Opportunities for Innovation

Innovations resulting from NMT functionality improvements: micrometer-scale pH detection of living samples

Contributed by the editorial department of Zhongguancun NMT Industrial Alliance

Editor's note:

Fungal colonization can activate the activity of proton pumps in poplar roots under salt stress, promote the secretion of more H^+ and reduce the pH of the root surface, thereby effectively improving the unfavorable conditions that poplar roots absorb NO_3^- under salt stress. In addition to the dynamic detection of real-time of H^+ fluxes secreted by the root surface during this process, the non-invasive micro-test technology (NMT) can also innovatively observe the fine changes in pH on a micrometer scale on the root surface, which truly shows the "dynamic and static combination" monitoring of H^+.


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Amelioration of nitrate uptake under salt stress by ectomycorrhiza with and without a Hartig net

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Basic Information

Topic: NMT finds that colonizing fungi causes rhizosphere acidification to promote NO_3^- absorption, providing evidence that mycorrhizal promotes salt tolerance in host plants by maintaining nutrient uptake

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Title: Amelioration of nitrate uptake under salt stress by ectomycorrhiza with and without a Hartig net

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Test sample: gray poplar root

Ion/molecule detection indicators: H^+, NO_3^-

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Abstract: Salt stress is an important environmental factor that hinders poplar from absorbing nitrogen nutrients. This article describes the effect of salinity on proton-driven nitrate flux in ectomycorrhizal roots and the importance of a Hartig net for nitrate uptake. Two strains of *Paxillus involutus* were used for root colonization: one MAJ, which forms typical ectomycorrhizal structures (mantle and Hartig net), and one NAU, which colonizes roots with a thin, loose hyphal sheath.

Fungus-colonized and noncolonized *Populus* × *canescens* were exposed to NaCl and used to measure root surface pH, nitrate (NO_3^-) flux and transcription of NO_3^- transporters (NRTs; *PcNRT1.1, -1.2, -2.1*), and plasmalemma proton ATPases (HAs; *PcHA4, -8, -11*).

Paxillus colonization enhanced root NO_3^- uptake, decreased surface pH, and stimulated *NRTs* and *HA4* of the host regardless the presence or absence of a Hartig net. Under salt stress, noncolonized roots exhibited strong net NO_3^- efflux, whereas beneficial effects of fungal colonization on surface pH and HAs prevented NO_3^- loss. Inhibition of HAs abolished NO_3^- influx under all conditions.

It was found that stimulation of HAs was crucial for the beneficial influence of ectomycorrhiza on NO_3^- uptake, whereas the presence of a Hartig net was not required for improved NO_3^- translocation. Mycorrhizas may contribute to host adaptation to salt-affected environments by keeping up NO_3^- nutrition.

Key words: non-invasive micro-test technology, salt stress, mycorrhiza, nitrate, Hartig net, NRT

1. Experimental results of ion/ molecule flux

Since ^{15}N tracing revealed the identity of the transported N compounds, the study used Non-invasive Micro-test Technology (NMT) to determine NO_3^- fluxes in poplar in the presence or absence of fungal colonization and in response to salt stress. Along the root apex (100-2100 μm), the fluxes of NO_3^- were constant in the NO_3^- measuring solution (0.1 mM low NO_3^-, Figure 2a). The magnitude and direction of NO_3^- fluxes were significantly influenced by salt exposure and fungal colonization (Figure 1a). The root tips of non-mycorrhizal (NM) poplar showed moderate NO_3^- uptake, whereas fungus-colonized roots exhibited 7.4- to 11.8-fold higher uptake (Figure 1a). Apparently, a Hartig net was not required for this stimulation because root colonization with either *P. involutus* strain MAJ or NAU resulted in enhanced NO_3^- uptake compared with NM roots (Figure 1a). Furthermore, fungal-colonized roots maintained a net NO_3^- uptake under salt stress, while NM roots showed a net NO_3^- efflux under both short- and long-term salt stress (Figure 1a). Pure fungal mycelia of NAU and MAJ exhibited net NO_3^- influx irrespective of control or salt treatments (Figure 3).

NO_3^- uptake requires H^+ co-transport and is therefore dependent on the pH in the external environment. Here, they determined the pH values at NM and fungus-colonized root surfaces. The pH along NM roots was stable (Figure 2b) with a mean value of 5.41 (Figure 1b). Fungal colonization resulted in a more acidic surface pH, ranging from 5.05 to 5.12 (Figure 1b, Figure 2b). Long-term salinity caused a marked rise of pH in NM plants to about pH 5.8 ($P<0.001$). In fungus-colonized plants, salt exposure also caused pH increments resulting in pH values of *c*. 5.4 at the root surface similar to those of NM control roots (Figure 1b, Figure 2b). The salt-induced increase of pH was due to the decline of H^+ efflux from root surface. The surface pH of NAU did not differ from that of MAJ-colonized roots regardless of control conditions, or short- or long-term salt stress (Figure 1b).

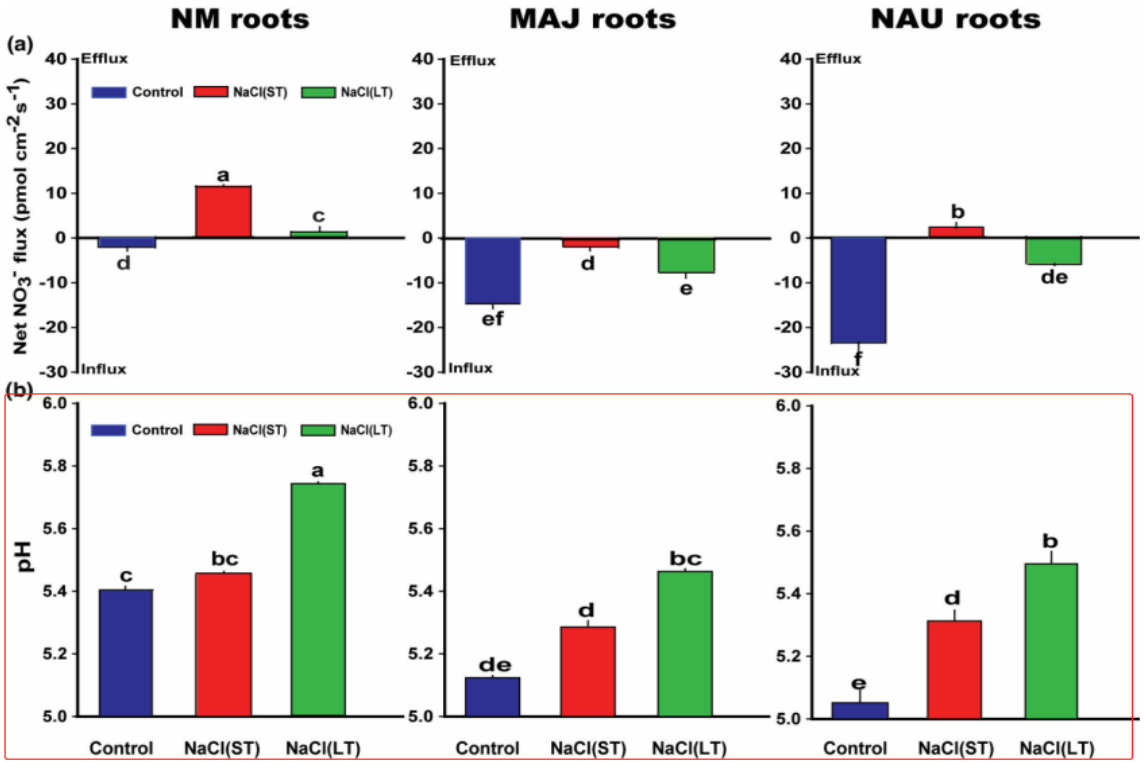

Figure 1. Effects of NaCl on steady-state fluxes of NO_3^- and root surface pH value in *Populus* × *canescens* colonized with or without strains. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

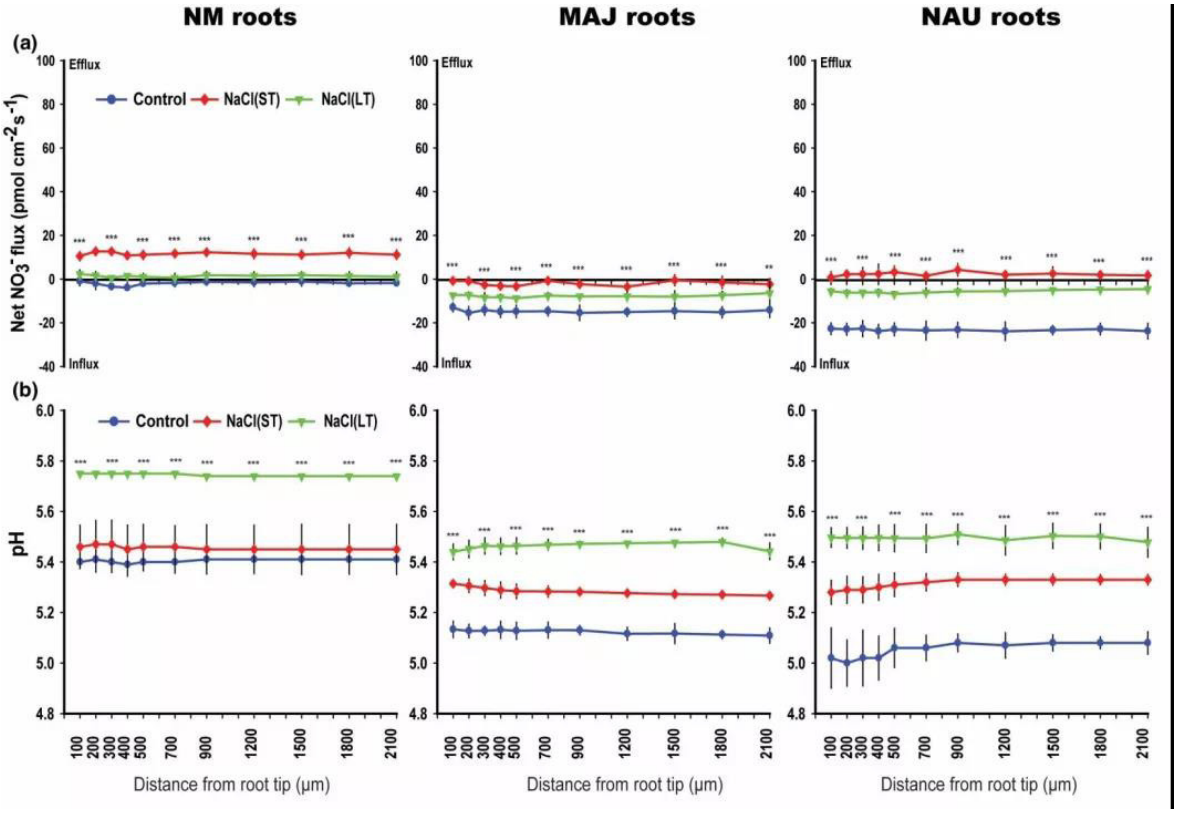

Figure 2. Effect of NaCl on steady-state fluxes of NO_3^- and root surface pH of poplar with and without mycorrhizal inoculation. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

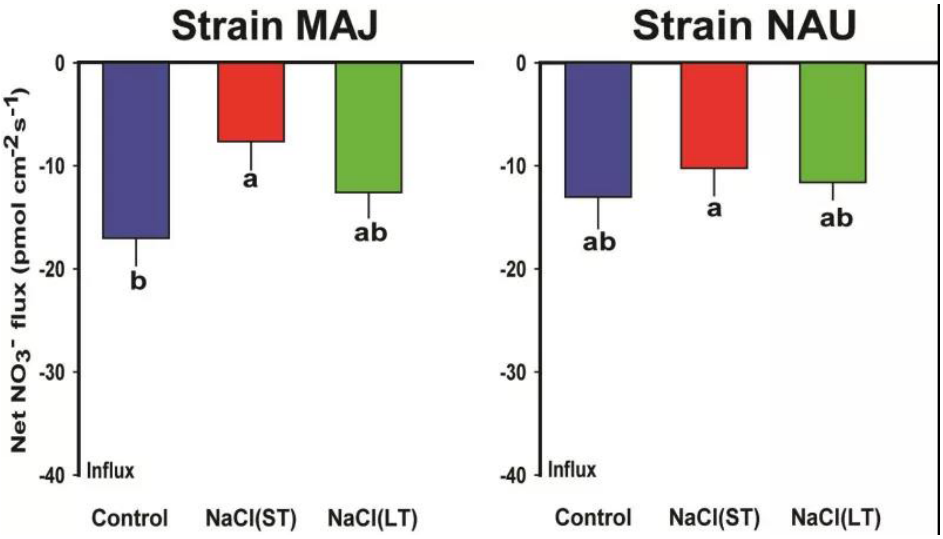

Figure 3. Effect of NaCl on steady-state fluxes of NO_3^- of MAJ and NAU strains. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

To test the importance of H^+ gradients for NO_3^- uptake, HAs were inhibited with orthovanadate (plasmalemma HA inhibitor). The inhibitor significantly increased the pH at the surface of NM and fungus-colonized roots (Figure 4b, Figure 5b), indicating that the H^+ pumps were effectively suppressed. Measurements of H^+ fluxes confirmed that the inhibitor orthovanadate shifted net H^+ efflux towards influx under control and saline conditions (Figure 6). This resulted in NO_3^- release regardless of the presence or absence of *Paxillus* or salt stress (Figure 4a, Figure 5a).

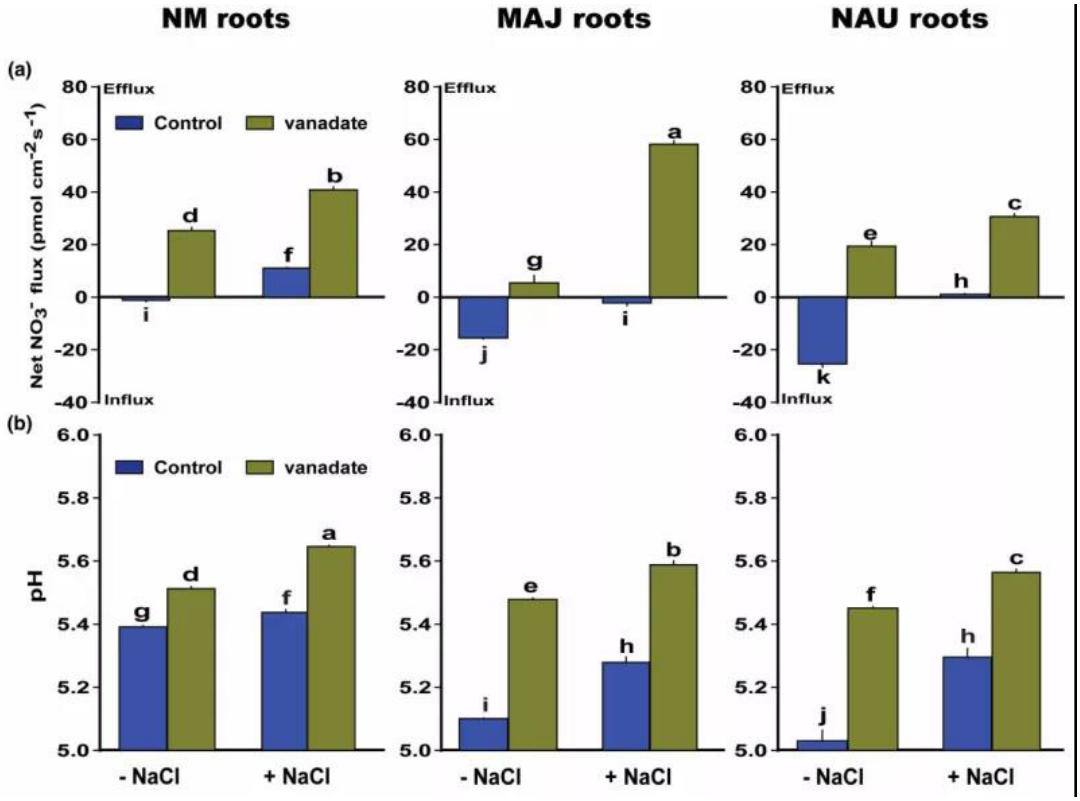

Figure 4. Effect of orthovanadate on steady-state fluxes of NO_3^- and root surface pH in *Populus* × *canescens* with and without mycorrhizal colonization and with or without NaCl. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

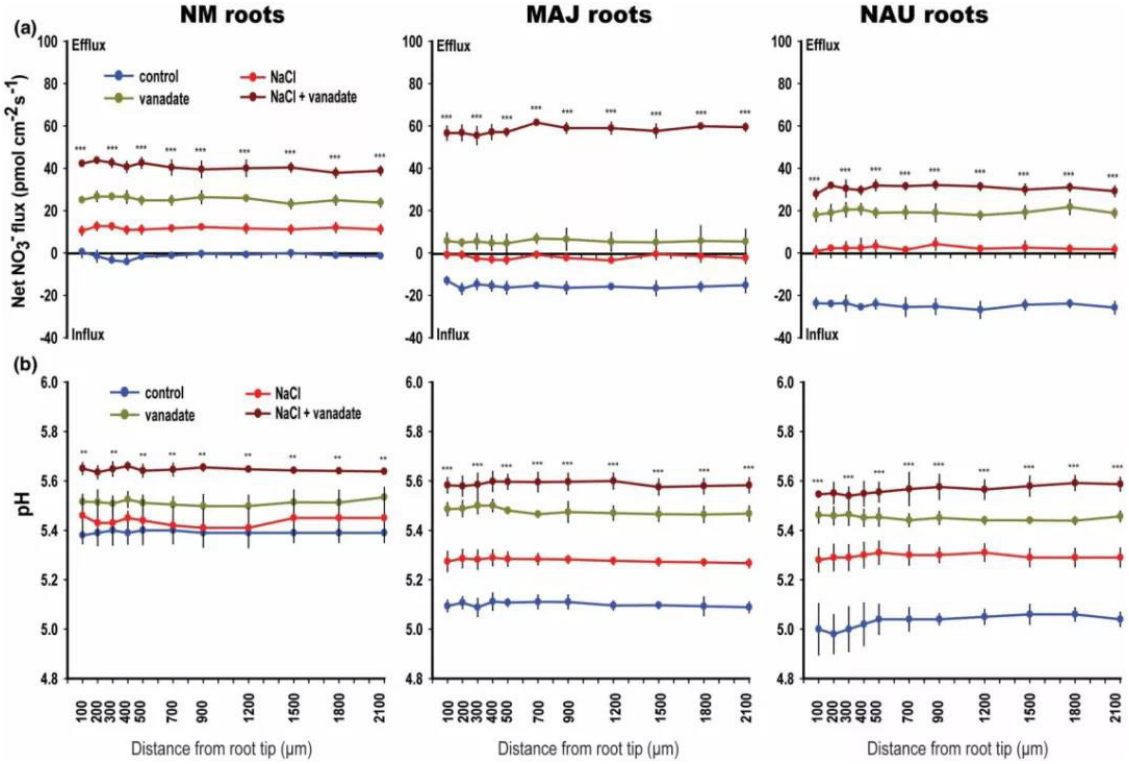

Figure 5. Effect of orthovanadate on steady-state fluxes of NO_3^- and root surface pH in poplars inoculated with and without mycorrhizal roots under NaCl stress. Positive values represent NO_3^- efflux, negative values represent NO_3^- influx.

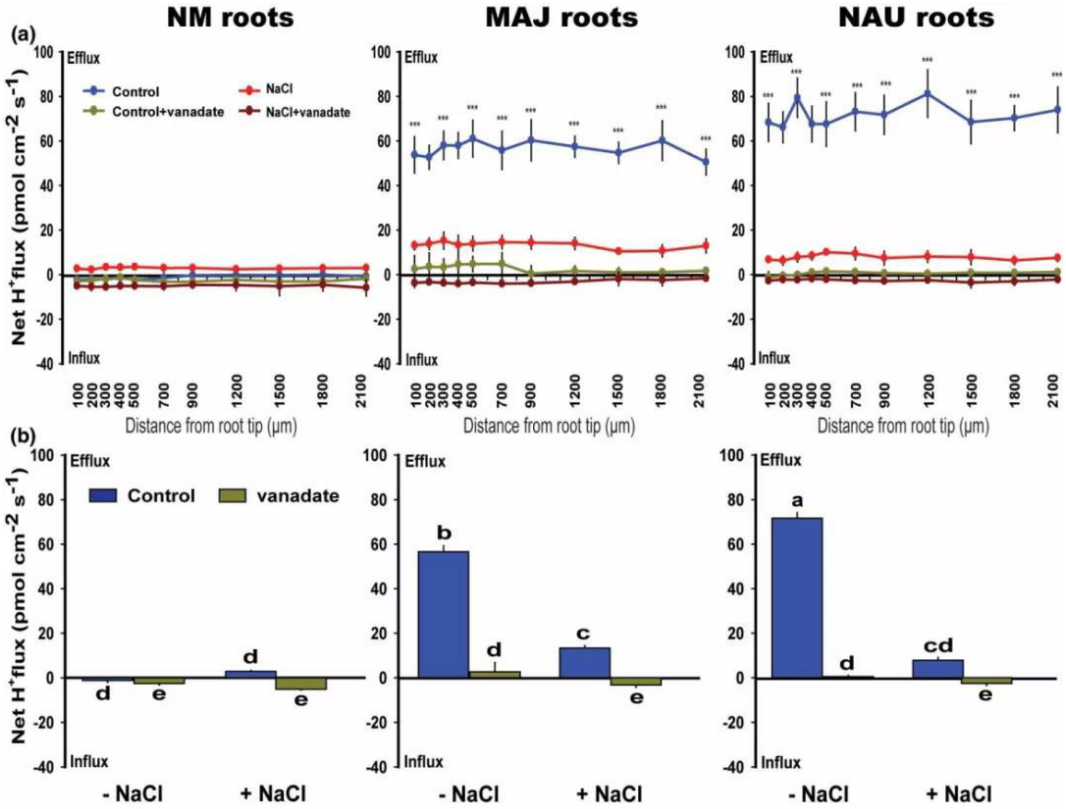

Figure 6. Effect of orthovanadate on steady-state fluxes of H^+ in poplars inoculated with and without mycorrhizae under NaCl stress. Positive values represent H^+ efflux, negative values represent H^+ influx.

2. Ion/molecule flux experimental treatment method

The roots of *P.* × *canescens* were inoculated with and without *P. involutus* strains (MAJ and NAU) for 30 days, and then exposed to 0 or 100 mM NaCl for 24 hours (short term, ST) or 7 days (long term, LT).

3. Other experimental results

1) Compared with NM poplar, the enrichment of ^{15}N in the roots and shoots of poplar colonized by MAJ or NAU was higher.

2) Fungal colonization and salt stress altered the transcript levels of low-affinity and high-affinity nitrate transporters (NRTs) in poplar roots.

3) Under long-term salt stress, the activities of NR (nitrate reductase) and NiR (nitrite reductase) showed a moderate decrease.

4) Despite the salt-induced decrease in the level of PcHA4 transcripts, the transcript levels of PcHA4 were still higher in fungus-colonized than in NM roots under salt stress.

5) *P. involutus*-colonized roots exhibited higher hyperpolarization of the plasma membrane compared with NM roots

6) Roots colonized by MAJ showed higher O_2 uptake at 500–900 μm from the tip.

4. Conclusion

The present study demonstrated that poplar roots colonized with the ectomycorrhizal fungus *P. involutus* exhibited enhanced net NO_3^- uptake and increased expression of several *NRTs* and *HAs*. Without a Hartig net, *P. involutus* strain NAU caused enhanced net NO_3^- fluxes and increases in *NRT* and *HA* transcript levels similar to those in roots colonized with MAJ that form typical ectomycorrhizal structures.

The study concluded that the beneficial effects of fungi on enhancing NO_3^- transport capacities were overruled by salt stress. However, the *Paxillus* strains MAJ and NAU fostered the maintenance of root NO_3^- homeostasis under salt stress due to higher surface acidity of fungus-colonized roots than of NM roots. Salt-stress-induced NO_3^- efflux from NM roots was combatted by mycorrhizal activated H^+-ATPases, which apparently caused an H^+ gradient across the plasma membrane sufficient for NO_3^- retention. The nature of how mycorrhizal fungi can influence H^+-pumping activities and the transcriptional regulation of host *PcNRTs* and *PcHAs* needs to be explored in the future, thus leaving room for more innovative opportunities for scientific researchers. Overall, the results of this study open new insights into the functioning of mycorrhizal symbioses for nutrient uptake and stress amelioration.

(Editor in charge: Xuefei Li)