluxes were measured using SIET. Six similar seedlings per treatment were selected to measure ion flux.

**Arabidopsis seeds were pre-cultured for 4 d (as described above for the ion flux test) and transferred to a 1/2 MS agar-medium (containing 0.25 mmol L\(^{-1}\) NH\(_4\)Cl + 0.75 mmol L\(^{-1}\) NaNO\(_3\)) for 10 d. Shoots and roots were harvested to isolate total RNA for qPCR analysis and measure the content of NH\(_4\)\(^+\) and NO\(_3\)\(^-\), as described by Ivančić and Degobbis (1984) and Downes (1978), respectively. Three biological replicates were used for each measurement. The wildtype was used as control in the above tests.**

**Statistical Analysis**

Microsoft Excel (Microsoft Corporation, USA) and SPSS 17 (SPSS Incorporation, Chicago, USA) were used to analyze the data. An one-way ANOVA was performed. SigmaPlot 11.1 (Jandel Scientific Software, San Rafael, CA, USA) was utilized to draw figures for data presentation. For gene expression analysis, Hem I software (Heatmap Illustrator, version 1.0) (Deng et al., 2014) was used to generate hierarchical cluster heat maps.

**RESULTS**

**Net Fluxes of NO\(_3\)\(^-\) and NH\(_4\)\(^+\) in Response to Treatment With Different N Forms and Levels**

After 7 d N-starvation, B. campestris roots were immersed in measuring solutions containing different N forms (1 mmol L\(^{-1}\) NH\(_4\)Cl, 1 mmol L\(^{-1}\) NaNO\(_3\), 0.25 mmol L\(^{-1}\) NH\(_4\)Cl + 0.75 mmol L\(^{-1}\) NaNO\(_3\)) to monitor net NO\(_3\)\(^-\) and NH\(_4\)\(^+\) fluxes. Net NO\(_3\)\(^-\) and NH\(_4\)\(^+\) flux curves are shown in Figures 1A–C. Net NO\(_3\)\(^-\) fluxes fluctuated gently in sole NO\(_3\)\(^-\) (Figure 1A) or mixed N (Figure 1C). In contrast, net NH\(_4\)\(^+\) fluxes increased transitorily, then decreased gradually and subsequently increased in sole NH\(_4\)\(^+\) (Figure 1B), whereas net NH\(_4\)\(^+\) fluxes changed stably in the mixed N treatment (0.25 mmol L\(^{-1}\) NH\(_4\)Cl + 0.75 mmol L\(^{-1}\) NaNO\(_3\)) (Figure 1C). Compared with fluxes in sole N source, NO\(_3\)\(^-\) fluxes were decreased in mixed N forms and NH\(_4\)\(^+\) fluxes were close to the fluxes of sole NH\(_4\)\(^+\) (1 mmol L\(^{-1}\) NH\(_4\)Cl) which did not decrease with increasing NH\(_4\)\(^+\) concentration (Figures 1A–C). Thus, the mixed N treatment significantly enhanced total N fluxes (Figure 1D) under the same total N conditions (i.e. 3.53-fold for sole NO\(_3\)\(^-\), 1.25-fold for sole NH\(_4\)\(^+\)).
To eliminate the effect of N concentration on N fluxes, we measured the net NO$_3^-$ and NH$_4^+$ fluxes under different N levels. The net NO$_3^-$ fluxes increased significantly with an increase in N concentration, while NH$_4^+$ influx rates in 1 mmol L$^{-1}$ N were 2.66-fold and 1.33-fold of those in 0.25 mmol L$^{-1}$, respectively (Figures 2A, B). In addition, NH$_4^+$ influx rates were 1.42 and 2.88 times higher than those of NO$_3^-$ at N levels of 0.25 and 1 mmol L$^{-1}$, respectively. This indicated that the roots of *B. campestris* showed a preference for NH$_4^+$ over NO$_3^-$.

The absorption of NH$_4^+$ and NO$_3^-$ are mediated by AMTs and NRTs, respectively. To investigate how the expression of the N transporter genes was affected in response to the addition of NH$_4^+$ or NO$_3^-$, we measured the mRNA levels of four *BcAMT* genes (*BcAMT1.1*, *BcAMT1.2*, *BcAMT1.3*, and *BcAMT1.5*) and five *BcNRT* genes (*BcNRT1.1*, *BcNRT1.8*, *BcNRT2.1*, *BcNRT3.1*, and *BcNAXT1*) using qPCR. After a 7-d period of N-starvation, the addition of different N levels had significant effects on the expression levels of *BcAMT* and *BcNRT* genes. Compared with the expression levels at nitrogen starvation (0 mmol L$^{-1}$ N), the expression levels of *BcAMT1.1*, *BcAMT1.3*, and *BcAMT1.5* decreased in response to NH$_4^+$ (0.25 and 1 mmol L$^{-1}$) and 0.25 mmol L$^{-1}$ NO$_3^-$, but they increased in response to 1 mM NO$_3^-$ treatment (i.e. 1.30–1.88 times) (Figure 2C). In contrast, *BcAMT1.2* expression increased significantly under 1 mM NH$_4^+$ (i.e. 2.30 times higher), and it was also significantly enhanced with an increase in NO$_3^-$ concentration (i.e. 2.01 and 6.51 times higher in response to 0.25 mmol L$^{-1}$ and 1 mmol L$^{-1}$ NO$_3^-$ treatment, respectively) (Figure 2C). *BcAMTs* expression levels were increased by supplying 1 mmol L$^{-1}$ NO$_3^-$, with the expression of *BcAMT1.1*, *BcAMT1.2*, *BcAMT1.3*, and *BcAMT1.5* being 2.36, 2.83, 3.12, and 2.41 times higher, respectively, than that with the same NH$_4^+$ concentration (Figure 2C). In contrast to 0.25 mmol L$^{-1}$ NH$_4^+$, adding NO$_3^-$ enhanced *BcAMT1.2* expression levels (Supplementary Figure S3A).
Compared with the expression in nitrogen starvation, BcNRT1.1 expression was lower following treatment with 0.25 mmol L\(^{-1}\) NH\(_4^+\), although it did not appear to be affected by treatment with 1 mmol L\(^{-1}\) NH\(_4^+\). In contrast, although the expression of other BcNRTs was not affected by treatment with 0.25 mmol L\(^{-1}\) NH\(_4^+\), the expression was significantly enhanced in response to treatment with 1 mmol L\(^{-1}\) NH\(_4^+\) (Figure 2D). Except for BcNRT1.1, the expression of other BcNRTs increased gradually with the concentration of NO\(_3^-\) (Figure 2D). BcNRTs expression was increased by supplying 1 mmol L\(^{-1}\) NO\(_3^-\), with the expression of BcNRT1.1, BcNRT1.8, BcNRT2.1, BcNRT3.1, and BcNAXT1 being 2.74, 2.03, 3.06, 2.68, and 1.20 times higher than that with the same NH\(_4^+\) concentration, respectively (Figures 2C, D). In contrast to treatment with 1 mmol L\(^{-1}\) NO\(_3^-\), adding a mixture of 0.25 mmol L\(^{-1}\) NH\(_4^+\) and 1 mmol L\(^{-1}\) NO\(_3^-\) decreased the expression levels of BcNRT1.8, BcNRT2.1, BcNRT3.1, and BcNAXT1 (Supplementary Figure S3B).

Interactions Between NH\(_4^+\) and NO\(_3^-\) in Roots of B. Campestris

To elucidate the interaction between NH\(_4^+\) and NO\(_3^-\), we undertook dynamic monitoring of NH\(_4^+\) fluxes after adding NH\(_4^+\) to the bathing solution either with or without NO\(_3^-\). Before adding NH\(_4^+\), net NH\(_4^+\) influxes of bathing solution with NO\(_3^-\) were higher than that of bathing solution without NO\(_3^-\) (Figure 3A). Regardless of whether the bathing solution contained NO\(_3^-\) or not, net NH\(_4^+\) influxes rates after adding NH\(_4^+\) increased markedly for 30 to 90 s (t1 stage), then decreased quickly for 180 s (t2 stage), then increased gradually (t3 stage), followed by a slow relaxation to the stable level (t4 stage) (Figure 3A). With the exception of several time points in the t2 stage, net NH\(_4^+\) influxes of the solution with NO\(_3^-\) was higher than that of the solution without NO\(_3^-\). There was no obvious difference between NH\(_4^+\) flux rates in the bathing solution with or without Na\(^+\), indicating that adding Na\(^+\) had no obvious effect on NH\(_4^+\) flux in this study (Supplementary Figure S4). It indicated that NO\(_3^-\) influenced NH\(_4^+\) flux rates.

Before adding NO\(_3^-\), NO\(_3^-\) fluxes of the bathing solution without NH\(_4^+\) showed net influxes, whereas those with NH\(_4^+\) showed net effluxes (Figure 3B). Net NO\(_3^-\) influx began to increase rapidly for 60 s (t1 stage) after adding NO\(_3^-\), and decreased gradually for 330–420 s (t2 stage). Subsequently, net NO\(_3^-\) influx rates increased slowly for approximately 210 s (t3 stage) and remained stable (t4 stage). During the stages t1 and t2, net NO\(_3^-\) influx rates of the bathing solution with NH\(_4^+\) were lower than those for the bathing solution without NH\(_4^+\). There

FIGURE 2 | NO\(_3^-\) and NH\(_4^+\) net fluxes and expressions of BcAMTs and BcNRTs in B. campestris roots in response to treatments with different N levels. (A) Net NH\(_4^+\) fluxes in different NH\(_4^+\) levels (0.25, and 1 mmol L\(^{-1}\) NH\(_4^+\)). (B) Net NO\(_3^-\) fluxes in different NO\(_3^-\) levels (0.25, and 1 mmol L\(^{-1}\) NO\(_3^-\)). (C, D) BcAMTs and BcNRTs expression in different N levels, respectively (0.25, and 1 mmol L\(^{-1}\) NH\(_4^+\)/NO\(_3^-\)). GAPDH was used as internal control. The data represent the mean ± SE (n = 6 in A–B, n = 3 in C–D). Significant differences (P < 0.05) between treatments are indicated by different letters.
FIGURE 3 | Interaction between NO$_3^-$ and NH$_4^+$ fluxes on root surfaces of B. campestris. (A) Influence of NO$_3^-$ on net NH$_4^+$ fluxes after adding 1 mmol L$^{-1}$ NH$_4^+$ to the bathing solution with or without 1 mmol L$^{-1}$ NO$_3^-$; (B) Influence of NH$_4^+$ on net NO$_3^-$ fluxes after adding 1 mmol L$^{-1}$ NO$_3^-$ to the bathing solution with or without 1 mmol L$^{-1}$ NH$_4^+$. Changes in net NH$_4^+$/NO$_3^-$ fluxes in roots at 30 s intervals are presented. The vertical arrow indicates the point at which 1 mmol L$^{-1}$ NH$_4^+$ or NO$_3^-$ was added. t1–t4 represent the stages of net NH$_4^+$/NO$_3^-$ fluxes after adding NH$_4^+$/NO$_3^-$ to the bathing solution. The data represent the mean ± SE (n = 4–6) during the measurement period.
was no obvious difference between the bathing solution with and without \( \text{NH}_4^+ \) during the stages t3 and t4, indicating that \( \text{NH}_4^+ \) affected net \( \text{NO}_3^- \) influxes.

**BcAMTs and BcNRTs Expression in Response to Treatment With Adding NH\(_4^+\) or NO\(_3^-\) in *B. campestris* Roots**

Compared with the expression in N deficiency, adding \( \text{NH}_4^+ \) without \( \text{NO}_3^- \) markedly reduced the expression levels of *BcAMT1.1*, *BcAMT1.3*, and *BcAMT1.5*, whereas it induced the expression of *BcAMT1.2* after 20 min (Figure 4A). Moreover, adding \( \text{NH}_4^+ \) with \( \text{NO}_3^- \), resulted in a sharp increase in the expression of *BcAMT1.2* and a weak transient increase in the expression of *BcAMT1.1*, *BcAMT1.3*, and *BcAMT1.5* (Figure 4A). Sole \( \text{NO}_3^- \) treatment increased the expression of *BcAMT1.2* and *BcAMT1.5* and decreased that of *BcAMT1.1* and *BcAMT1.3* (Figures 4A, B), whereas adding \( \text{NO}_3^- \) to the nutrient solution containing \( \text{NH}_4^+ \) resulted in a decrease in the transcript levels of four *BcAMT1s* (Figure 4B).

In terms of NRTs expression, sole \( \text{NH}_4^+ \) treatment resulted in a slight increase in the expression of *BcNRT1.1*, *BcNRT1.8*, *BcNRT2.1*, and *BcNRT3.1*, and clearly increased the expression of *BcNAXT1* compared with N starvation (Figure 4A), whereas sole \( \text{NO}_3^- \) treatment resulted in a marked increase of five NRTs transcripts (Figure 4B). However, the effect of adding \( \text{NO}_3^- \) was more pronounced than that obtained with the combined addition of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) (Figure 4A). The transcript levels of five NRTs were upregulated in response to the addition of \( \text{NO}_3^- \), whereas *BcNRT1.1*, *BcNRT2.1*, and *BcNRT1.8* expression levels were clearly downregulated by adding \( \text{NH}_4^+ \) and slightly
upregulated by the subsequent addition of NO$_3^-$ (Figure 4B). However, the expression levels were lower than those obtained in response to the N mixture in which NO$_3^-$ was added for 10 min and NH$_4^+$ was added for another 10–20 min (Figure 4A).

Most BcNRTs transcripts were induced by NO$_3^-$ and inhibited by NH$_4^+$, whereas BcAMT1s transcripts were inhibited by NH$_4^+$ except for BcAMT1.2, which was induced by adding NH$_4^+$ and the effect was strengthened by adding NO$_3^-$.

Regarding the analysis of AMT1s and NRT1 transcripts, we speculated that BcAMT1.2 might play an important role in the coexistence of NO$_3^-$ and NH$_4^+$.

Cloning of a Putative ORF Encoding an AMT1.2 Homolog From B. campestris

To isolate the AMT1.2 gene from B. campestris, we designed primers based on the sequence of AMT1.2 from B. rapa (accession no. XM_009113156.1) (Supplementary Table S1), we obtained the homologous sequence using cDNA from B. campestris, designated BcAMT1.2 (GenBank accession no. MF966937.1). The complete ORF of BcAMT1.2 consisted of 1539 nucleotides and encoded a 54.94 kD polypeptide. Phylogenetic analysis of AMT1 and AMT2 subfamily members from other plant species showed that BcAMT1.2 belonged to the AMT1 cluster (Figure 5A), shared high sequence identity with Populus trichocarpa and Arabidopsis AMT1.2, and shared 99% identity with B. rapa AMT1.2 (Figure 5B). It was predicted to be a member protein exhibiting nine transmembrane domains with an N-terminus outside and C-terminus inside the cytoplasm (Figure 5B). The subcellular location in onion cells also showed that BcAMT1.2 was located in the plasma membrane (Supplementary Figure S5).

To investigate whether BcAMT1.2 is a functional ammonium transporter, we recombined the ORF of BcAMT1.2 into pYES2 vector, and transformed this into yeast mutants 31019b. Negative control cells transformed into pYES2 did not grow normally on a solid medium with 2 mmol L$^{-1}$ NH$_4^+$ as the only N source, whereas recombinant strains harboring pYES2-BcAMT1.2 grew normally (Figure 5C). This indicated that BcAMT1.2 may be a
functional ammonium transporter. *BcAMT1.2* was constitutively expressed throughout the growth period, mainly in roots and leaves, whereas the expression in stems and flowers was lower (Supplementary Figure S6A). In roots and leaves, *BcAMT1.2* expression decreased significantly as N starvation progressed (Supplementary Figures S6B, C).

We subsequently investigated that histochemical staining for *BcAMT1.2pro::GUS* transformants that were treated with NH$_4$\(^+\), NO$_3$\(^-\), or N-deficiency and stained for GUS activity. In leaves and roots, GUS activity was greater in response to treatment with NH$_4$\(^+\) or NO$_3$\(^-\) (Figures 6C–F) than that with N-deficiency (Figures 6A, B). GUS was mainly expressed in the vascular tissues of roots and shoots (Figures 6A–F). Two lines showed a similar pattern in response to N-deficiency and a low concentration of NH$_4$\(^+\) or NO$_3$\(^-\) after N-deficiency.

**Heterologous Expression of *BcAMT1.2* in *Arabidopsis***

To gain an insight into the possible function of *BcAMT1.2* in NH$_4$\(^+\) transportation and utilization in plants, *BcAMT1.2* was overexpressed in the *Arabidopsis* wildtype line (Col-0), which was supplied with 0.25 mmol L\(^{-1}\) NH$_4$\(^+\) as the sole N source. Several independent homozygous lines harboring *BcAMT1.2* were constructed and the expression of *BcAMT1.2* in *Arabidopsis* was confirmed by qPCR (Figure 7A). These seedlings were grown for 10 d on vertical agar plates containing 0.25 mmol L\(^{-1}\) NH$_4$Cl after a 4-d pre-culture on 4 mmol L\(^{-1}\) NaNO$_3$. The growth phenotype of transgenic lines showed that the overexpression of *BcAMT1.2* could promote the growth of *Arabidopsis* seedlings at a low concentration of NH$_4$\(^+\) (Figure 7B). Compared with the biomass in the wildtype, three *BcAMT1.2*-overexpressing (*BcAMT1.2-ox*) lines significantly increased the biomass of shoots and roots (Figure 7C), and the length of primary root (Figure 7D). Furthermore, NH$_4$\(^+\) content was increased by 17.9–32.0% in *BcAMT1.2-ox* lines (Figure 7E).

**Ion Fluxes of Overexpression *BcAMT1.2* Lines in *Arabidopsis* Under Coexistence of NH$_4$\(^+\) and NO$_3$\(^-\)**

To examine how *BcAMT1.2-ox* lines affected the absorption of NH$_4$\(^+\) and NO$_3$\(^-\), we measured ion flux rates of *Arabidopsis* seedlings in response to the mixture of N (0.25 mmol L\(^{-1}\) NH$_4$\(^+\) and 0.75 mmol L\(^{-1}\) NO$_3$\(^-\)) using SIET. *BcAMT1.2-ox* lines OX-6 and OX-9 showed larger net NH$_4$\(^+\) influxes than the wildtype, but had little difference in the last minutes of the experiment (Figure 8A). *BcAMT1.2-ox* lines influenced NO$_3$\(^-\) flux, which was changed significantly from net influxes to net effluxes in the *BcAMT1.2-ox* line (Figure 8B). During the test process, *BcAMT1.2-ox* lines increased 32.8–45.7% in net NH$_4$\(^+\) influx and 2.50–2.72-fold in net NO$_3$\(^-\) efflux in response to a mixture of NH$_4$\(^+\) and NO$_3$\(^-\) (Figures 8A–C). These observations indicated that overexpression of *BcAMT1.2* increased NH$_4$\(^+\) influxes and NO$_3$\(^-\) effluxes in *Arabidopsis*. The results of NO$_3$\(^-\) content showed a similar tendency (Figure 8D); however, *BcAMT1.2-ox* lines had little influence on NH$_4$\(^+\) content and even reduced it (Figure 8D).

To understand if the overexpression of *BcAMT1.2* will affect N assimilation, we investigated the expression levels of five N assimilation genes in *Arabidopsis* under a mixture of NH$_4$\(^+\) and NO$_3$\(^-\). GLN, GDH and GLT encode glutamine synthetase (GS),...
glutamate dehydrogenase (GDH), and NADH-dependent glutamate synthase (GOGAT), respectively. In roots, the transcript levels of \textit{AtGLN1.2} and \textit{AtGLT1} were $5.73 \text{--} 8.88$-fold and $2.85 \text{--} 3.83$-fold higher in \textit{BcAMT1.2-ox} lines than those in the wildtype (Figure 8E), respectively; in leaves, \textit{AtGLN1.2} and \textit{AtGLN2} transcript levels were $2.67 \text{--} 2.76$-fold and $2.71 \text{--} 4.61$-fold higher in both \textit{BcAMT1.2-ox} lines than those in the wildtype, respectively (Figure 8F). Other genes were affected little, either significantly or inconsistently, between two \textit{BcAMT1.2-ox} lines (Figures 8E, F). Elevated transcription of N assimilation genes (i.e. \textit{GLN1.2}, \textit{GLN2}, and \textit{GLT1}) might be physiologically crucial for the plants to effectively assimilate and utilize the higher levels of NH$_4^+$ induced by overexpressing \textit{BcAMT1.2}, to retain NH$_4^+$ at a relatively stable level.

**DISCUSSION**

**Characteristics of NH$_4^+$, NO$_3^-$ Fluxes, and Related Genes Expression in the Roots of \textit{B. Campestris}**

Compared with the growth with a sole N source, a mixture of NO$_3^-$ and NH$_4^+$ accelerates plant growth (Supplementary Figure S1) (Wang and Shen, 2011; Song et al., 2012). Plants often show a preference for the uptake of NH$_4^+$ or NO$_3^-$ (Song et al., 2016). Previous studies have shown that molecule-specific activities associated with net NO$_3^-$ and NH$_4^+$ fluxes can be evaluated non-invasively using SIET (Xu et al., 2006). In this study, we observed that the total N influx of the NH$_4^+$ and NO$_3^-$ mixture was higher than that of sole NH$_4^+$ or NO$_3^-$ at the same N amount (Figures 1A–D), which is consistent with previous studies on wheat (Zhong et al., 2015) and tea (Ruan et al., 2016). However, it is contrary to the results reported by Arkon et al. (2012), who show a significant decrease of total N uptake in \textit{B. napus} by an NH$_4^+$ and NO$_3^-$ mixture. NH$_4^+$ or NO$_3^-$ uptake is affected by the depolarization of electrical membrane potential which increases with the increase in NH$_4^+$ or NO$_3^-$ concentration, reaches the peak and changes to be steady, according to the Michaelis-Menten equation (Wang et al., 1994). We observed similar results in Figures 2A, B. However, at the same concentration, the net influx of NH$_4^+$ was greater than that of NO$_3^-$ in the roots of \textit{B. campestris} (Figures 1A–D; Figures 2A, B), and at the concentrations of 0.25 mmol L$^{-1}$ and 1 mmol L$^{-1}$, net NH$_4^+$ uptake was 1.42-fold and 2.88-fold higher than net NO$_3^-$ uptake, respectively (Figures 2A, B). This indicated that \textit{B. campestris} exhibited a preference for NH$_4^+$ over NO$_3^-$.

Previous studies have made similar observations (Zhong et al., 2015; Ruan et al., 2016). Indeed, many plants use NH$_4^+$ as their preferred N form (Socci and Templer, 2011) and...
most plants prefer to absorb NH$_4^+$ rather than NO$_3^-$ when NH$_4^+$ and NO$_3^-$ are supplied at the same concentration (Zhong et al., 2015; Ruan et al., 2016). Arkon et al. (2012) reported that N uptake and plant growth in B. napus are no significantly affected by adding NH$_4^+$ or mixed N during the first 24–72 h, whereas causes N uptake and plant growth to decrease after 15 days of treatment compared with NO$_3^-$ treatment. This may be associated with ammonium toxicity (Arkon et al., 2012; Hachiya et al., 2012; Hachiya and Sakakibara, 2017). Therefore, B. campestris plant prefers NH$_4^+$ to NO$_3^-$ on the premise that ammonium toxicity cannot affect plant cells in a short time.

In plants, the absorption of NH$_4^+$ or NO$_3^-$ is mainly regulated by AMT or NRT genes, respectively (Glass et al., 2002), and their expression levels are regulated by N status and forms (Gazzarrini et al., 1999; Yuan et al., 2007). In this study, compared with the transcripts in N-deficiency, BcAMT1.1, BcAMT1.3, and BcAMT1.5 transcripts were repressed by adding NH$_4^+$ and affected slightly by NO$_3^-$, whereas BcAMT1.2 expression was induced by both NH$_4^+$ and NO$_3^-$ (Figure 2C). The response of BcAMT1.1, BcAMT1.3, and BcAMT1.5 to NH$_4^+$ was similar to the results in Arabidopsis (Gazzarrini et al., 1999; Yuan et al., 2007). Those of BcAMT1.2 to NH$_4^+$ and NO$_3^-$ were consistent with previous results (Pearson et al., 2002; Yusuf and Deepa, 2017). BcNRTs transcripts were more affected by NO$_3^-$ than NH$_4^+$, as they were upregulated with an increase in NO$_3^-$ concentration (Figure 2D). This is consistent with previous studies (Fan et al., 2016; Qu et al., 2016). Consequently, we conclude that N status and form influence AMT and NRT...
transcripts and that these genes are involved in the regulation of NH$_4^+$ and NO$_3^-$ fluxes, respectively.

**NO$_3^-$ Accelerates Net NH$_4^+$ Influxes in *B. campestris***

Previous studies have reported that NH$_4^+$ and NO$_3^-$ might interact with each other under coexistence (Hachiya et al., 2017). Net N fluxes include total N influxes and total N efluxes. When net N influxes increased, total N influxes were enhanced, and/or total N efluxes were reduced (Hachiya and Sakakibara, 2017). In this study, net NH$_4^+$ influxes, with and without containing NO$_3^-$, increased sharply, then decreased rapidly, and slowly relaxed to a stable level with the addition of NH$_4^+$ (Figure 3A). Drastic initial changes in NH$_4^+$ fluxes may be caused by depolarization and polarization which are affected by electrical membrane potential after adding more NH$_4^+$ (Wang et al., 1994).

In addition, at a high external concentration of NH$_4^+$, plants may activate the NH$_4^+$ efflux system to cope with high NH$_4^+$ influx (Britto and Kronzucker, 2001; Babourina et al., 2007; Hachiya and Sakakibara, 2017). However, to date there have been no reports of any gene that encodes protein that is specifically involved in the NH$_4^+$ efflux system (Babourina et al., 2007). NH$_4^+$ influxes may be mediated via aquaporin channels or non-selective K$^+$ channels (Hachiya and Sakakibara, 2017). Babourina et al. (2007) reported that K$^+$ net fluxes are not correlated with net NH$_4^+$ fluxes. Moreover, before adding NH$_4^+$, net NH$_4^+$ influxes in bathing solution containing NO$_3^-$ were higher than those lacking NO$_3^-$, which was observed after adding NH$_4^+$ (Figure 3A). This indicated that the presence of NO$_3^-$ might have a positive effect on net NH$_4^+$ uptake, which is consistent with previous studies performed on other species (Kronzucker et al., 1999; Babourina et al., 2007; Luo et al., 2013); however, it is contrary to the results reported by Arkon et al., 2012. Using isotope labeling, Kronzucker et al. (1999) reported that a larger proportion of $^{15}$NH$_4^+$ signal is allocated to the xylem in the presence of both NH$_4^+$ and NO$_3^-$ than that with sole NH$_4^+$. NO$_3^-$ may influence the expression of AMT1-type homologous genes isolated from *B. campestris* (Yuan et al., 2007), AtAMT1.1, AtAMT1.3, and AtAMT1.5 were located in rhizodermal cells, and AtAMT1.2 is located in root endodermal and cortical cells (Yuan et al., 2007). Specific localization in the root zone of AMTs determines the pathways of NH$_4^+$ uptake, transport and allocation to shoots (Duan et al., 2018). When external NH$_4^+$ is high, apoplastic transport mediated by AtAMT1.2 prevails at the root endodermis (Yuan et al., 2007; Duan et al., 2018). AtAMT1.2 exclusively regulates NH$_4^+$ flux into the vasculature (Yuan et al., 2007; Straub et al., 2017) and favors N allocation to the shoot (Duan et al., 2018). BcAMT1.2$_{pro}$::GUS activity, which was expressed mainly in the vascular tissues in *Arabidopsis*, was enhanced by adding NH$_4^+$ or NO$_3^-$ compared with that in N-deficiency (Figures 6A–F). Therefore, we speculated that BcAMT1.2 may participate in the interaction of NH$_4^+$ and NO$_3^-$.

**NH$_4^+$ Decreases Net NO$_3^-$ Influxes in *B. campestris***

NH$_4^+$ had an influence on NO$_3^-$ influxes. Before and after adding NO$_3^-$, net NO$_3^-$ influxes of bathing solution containing NH$_4^+$ were lower than those without NO$_3^-$, whereas net NO$_3^-$ influxes of bathing solution with NH$_4^+$ were lower than those without NH$_4^+$ (Figure 3B). This indicated that NH$_4^+$ might decrease net NO$_3^-$ influxes, which is consistent with the discoveries in other plants (Kronzucker et al., 1999; Arkon et al., 2012; Luo et al., 2013). BcNRT1.1 and BcNRT2.1 are dual-affinity transport system and high affinity transport system, respectively, were downregulated by NH$_4^+$ (Figures 4A, B). Furthermore, the expression of BcNRT1.8, which regulates the xylem loading of NO$_3^-$, was decreased by NH$_4^+$, whereas that of BcNAXT1, which regulates NO$_3^-$ efflux system, was increased (Figures 4A, B). The addition of NH$_4^+$ not only decreased NO$_3^-$ absorption, but also NO$_3^-$ xylem loading, and consequently NO$_3^-$ influxes were decreased or NO$_3^-$ efluxes were increased. Previous studies have reported that the acidification of the rhizosphere caused by NAXT1 inhibits NO$_3^-$ absorption (Hachiya and Sakakibara, 2017). Furthermore, the overexpression of OsNRT2.3b enhances NO$_3^-$ uptake in response to sole NO$_3^-$ treatment, whereas OsNRT2.3b expression is inhibited in response to treatment with mixtures of NH$_4^+$ and NO$_3^-$ (Fan et al., 2016). Therefore, NH$_4^+$ may affect the absorption of NO$_3^-$ by regulating NRT transcripts in the coexistence of NH$_4^+$ and NO$_3^-$.

**BcAMT1.2 Mediated the Interaction of NH$_4^+$ and NO$_3^-$ Coexistence***

One AMT1-type homologous gene, namely BcAMT1.2, was isolated from *B. campestris* (Figures 5A, B). The protein encoded by BcAMT1.2, which is located in the plasma membrane, may be a functional AMT (Supplementary Figure S4). In a low concentration of NH$_4^+$, overexpressing BcAMT1.2 lines accelerated the growth of *Arabidopsis* which increased NH$_4^+$ content compared with the wildtype (Figures 7B–E). This is consistent with overexpressing AtAMT1.2 in *Arabidopsis* mutant lines (Yuan et al., 2007). In the NH$_4^+$ and NO$_3^-$ mixture, net NH$_4^+$ influxes of BcAMT1.2-ox lines were obviously increased (Figure 8A), and net NO$_3^-$ influxes were decreased and changed from net influxes to net effluxes (Figure 8B), NO$_3^-$ content of BcAMT1.2-ox lines was lower than that of the wildtype.

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February 2020 | Volume 10 | Article 1776
13
Further studies are required to clarify whether BcAMT2s play a similar role in the interaction between NH$_4^+$ and NO$_3^-$ in *B. campestris*. It may be related to NO$_3^-$ signaling, uptake, and reduction during the interaction of NH$_4^+$ and NO$_3^-$ (Hachiya et al., 2012). How AMT1.2 affects the interaction between NH$_4^+$ and NO$_3^-$ to exert its effects, and whether other proteins and signaling cascades are involved, are interesting questions that await future research.

**DATA AVAILABILITY STATEMENT**

All datasets for this study are included in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

SS and RC conceived and designed the research. YZ and XH carried out the experiments. WS analyzed the data. YH, GS, and HL reviewed and edited the manuscript.

**FUNDING**

This work was supported by the National Natural Science Foundation of China (31972481, 31401855) and the China Agriculture Research System (CARS-25-C-04).

**ACKNOWLEDGMENTS**

We are grateful to Dr. Bruno André (Université Libre de Bruxelles, Belgium) for providing the yeast mutant strain 31019b.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.01776/full#supplementary-material

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