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Ion Flux in Salinized Ectomycorrhizal Poplar Roots

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Paxillus involutus strains MAJ and NAU mediate K^+/Na^+ homeostasis in ectomycorrhizal *Populus* × *canescens* under NaCl stress[1]

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ABSTRACT

Salt-induced fluxes of H^+, Na^+, K^+, and Ca^{2+} were investigated in ectomycorrhizal (EM) associations formed by *Paxillus involutus* (strains MAJ and NAU) with the salt-sensitive poplar hybrid *Populus* × *canescens*. A scanning ion-selective electrode technique was used to measure flux profiles in non-EM roots and axenically grown EM cultures of the two *P. involutus* isolates to identify whether the major alterations detected in EM roots were promoted by the fungal partner. EM plants exhibited a more pronounced ability to maintain K^+/Na^+ homeostasis under salt stress. The influx of Na^+ was reduced after short-term (ST; 50 mM NaCl, 24 h) and long-term (LT; 50 mM NaCl, 7 d) exposure to salt stress in mycorrhizal roots, especially in NAU associations. Flux data for *P. involutus* and susceptibility to Na^+-transport inhibitors indicated that fungal colonization contributed to active Na^+ extrusion and H^+ uptake in the salinized roots of *Populus* × *canescens*. Moreover, EM plants retained the ability to reduce the salt-induced K^+ efflux, especially under LT salinity. Our study suggests that *P. involutus* assists in maintaining K^+ homeostasis by delivering this nutrient to host plants and slowing the loss of K^+ under salt stress. EM poplar plants exhibited an enhanced Ca^{2+} uptake ability, whereas ST and LT treatments caused a marked Ca^{2+} efflux from mycorrhizal roots, especially from NAU-colonized roots. We suggest that the release of additional Ca^{2+} mediated K^+/Na^+ homeostasis in EM plants under salt stress.

Soil salinization is a serious factor restricting the expansion of agriculture and forestry around the world. Among the novel biotechnological tools that can enhance salt resistance, inoculation with ectomycorrhizal (EM) fungi has been suggested to be an important measure for enhancing the performance of and ensure biomass production by woody species in saline environments (Luo et al., 2009). In general, EM fungi enhance the growth of host plants by increasing mineral nutrition and reducing the uptake of sodium (Na^+) ions under salt stress (Smith and Read, 2008; Hall, 2002; Polle and Schützendübel, 2003). For example, *Scleroderma bermudense* significantly increases P and K^+ levels but decreases Na^+ and Cl^- concentrations in *Coccoloba uvifera* plants (Bandou et al., 2006). Similarly, *Hebeloma crustuliniforme* and *Laccaria bicolor* reduce tissue Na^+ and Cl^- concentrations and alleviate salt injury in white spruce (*Picea glauca*), black spruce (*Picea mariana*), and jack pine (*Pinus banksiana*) seedlings (Muhsin and Zwiazek, 2002; Nguyen et al., 2006). In contrast to conifers, EM associations did not decrease tissue concentrations of Na^+ and Cl^- in NaCl-treated trembling aspen and paper birch (Yi et al., 2008), showing that the fungal effect on salt accumulation varies with EM fungus and host plant species.

Paxillus involutus strains MAJ and NAU have been identified as highly salt-tolerant fungi (Gafur et al., 2004; Langenfeld-Heyser et al., 2007; Zhang et al., 2008). Colonization with *P. involutus* strain MAJ reduces the buildup of Na^+ but enhances K^+ accumulation in the leaves of a salt-sensitive poplar, *Populus* × *canescens* (Langenfeld-Heyser et al., 2007, Luo et al., 2011). The maintenance of a high K^+/Na^+ ratio is critical for salt-tolerance in herbaceous plants (Shabala, 2000; Tester and Davenport, 2003; Chen et al., 2007; Shabala and Cuin, 2008) and woody species, including *Populus* spp. (Chen et al., 2001, 2002a, 2002b, 2003; Sun et al., 2009a, 2009b, 2010, 2012). At the cellular level, salinized plants avoid Na^+ toxicity in the cytosol by compartmentalizing Na^+ into the vacuole and excreting Na^+ into the external environment or the apoplast (Blumwald et al., 2000; Hasegawa et al., 2000; Zhu, 2001, 2003; Ottow et al., 2005; Apse and Blumwald, 2007; Munns and Tester, 2008; Chen and Polle, 2010). The driving force for Na^+/H^+ antiporters is provided by H^+-ATPases, which make an important contribution to the maintenance of low Na^+

levels in the cytosol (Chen and Polle, 2010). In addition, NaCl-induced K^+ deficiency in plants is regulated by depolarization-activated outward rectifying K^+ channels (DA-KORCs) and depolarization-activated non-selective cation channels (DA-NSCCs) (Shabala et al., 2005, 2006a; Demidchik and Maathuis, 2007; Shabala and Cuin, 2008). Moreover, salinity is known to cause oxidative stress (Zhu, 2003; Demidchik et al., 2010), and a large proportion of NSCCs are ROS-activated (Demidchik et al., 2002, 2003). Roots of EM plants accumulate more, and leaves less, Na^+ than the respective tissues of non-EM plants, probably due to decreased xylem loading (Langenfeld-Heyser et al., 2007). EM roots increase the supply of K^+ under salt stress (Langenfeld-Heyser et al., 2007). However, how EM fungi assist plants by improving Na^+ and K^+ relationships after exposure to salinity is not yet clear.

It has been shown that different strains of *P. involutus* differ in their abilities to form typical mycorrhizal structures with poplar roots (Gafur et al., 2004). While the fungal strain MAJ forms a typical hyphal mantle and Hartig net with roots of *Populus* × *canescens*, NAU induces defense reactions, such as cell wall thickening, and is unable to intrude between the host cells (Gafur et al., 2004). Clarifying whether and how the incompatible fungal isolate affects the salt tolerance of host plants is necessary.

Populus × *canescens* roots exhibit increased Ca^{2+} enrichment during mycorrhizal symbiosis with *P. involutus* strain MAJ (Langenfeld-Heyser et al., 2007). Ramos et al. (2009) found that EM roots are more efficient than non-EM roots in taking up Ca^{2+} from the external medium. Under NaCl stress, Ca^{2+} regulates K^+/Na^+ homeostasis in a *sos3* mutant and wild-type *Arabidopsis* (Liu and Zhu, 1997). Ca^{2+} has been suggested to restrict Na^+ uptake via VI-NSCCs (Demidchik and Tester, 2002; Tester and Davenport, 2003) and restrain K^+ loss through DA-KORCs and DA-NSCCs (Shabala et al., 2006a; Sun et al., 2009b; Chen and Polle, 2010). Although Ca^{2+} is well known to ameliorate salt stress, Ca^{2+} possibly impacts K^+/Na^+ homeostasis via EM associations is unclear.

Using ion selective vibrating microelectrodes, significant correlations between anion, Ca^{2+} and H^+ fluxes were found on root surfaces with increased fluxes after

colonization of different hosts with either EM or arbuscular mycorrhizas (AM) (Ramos et al., 2008, 2009). The goal of the present study was to examine the role of EM in ion homeostasis under salt stress. We used the scanning ion-elective electrode technique (SIET) to measure steady and transient profiles of ion fluxes (Na^+, H^+, K^+, and Ca^{2+}) in *Populus* × *canescens* – *P. involutus* associations, non-EM roots, and fungal mycelia of the two *P. involutus* isolates MAJ and NAU. We also examined the effects of Ca^{2+} on K^+ and Na^+ fluxes in poplar roots; *Populus* × *canescens* exhibited Ca^{2+} enrichment upon colonization with the EM fungus *P. involutus*.

RESULTS

Na^+, K^+, and Ca^{2+} Concentrations in Roots and Leaves

SEM-EDX was used to measure relative changes in the Na^+, K^+, and Ca^{2+} concentrations in the cross sections of roots and leaves. NaCl treatment (50 mM) for one week significantly increased Na^+ in the roots and leaves, with the exception of plants colonized with *P. involutus* strain NAU (Fig. 1). In contrast to Na^+, root and leaf K^+ concentrations decreased in salt-treated plants, especially non-mycorrhizal (NM) plants (Fig. 1). As a result, the K^+/Na^+ ratio in roots and leaves was markedly reduced by salt stress, with a more pronounced effect in NM plants compared to mycorrhizal (EM) plants (Fig. 1). *P. involutus* mycorrhization increased Ca^{2+} by 19-24% in the roots and 53-72% in the leaves of non-stressed plants (Fig. 1). Salinized *Populus* × *canescens* had a reduced Ca^{2+}/Na^+ ratio in the roots and leaves; however, the salt effect was less evident in NAU-mycorrhizal plants compared to NM and MAJ-mycorrhizal plants (Fig. 1).

Steady and Transient Ion Fluxes in Roots and EM Fungus

Na^+ flux

SIET analyses showed that the pattern of Na^+ fluxes in EM roots differed from that of NM roots after short-term (ST, 24 h) and long-term (LT, 7 d) exposure to 50 mM NaCl (Fig. 2). In NM roots, ST stress caused a net Na^+ influx at the region 1,400

to 2,000 μm from the apex, whereas LT salinity resulted in a stable and constant influx along the whole measured distance from 50 to 2,000 μm (Fig. 2). Compared to NM roots, the salt-induced entry of Na^+ was less pronounced in MAJ- and NAU-mycorrhizal roots when considering both spatially resolved values along the scanned surface (Fig. 2) and mean values (Fig. 3A). A salt-induced efflux of Na^+ was detected in some regions along these mycorrhizal roots (Fig. 2).

The mycelia of the two *P. involutus* strains, MAJ and NAU, exhibited a marked Na^+ efflux under ST and LT stress (Fig. 3, B and C). The NaCl-induced Na^+ efflux was typically higher under conditions of high salinity (400 mM NaCl) compared to lower salinity (50, 100, and 200 mM NaCl) (Fig. 3, B and C). However, the two *P. involutus* strains displayed different capacities to sustain the Na^+ efflux at high salt concentrations (400 mM NaCl). LT-treated NAU exhibited a flux rate similar to that of ST-treated hyphae, whereas a reduced Na^+ efflux was detected in MAJ under LT salinity (Fig. 3, B and C). Pharmacological experiments showed that the high salt-induced Na^+ efflux in the two fungal strains was significantly reduced by the Na^+/H^+ antiporter inhibitor amiloride, or the plasma membrane H^+-ATPase inhibitor sodium orthovanadate (Fig. 4A).

H^+ flux

SIET measurements of root apices revealed a net H^+ influx into NM roots in the absence of salt stress (Fig. 5; mean values are shown in Fig. 6A). In contrast, EM roots were characterized by stable and constant H^+ effluxes along the measured regions (Figs. 5 and 6A). ST and LT salinity caused a typical shift of H^+ efflux towards an influx in EM roots (Figs. 5 and 6A). In the absence of fungal colonization, NaCl did not significantly change the H^+ flux profile along the root axis after ST or LT exposure, though the H^+ flux oscillated in the measured regions (Fig. 5).

MAJ and NAU mycelia exhibited a net H^+ efflux under control conditions similar to that of EM roots (Fig. 6, A-C). ST and LT salinity reduced the efflux of H^+ into strain MAJ (Fig. 6B). A similar trend was observed in the salinized hyphae of strain NAU, though H^+ fluxes in ST-treated NAU varied with salt concentration (Fig.

6C). The salinized fungus NAU exhibited a temporary influx (approximately 5-10 min) during the period of recording (30 min), especially under LT treatment, though the mean value indicated outward rectification (data not shown).

The salt-induced transient H^+ kinetics in roots and fungal mycelia were also examined. In the absence of salt stress, H^+ efflux was detected in EM roots instead of H^+ influx in NM roots (Fig. 6D). Salt shock (50 mM NaCl) caused a pronounced shift in the H^+ efflux toward an influx in EM roots (Fig. 6D), but no significant changes in the H^+ kinetics of NM roots were observed during the recording period (Fig. 6D). The responses of the transient H^+ kinetics to salt shock were compared between the two *P. involutus* strains. After exposure to NaCl (50 to 400 mM), hyphae exhibited an instantaneous decrease in the H^+ efflux, which then remained constant during the period of recording (40 min) (Fig. 6, E and F). Compared to strain NAU, the shock-induced reduction in H^+ efflux was more pronounced in strain MAJ over the concentration range of 50 to 400 mM NaCl (Fig. 6, E and F).

K^+ flux

Although the K^+ flux varied along the root axis (Fig. 7), ST and LT salt treatments resulted in an overall net K^+ efflux from NM roots (Fig. 8A). Mycorrhization of poplar roots with *P. involutus* reduced the K^+ efflux under ST and LT treatments, with the exception of ST-stressed MAJ-mycorrhizal roots (Figs. 7 and 8A). ST and LT treatment caused a net K^+ efflux from the mycelia of both NAU and MAJ, but the effect was more pronounced in MAJ, especially under ST salinity (Fig. 8, B and C). TEA, a K^+ channel blocker, significantly decreased the salt-induced K^+ efflux from the hyphae of the two strains (Fig. 4B).

In salinized poplar roots, the transient K^+ kinetics in response to salt shock followed a trend similar to the steady state measurements (Fig. 8, A and D). Salt shock caused an evident K^+ efflux in non-mycorrhizal roots, but the flux rate was lower in MAJ- and NAU-mycorrhizal roots (Fig. 8D). In the hyphae of the two strains, the rate of K^+ efflux was reduced after the addition of NaCl (50-400 mM) (Fig. 8, E and F). An

instantaneous increase in the K^+ influx was detected in MAJ and NAU after the onset of salt shock (Fig. 8, E and F).

Ca^{2+} flux

Steady state flux measurements showed that ST and LT treatment accelerated Ca^{2+} efflux along mycorrhizal roots, especially in NAU-mycorrhizal plants (Fig. 9, mean values are shown in Fig. 10A). In the absence of salt stress, the hyphae of the two strains exhibited Ca^{2+} influx, with a higher flux rate in MAJ than in NAU (Fig. 10, B and C). ST-treated MAJ maintained the Ca^{2+} influx, but the flux rate decreased with increasing NaCl concentrations (Fig. 10B). In strain NAU, ST salinity reduced the influx under low salt conditions (50, 100 mM NaCl) and shifted towards an efflux at high salinity (200, 400 mM NaCl) (Fig. 10C). Under LT stress, the pattern of Ca^{2+} flux was similar in the two strains. LT salinity reduced the influx or reversed the rectification toward an efflux (Fig. 10, B and C).

When subjected to salt shock, EM roots exhibited a transient increase in Ca^{2+} efflux, but no corresponding changes were observed in non-mycorrhizal roots (Fig. 10D). The shock-induced Ca^{2+} efflux was more pronounced in MAJ-mycorrhizal roots than NAU-colonized roots (Fig. 10D). $LaCl_3$, an inhibitor of Ca^{2+}-permeable channels, did not markedly restrict the high rate of Ca^{2+} efflux from salt-shocked MAJ and NAU roots (Supplemental Fig. S1). In the absence of salt stress, the mycelia of the two strains exhibited a stable and steady influx of Ca^{2+}, typically with higher flux rates in MAJ than NAU (Fig. 10, E and F). NAU hyphae exhibited a Ca^{2+} efflux immediately after the addition of NaCl (50-400 mM) (Fig. 10F). Similarly, salt shock reduced the Ca^{2+} influx in strain MAJ, though a transient increase in Ca^{2+} influx was observed in the presence of 400 mM NaCl (Fig. 10E).

Effects of Ca^{2+} on Salt-Induced K^+ and Na^+ Fluxes in *Populus* × *canescens* Roots

The effect of Ca^{2+} on K^+ and Na^+ fluxes was examined in salinized roots. The aim was to investigate whether Ca^{2+} released from salt-treated mycorrhizal associations benefits root cells in the control of K^+/Na^+ homeostasis. Ca^{2+} application

(10 mM) markedly limited the salt-induced K^+ efflux and enhanced the apparent Na^+ efflux in *Populus* × *canescens* roots (Fig. 11). More profound effects were found in the meristematic zone (K^+ and Na^+) and elongation region (Na^+) than in other parts of the root.

DISCUSSION

EM Fungal Colonization Ameliorated K^+/Na^+ Homeostasis under Salt Stress

Colonization of the salt-sensitive poplar *Populus* × *canescens* with EM fungus *P. involutus* (strain MAJ) was previously found to improve growth, prime for increased stress tolerance, and increase nutrition under salt stress (Langenfeld-Heyser et al., 2007; Luo et al., 2009, 2011). Similar findings were observed in the present study. NM plants abscised old leaves from the lower shoots and upper leaves displayed salt damage after 7 days of NaCl treatment (50 mM; Supplemental Fig. S2D). However, the salt injury was alleviated by ectomycorrhizal colonization Supplemental Fig. S2, D-F). Salinized poplars exhibited an enhanced capacity to maintain K^+/Na^+ homeostasis in the presence of fungal colonization (strains MAJ and NAU). The maintenance of K^+/Na^+ homeostasis is crucial for a poplar to tolerate saline conditions (Chen et al., 2001, 2002a, 2002b, 2003; Sun et al., 2009a, 2009b, 2010; Chen and Polle, 2010). SEM-EDX microanalysis data indicate that the high K^+/Na^+ ratio in mycorrhizal plants was the result of less Na^+ accumulation and K^+ reduction under NaCl stress than in NM plants (Fig. 1). This finding is in accordance with our previous finding that MAJ-mycorrhizal poplar had diminished leaf Na^+ but increased the K^+ supply under salt stress (Langenfeld-Heyser et al., 2007; Luo et al., 2009). Unexpectedly, we found that NAU-colonized plants exhibited a higher ability to maintain K^+/Na^+ homeostasis than MAJ, though NAU does form a Hartig net with poplar and ensheathes the root tip with a loose mycelial network (Supplemental Fig. S2; Gafur et al., 2004). Thus, the formation of a physical barrier by the mantel formed by MAJ is likely not involved in modifying salt uptake. The increased capacity for ion balance control in the salt-sensitive host plant, which was promoted by fungal colonization, was probably due to the modulation of transport systems.

EMs Enhance Na^+ Extrusion

The reduction in shoot Na^+ uptake has been suggested to be an important resistance mechanism in ectomycorrhizal plants growing in salinized soil (Muhsin and

Zwiazek, 2002; Nguyen et al., 2006). Leaf Na^+ in mycorrhizal poplar was 6% (MAJ) and 76% (NAU) lower than in non-mycorrhizal plants after 1 week of exposure to 50 mM NaCl (Fig. 1), which is in agreement with our previous findings (Langenfeld-Heyser et al., 2007). Similarly, the ectomycorrhizal fungi *Hebeloma crustuliniforme* and *Laccaria bicolor* has been shown to reduce tissue Na^+ concentrations and alleviate salt injury in several conifers (Muhsin and Zwiazek, 2002; Nguyen et al., 2006). The diminished buildup of salt in shoots is likely the result of salt uptake and transport restriction in roots (Chen et al., 2002a, 2003). Our SIET data show that the salt-induced entry of Na^+ was markedly impeded in EM plants, especially in NAU-colonized roots (Figs. 2 and 3). The same trend was shown in fungal hyphae, which exhibited a steady Na^+ efflux under ST and LT stress. As amiloride, a Na^+/H^+ antiporter inhibitor, reduced Na^+ efflux in the salt-treated mycelia of both strains (Fig. 4), our data suggest that *P. involutus* contributed to active Na^+ extrusion in the salinized roots of *Populus* × *canescens*.

Notably, the salt-enhanced Na^+ efflux was always associated with an H^+ influx into EM roots (Figs. 2, 3, 5, and 6), whereas EM roots exhibited a net H^+ efflux in the absence of salt stress (Figs. 5 and 6). The H^+ efflux in EM roots apparently resulted from the root-ensheathing fungus as MAJ and NAU hyphae exhibited an evident H^+ efflux (Fig. 6). Our data agree with the findings of Ramos et al. (2009), who reported significant H^+ efflux from *Eucalyptus globulus* roots (the apex, meristematic, and elongation zones) colonized with *Pisolithus* sp. The mycorrhiza-stimulated H^+ efflux is due to the activity of PM H^+-ATPase in the ectomycorrhizal associations. In the roots of *Pinus sylvestris-Laccaria laccata*, Lei and Dexheimer (1988) showed the localization of ATPase activity along the Hartig net hyphal plasma membranes and plasma membranes of contiguous living cortical cells. AM mycorrhizal symbioses induced the expression of two genes (*pma*2 and *pma*4) responsible for de-novo H^+-ATPase activity in the periarbuscular membrane of invaded cells (Gianinazzi-Pearson et al., 2000). Some host PM H^+-ATPase isoforms show high activity in arbuscular mycorrhizal associations (Ramos et al., 2005; Rosewarne et al., 2007). In addition, three PM H^+-ATPase genes (LHA1, LHA2, and LHA4) were found to be regulated by

arbuscular mycorrhiza in tomato plants (Ferrol et al., 2002). The molecular analysis of ATPases in EM roots appears to be missing. In our study, salt exposure reversed the rectification of H^+ from efflux to influx in the EM roots of poplar (Figs. 5 and 6). In accordance, the H^+ efflux from hyphae was diminished by various salt treatments (SS, ST, and LT, with a few exceptions) or shifted to a net influx during the period of recording. In the presence of an inhibitor of the PM proton pump, sodium orthovanadate, the salt-enhanced Na^+ efflux was reduced in the two strains (Fig. 4). Taken together, the data suggest that colonization with *P. involutus* stimulates the H^+-ATPase activity in *Populus* × *canescens*-ectomycorrhizal associations, which pumps protons to promote the secondary active Na^+/H^+ antiport at the PM (Blumwald et al., 2000; Zhu, 2003). We have noticed that under salt exposure the efflux of H^+ was not equivalent to the influx of Na^+. The flux inconsistency of Na^+ and H^+ is mainly due to the superimposition of two effects: salt-induced H^+ excretion, as salinity stress is usually associated with increased H^+ efflux and at the same time, higher SOS1 Na^+/H^+ exchanger activity of EM plants that leads to an accelerated H^+ uptake. As such, the net H^+ flux will not be changed.

For Na^+ flux measurements, special attentions need to be paid to Na^+ microelectrodes because of non-ideal selectivity of the commercially available Na^+ LIX (Chen et al. 2005). Na^+ electrodes produced signals when we calibrated Na^+ LIX in a range of K^+ or Ca^{2+} standards, in accordance to the finding by Chen et al. (2005). To reduce interfering effects of K^+ on Na^+ flux, K^+ was omitted from the bathing medium (Cuin et al. 2011). In our study, Ca^{2+} and K^+ concentrations in the measuring solution were set to low concentrations, 0.1 mM and 0.5 mM, respectively. To estimate the interference of K^+ and Ca^{2+} on Na^+ flux in plant materials, we measured root Na^+ flux in control and salinized *P. canescens* plants (50 mM NaCl for 24 h) in the presence and absence of interfering ions (K^+, Ca^{2+}). We found that 0.5 mM K^+ and 0.1 mM Ca^{2+} had no significant effects on root Na^+ flux in no-salt controls (data not shown). However, in salt-treated roots, the absence of K^+ and Ca^{2+} in measuring buffer resulted in higher signals but did not change the tendency of root flux (data not shown). We found that the detected Na^+ signals in salinized roots were unstable and

fluctuated greatly during the period of recording. This is presumably the plant response to nutrient deficiency in the root medium. In our study, the presence of K^+ and Ca^{2+} may not affect the accuracy of our conclusions relating to Na^+ fluxes in mycorrhizal roots and axenic EM cultures. The experimental evidence and explanations are briefly listed below. (1) Na^+ electrodes exhibited much higher selectivity for Na^+ relative to K^+ and Ca^{2+} in the presence of both Na^+ and interfering ions (Supplemental Table S1). Moreover, the released Na^+ from salinized mycorrhizal roots and hyphae would increase the ratio of Na^+ to K^+ and Ca^{2+} near the electrode, thus increasing the selectivity of Na^+ electrode during the period of recording. (2) Treatment of amiloride (an inhibitor of Na^+/H^+ antiporter) significantly decreased the Na^+ efflux in axenic mycelia (Fig. 4), suggesting that the detected signals were largely carried by Na^+ across the plasma membrane. (3) The different trends of Na^+, K^+ and Ca^{2+} fluxes suggest that the selectivity of Na^+ LIX was sufficient for Na^+/K^+ and Na^+/Ca^{2+} discriminations. Salinized axenic mycelia exhibited an outward rectification of Na^+ and K^+ but with different patterns with increasing the NaCl concentrations (Figs. 3 and 8). Salt treatment caused an evident Na^+ efflux in EM cultures, while the detected Ca^{2+} efflux was low and an inward rectification was usually seen in salinized mycelia (Figs. 3 and 10). Salinity induced an evident Ca^{2+} efflux in mycorrhizal roots, whereas there was no equivalent Na^+ flux corresponding to the Ca^{2+} efflux in these roots (Figs. 2, 3, 9 and 10).

EM Ameliorates K^+ Homeostasis

In our study, K^+ concentrations in the roots and leaves were reduced by salt treatment to a lesser extent in mycorrhizal plants than NM plants. This finding agrees with the results of Langenfeld-Heyser et al. (2007), who found that MAJ-mycorrhizal plants contained higher leaf K^+ levels than non-EM plants. Our studies show that *P. involutus* fungi assist host plants in the maintenance of K^+ homeostasis by delivering the nutrient to the plant and slowing the loss of K^+ under NaCl stress. Zhang et al. (2008) reported that *P. involutus* mycelium, especially strain MAJ, increased the uptake of K^+ after exposure to salt treatment. Salt shock caused an instantaneous

influx of K^+ into the fungal mycelium; however, ST- and LT-stressed hyphae exhibited a K^+ efflux (Fig. 8). Our data indicate that prolonged NaCl treatment resulted in K^+ loss from *P. involutus*. Currently, we cannot conclude that K^+ efflux is mediated by cation channels, though the K^+ efflux caused by NaCl (400 mM) was significantly reduced by the K^+ channel blocker (TEA) in the two tested strains (Fig. 4). The high rate of K^+ efflux detected in the control and salinized mycelia (ST and LT) relates, to some extent, to the concentrations of the K^+ sources (Fig. 8). The enriched K^+ in ectomycorrhizal hyphae is thought to be transferred to the host during the period of salt treatment, which caused a lesser reduction of K^+ in the roots and leaves of EM plants (Fig. 1). Rygiewicz and Bledsoe (1984) reported that external hyphae in EM symbiosis have a high capacity to take up K^+ and deliver the nutrient to the host plant. In addition to stimulating K^+ uptake, *P. involutus* colonization benefits the salinized hosts by slowing down the rate of K^+ loss. Steady and transient flux data for poplar roots indicate that the salt-induced K^+ efflux was decreased by *P. involutus* colonization (Figs. 7 and 8). The reduced rate of K^+ efflux in ST- and LT-treated EM roots is partly the result of a delayed K^+ loss from inner host cells. In the fungus ensheathed roots, K^+ in the compatible (MAJ) or incompatible (NAU) hyphae was replaced by Na^+ before K^+ exchange with the root cells of the host was expected. Moreover, the released Ca^{2+} ions that are replaced by Na^+ can assist plants in maintaining K^+ homeostasis under salt stress.

Mediation of Ca^{2+} in K^+/Na^+ Homeostasis in EM Plants

In our study, ectomycorrhizal *Populus* × *canescens* plants exhibited an enhanced Ca^{2+} uptake ability in the absence of salinity stress (Fig. 1). The Ca^{2+} enrichment was caused by colonization with *P. involutus* because the mycelium also displayed a stable Ca^{2+} influx under these conditions (Fig. 10). Similarly, the EM roots of *Eucalyptus globules* colonized by *Pisolithus* sp. exhibit a high uptake capacity for Ca^{2+} from the external medium (Ramos et al., 2009). Transient and steady flux measurements of mycorrhizal roots revealed a significant Ca^{2+} efflux under salt stress, suggesting that the Ca^{2+} accumulated in mycorrhizal roots could be replaced by

Na^+ (Figs. 9 and 10). The salt-induced Ca^{2+} flux may originate from the cell wall (Arif et al., 1995, Shabala and Newman, 2000); $LaCl_3$, an inhibitor of Ca^{2+}-permeable channels, did not markedly restrict the high rate of Ca^{2+} efflux from salt-shocked MAJ and NAU roots (Supplemental Fig. S1). A large amount of cell wall Ca^{2+} has been shown to be exchangeable in mycorrhizal roots (Peterson and Enstone, 1996; Kuhn et al., 2000; Bücking et al., 2002). The increase in leaf Ca^{2+} as a consequence of NaCl treatment indicates that the root-derived element could be transported to the shoots in addition to being released from the root surface. Thus, freed Ca^{2+} is able to assist plants in ameliorating the K^+/Na^+ ratio under salt stress. Ca^{2+} inhibits DA-KORC and DA-NSCCs to reduce K^+ efflux in Arabidopsis under saline conditions (Shabala et al., 2006a; Sun et al., 2009b). Exogenous Ca^{2+} improved the K^+/Na^+ balance by inhibiting K^+ efflux and increasing the apparent Na^+ efflux in *Populus* × *canescens* roots (Fig. 11). These results are consistent with our previous findings in non-mycorrhizal roots of another salt-sensitive poplar, *P. popularis* (Sun et al., 2009b). Therefore, we conclude that the increased availability of free Ca^{2+}, which was released by Ca^{2+}/Na^+ exchange from EM roots, favored the establishment of K^+/Na^+ homeostasis in *Populus* × *canescens* under salt treatment.

Differences in K^+/Na^+ maintenance between MAJ- and NAU-colonized roots are clear (Fig. 1), which might have resulted from differences in the release of exchangeable Ca^{2+} from mycorrhizal associations. MAJ-mycorrhizal roots lost a large amount of Ca^{2+} after being subjected to salt shock (Fig. 10), whereas NAU-colonized roots exhibited a long-sustained release of free Ca^{2+} during ST and LT salt treatment (Figs. 9 and 10). Consequently, the Ca^{2+} ions released from the mycorrhizal associations would benefit inner root cells controlling K^+/Na^+ homeostasis in terms of the effects of Ca^{2+} on K^+ and Na^+ fluxes in *Populus* × *canescens* roots. We noticed that MAJ-mycorrhizal roots displayed a significant Ca^{2+} efflux under salt stress; however, a net Ca^{2+} influx was usually recorded in the fungal mycelium of this strain after exposure to salt shock and ST treatment (Figs. 9 and 10). Therefore, the salt-induced Ca^{2+} efflux that we detected from MAJ-mycorrhizal roots likely came from inner host cells, at least in part, as a result of Na^+/Ca^{2+} exchange. Compared to NAU,

the inner root cells might have been more accessible to Na^+ because MAJ forms a typical Hartig net with the roots of *Populus* × *canescens*, whereas NAU exhibits cell wall thickening (Supplemental Fig. S2; Gafur et al., 2004).

MATERIALS AND METHODS

Plant and Fungal Cultures for Ectomycorrhizal Colonization

The EM fungi used in this study were the *Paxillus involutus* isolates MAJ and NAU. Plants, fungal cultures, and the technique for synthesizing ectomycorrhizae followed the procedures described by Gafur et al. (2004). In brief, the two isolates were grown on 2% modified Melin Norkrans (MMN) agar medium [the medium contains the following components in $g \cdot L^{-1}$: KH_2PO_4 0.5, $(NH_4)_2SO_4$ 0.25, $MgSO_4 \cdot 7H_2O$ 0.15, $CaCl_2 \cdot 2H_2O$ 0.05, NaCl 0.025, $FeCl_3 \cdot 6H_2O$ 0.01, thiamine HCl 0.0001, glucose 10, malt extract 3, pH 5.0] (Gafur et al., 2004). After fungal inoculation, the Petri dishes (diameter 90 mm) were sealed with a strip of Parafilm^{TM} and kept in permanent darkness at 23°C.

In this study, we used a Petri dish culture system for the colonization of *Populus* × *canescens* with the *P. involutus* strains MAJ and NAU (Gafur et al., 2004). In brief, regenerated plantlets of *Populus* × *canescens* were grown for 2 to 3 weeks on Murashige and Skoog (MS) rooting medium. The fungi were pre-grown on the agar culture medium for 1 week prior to colonization. Vigorous plantlets with sufficient roots were used for ectomycorrhization. Rooted plantlets from sterile culture were carefully freed from agar particles. Rooted plantlets were placed on the MMN agar medium in the presence or absence of ectomycorrhizal mycelium. During the period of incubation, the room temperature was 24±1°C with a light period of 14 h (6:00-20:00). Photosynthetic active radiation of 200 $\mu mol\ m^{-2}s^{-1}$ was supplied by cool white fluorescent lamps. Root-fungal associations were formed during one month after colonization. All plantlets exhibited well-developed shoots and roots (Supplemental Fig. S2, A-C). Some leaves at lower shoots became dry at the tips (Supplemental Fig. S2, A-C). Samples of EM- and NM root tips for anatomical investigations were embedded, stained and photographed as previously described (Gafur et al., 2004). Anatomical analyses of mycorrhizal roots showed that the hyphae of strain MAJ penetrated into the cell walls of the cortex, whereas NAU hyphae were only detected

on the outer epidermal cell walls (Supplemental Fig. S2, G-I). Uniform mycorrhizal and non-mycorrhizal plants were used for acclimation and salt treatment.

Liquid Culture of Fungi

Liquid culture of *P. involutus* was grown at the College of Biological Sciences and Technology, Beijing Forestry University (China). Strains MAJ and NAU were obtained from the Büsgen Institute: Institute of Forest Botany and Tree Physiology, Göttingen University (Germany). For liquid culture, agar was absent and the medium was buffered with citrate (Ott et al., 2002). Mycelium from the agar plate was homogenized, transferred into 150 mL of liquid medium in flasks, and incubated on a rotary shaker in darkness (150 r/min, 23°C) (Langenfeld-Heyser et al., 2007). *P. involutus* in submerged culture grew in the form of compact spherical masses of mycelium (pellets). For salt treatment, sterile filtered NaCl solutions were added to achieve final concentrations of 0, 50, 100, 200, or 400 mM. After short-term (ST, 24 h) or long-term (LT, 7 d) treatment, axenic cultures of MAJ and NAU were used for steady flux measurements of Na^+, H^+, K^+, and Ca^{2+}.

Plant Acclimation and Salt Treatments

Prior to salt treatment, mycorrhizal and non-mycorrhizal plants were carefully removed from MMN agar medium, planted in individual pots containing fine sand, and grown in a greenhouse at the College of Biological Sciences and Technology, Beijing Forestry University, China. The room temperature was 24±1°C with a light period of 14 h (6:00-20:00), and photosynthetic active radiation of 200 μmol m^{-2} s^{-1} was supplied by cool white fluorescent lamps. Mycorrhizal and non-mycorrhizal plantlets were exposed to 50 mM NaCl for ST (24h) or LT (7d). The required NaCl concentrations were added to the LN nutrient solution (Langenfeld-Heyser et al., 2007). Control plantlets received LN solution without NaCl. On days 1 and 7, roots with apices of 1-2 cm were sampled from mycorrhizal and non-mycorrhizal plants and used for steady state measurements of the net Na^+, H^+, K^+, and Ca^{2+} fluxes. At the final harvest, leaf segments (2-3 mm long and 1-2 mm wide) and root segments with

1.0 cm apices were sampled from mycorrhizal and non-mycorrhizal plantlets, freeze-dried, and used for X-ray microanalysis with a scanning electron microscope equipped with an energy dispersive X-ray spectrometer (SEM-EDAX).

$CaCl_2$ Treatment

Plantlets of *Populus* × *canescens* were multiplied by micropropagation as described by Leplé et al. (1992). Rooted plantlets were cultivated in hydroponic LN-nutrient solutions with low nitrogen supply (Gafur et al., 2004). To acclimate the plants to ambient conditions, plantlets were covered with plastic bags and illuminated with low light (50 μmol m^{-2} s^{-1}) supplied by cool white fluorescent lamps. The plastic bags were gradually opened over 1 week. Acclimated plants were subjected to NaCl (50 mM) or NaCl (50 mM) plus $CaCl_2$ (10 mM). The required amount of NaCl and $CaCl_2$ was added to the nutrient solution. Control plants were treated in the same manner without the addition of NaCl. Plants were continuously aerated by passing air to hydroponic LN-nutrient solution, which was regularly renewed. Steady fluxes of K^+ and Na^+ in meristematic, elongation, and maturation zones were measured after 24 h and 7 days of treatment.

Inhibitor Treatments

ST-treated (400 mM NaCl) *P. involutus* isolates MAJ and NAU were subjected to sodium orthovanadate (500 μM), amiloride (50 μM), or tetraethylammonium chloride (TEA, 50 μM) for 30 min in the measuring solutions. Prior to recording Na^+ flux, the measuring solutions containing sodium orthovanadate were replaced with 10 mL of fresh solution, but the measuring solution with amiloride and TEA was not renewed (amiloride and TEA had no clear effect on the Nernstian slopes of Na^+ and K^+ electrodes) (Sun et al., 2009a, 2009b). Steady-state fluxes of Na^+ (orthovanadate or amiloride treatment) and K^+ (TEA treatment) were measured in axenic mycelia (pelleted hyphae) pre-treated with or without inhibitors at pH 6.0.

Steady State Measurements of Net Na^+, H^+, K^+, and Ca^{2+} Fluxes

The net fluxes of Na^+, H^+, K^+, and Ca^{2+} in roots and mycelia were measured using a non-invasive scanning ion-selective electrode technique (SIET, BIO-001A, Younger USA Sci. & Tech. Corp., Amherst, MA) (Xu et al., 2006; Sun et al., 2009a, 2009b). The concentration of the ions and concentration gradients were measured by moving the ion-selective microelectrode between two positions close to the materials in a preset excursion (30 μm) at a programmable frequency in the range of 0.3-0.5 Hz. Ion-selective microelectrodes for the target ions were calibrated prior to flux measurements: (1) Na^+: 0.1, 0.5, 1.0 mM (Na^+ concentration was usually 0.1 mM in the measuring buffer for roots and axenic mycelia); (2) H^+: pH 4.0, 5.0, 6.0 (pH was 5.0 in the measuring buffer); (3) K^+: 0.1, 0.5, 1.0 mM (K^+ was 0.5 mM in the measuring buffer), and (4) Ca^{2+}: 0.1, 0.5, 1.0 mM (Ca^{2+} was 0.5 mM in the measuring buffer). All electrodes that used for steady and transient recordings were usually corrected 2-3 times by calibrations during the experiments. The ion flux rate was calculated using Fick's law of diffusion:

$$J=-D(dc/dx),$$

where J is the ion flux in the x direction, dc represents the ion concentration difference, dx is the microelectrode movement between two positions, dc/dx is the ion concentration gradient, and D represents the ion diffusion coefficient in a particular medium.

Flux oscillations

Rhythmic (ultradian) flux oscillations are ubiquitous in the measured plant species (Toko et al., 1990; Souda et al., 1990; Shabala et al., 1997, 2003, 2006b; Shabala and Knowles, 2002). H^+ flux oscillations have been widely reported in a variety of plant species. In our study, oscillations in the H^+ flux in poplar roots were not as noticeable as in herbaceous species. The H^+ oscillations were more like fluctuations, and oscillations in the Na^+, K^+, and Ca^{2+} fluxes displayed similar trends as H^+ (Supplemental Fig. S3). This finding is presumably due to a lower growth rate of woody roots compared to crop species. Toko et al. (1990) found that roots exhibit no oscillations when they have a slow growth speed (Toko et al., 1990). Salinity caused a significant decrease in the period of H^+ flux oscillations (Shabala, 2003). Our

data show that the oscillatory periods of measured ions (H^+, Na^+, K^+, and Ca^{2+}) in poplar roots are usually in the range of several minutes. In our scanning studies, fluxes were recorded for 8-10 min at each point, which is long enough to ensure the absence of oscillations.

Ion selectivity of Na^+ electrodes

We found that Na^+ microelectrodes were not able to record Na^+ flux in a measuring buffer containing high Na^+ due to the low signal/noise (S/N) ratio of Na^+ LIX (Fluka 71178; Sun et al., 2009). Na^+-selective microelectrodes were also found to be unsuitable for screening Na^+ fluxes because of the non-ideal selectivity of the commercially available Na^+ LIX (Chen et al., 2005). K^+ was omitted from the bathing medium to reduce the interfering effects of K^+ on Na^+ flux (Cuin et al., 2011). To determine the interfering effects of K^+ and Ca^{2+} on Na^+-selective electrodes, Na^+-selective microelectrodes were calibrated in Na^+ solution (0.1, 0.5, 1.0 mM) in the presence or absence of K^+ (0.1, 0.5, 1.0 mM) and Ca^{2+} (0.1, 0.5, 1.0 mM). The calibration characteristics (Nernst slope) of the Na^+ electrode was not altered by the interfering K^+ ion (concentration range 0.1 to 1.0 mM; Supplemental Table S1). In the presence of Ca^{2+}, the Nernst slope of the Na^+ electrodes was reduced, up to 24% at 1.0 mM Ca^{2+} (Supplemental Table S1). To reduce the interfering effects of Ca^{2+} on Na^+ electrodes, the Ca^{2+} concentration in the measuring solution was set to 0.1 mM. The Nernst slope and intercept of the Na^+ electrodes in the measuring solution (0.5 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$) were 50.0031±1.9249 and 73.1593±0.6543, similar to the values in the absence of K^+ and Ca^{2+} (Nernst slope: 55.7648±1.9751, Nernst intercept: 75.9074±1.8814).

Experimental Protocols for SIET Measurements

Roots sampled from mycorrhizal and non-mycorrhizal plants and mycelial hyphae collected from the liquid culture medium were rinsed with distilled water and incubated in the basic measuring solution (0.5 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$) to equilibrate for 30 min. To record the Na^+ flux in mycelial hyphae, the axenic EM cultures (pelleted form) were equilibrated for 60 min to reach a stable

flux rate (Supplemental Fig. S4). The roots or hyphae were transferred to Petri dishes containing 10 mL of fresh measuring solution. Prior to recording the flux, root and fungal samples were immobilized on the bottom. The Na^+, H^+, K^+, and Ca^{2+} fluxes in the roots were measured along the root apex (0-2,000 μm from the tip) at intervals of 50 to 300 μm. Ion fluxes in the mycelium were measured over a recording period of 30-40 min. Real-time flux measurements of NM roots, EM roots, and axenic mycelia (pelleted hyphae) are shown in Supplemental Figure S5.

Transient Flux Kinetics

Mycorrhizal and non-mycorrhizal roots were sampled from non-salinized plants. After equilibration to the basic measuring solution, the steady-state fluxes of H^+, K^+, and Ca^{2+} in the apical region (200 μm from the root apex) were recorded (5–6 min) prior to salt shock. NaCl (200 mM) was slowly added to the measuring solution until the final NaCl concentration in the buffer reached 50 mM. Ion flux recording was continued for 30-40 min. The effect of lanthanum chloride (200 μM) on salt shock-induced transient Ca^{2+} kinetics was examined in the roots of mycorrhizal (MAJ and NAU) and non-mycorrhizal (NM) *Populus* × *canescens* plants.

Fungal mycelia were exposed to 0, 50, 100, 200, or 400 mM NaCl to induce salt shock. H^+, K^+, and Ca^{2+} fluxes were monitored over a continuous recording period of 30-40 min. For transient flux kinetics, the data measured during the first 2 to 3 min was discarded due to the diffusion effects of stock addition.

X-ray Microanalysis

The samples from control and stressed plantlets were rapidly frozen in liquid nitrogen and vacuum freeze-dried at -100°C for 7 days. The freeze-dried samples were gold-coated in a high vacuum sputter coater and analyzed using a Hitachi S-3400N scanning electron microscope equipped with an energy dispersive X-ray spectrometer (EX-250, Horiba Ltd., Kyoto, Japan). Probe measurements of roots and leaves were made with a broad electron beam covering the whole cross section. The relative amount of K^+, Na^+, or Ca^{2+} was expressed as atomic mass fraction (%).

Data Analysis

Three-dimensional ionic fluxes were calculated using MageFlux, developed by Xu Yue (http://xuyue.net/mageflux). All mean data were subjected to analysis of variance. Significant differences between means were determined by Duncan's multiple range test. Unless otherwise stated, differences were considered significant when $P < 0.05$.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. Interfering effects of K^+ and Ca^{2+} on Na^+-selective electrodes.

Supplemental Figure S1. Effects of lanthanum chloride (200 μM) on salt shock-induced transient Ca^{2+} kinetics in the roots of mycorrhizal (MAJ and NAU) and non-mycorrhizal (NM) *Populus* × *canescens* plants.

Supplemental Figure S2. Ectomycorrhizal colonization of *Populus* × *canescens* and performance of mycorrhizal (MAJ and NAU) and non-mycorrhizal (NM) plants under salt stress.

Supplemental Figure S3. Fluctuations and oscillations in the net H^+ flux in the elongation zone of *Populus* × *canescens* roots.

Supplemental Figure S4. Kinetics of the net Na^+ efflux in axenic mycelia of *P. involutus* strains MAJ and NAU after being exposed to 400 mM NaCl for 24 h.

Supplemental Figure S5. Representative images showing real time flux measurements of non-mycorrhizal (NM) roots, EM roots and axenically grown EM cultures.

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FIGURE LEGENDS

Figure 1. Effect of NaCl (50 mM, 1 week) on Na^+, K^+, Ca^{2+}, K^+/Na^+, and Ca^{2+}/Na^+ in the leaves and roots of mycorrhizal (MAJ and NAU) and non-mycorrhizal (NM) *Populus* × *canescens* plants. Amount of K^+, Na^+ and Ca^{2+} was expressed as atomic mass fraction (%). Each column is the mean of three to four individual plants and bars represent the standard error of the mean. Columns labeled with asterisks indicate significant difference at $P < 0.05$ between control and NaCl treatments.

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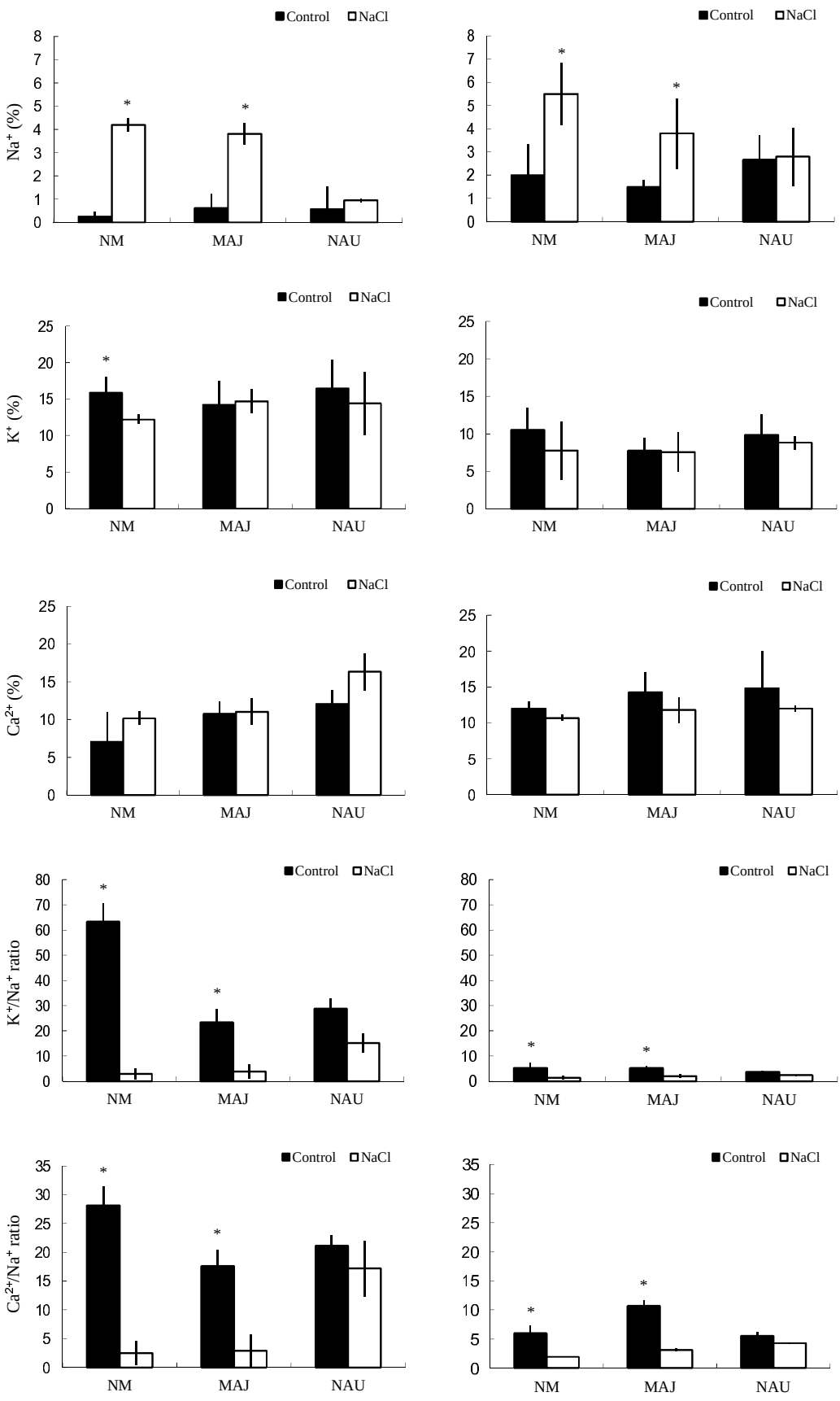
Figure 11. Effects of NaCl and Ca^{2+} supplementation on Na^{+} (A) and K^{+} (B) flux at the meristematic, elongation, and maturation zones of *Populus* × *canescens* roots. Plants were subjected to one week of NaCl stress (50 mM) supplemented with or without 10 mM $CaCl_2$. Control plants were well fertilized but treated without additional NaCl. Roots were sampled at 24 h and 7 d, and mean flux values are given. Steady fluxes at the meristematic, elongation, and maturation regions were measured along the root axes at an interval of 30-50 μm. Each column is the mean of six to eight individual plants and bars represent the standard error of the mean (for each plant, the average fluxes at the meristematic, elongation, and maturation zones were calibrated from measuring points in the measured region). Columns labeled with different letters, a, b, indicate significant difference at $P < 0.05$ between treatments. N.S., no significant difference.


Leaf
Root
Control
NaCl
Na+ (%)
K+ (%)
Ca2+ (%)
K+/Na+ ratio
Ca2+/Na+ ratio
NM
MAJ
NAU

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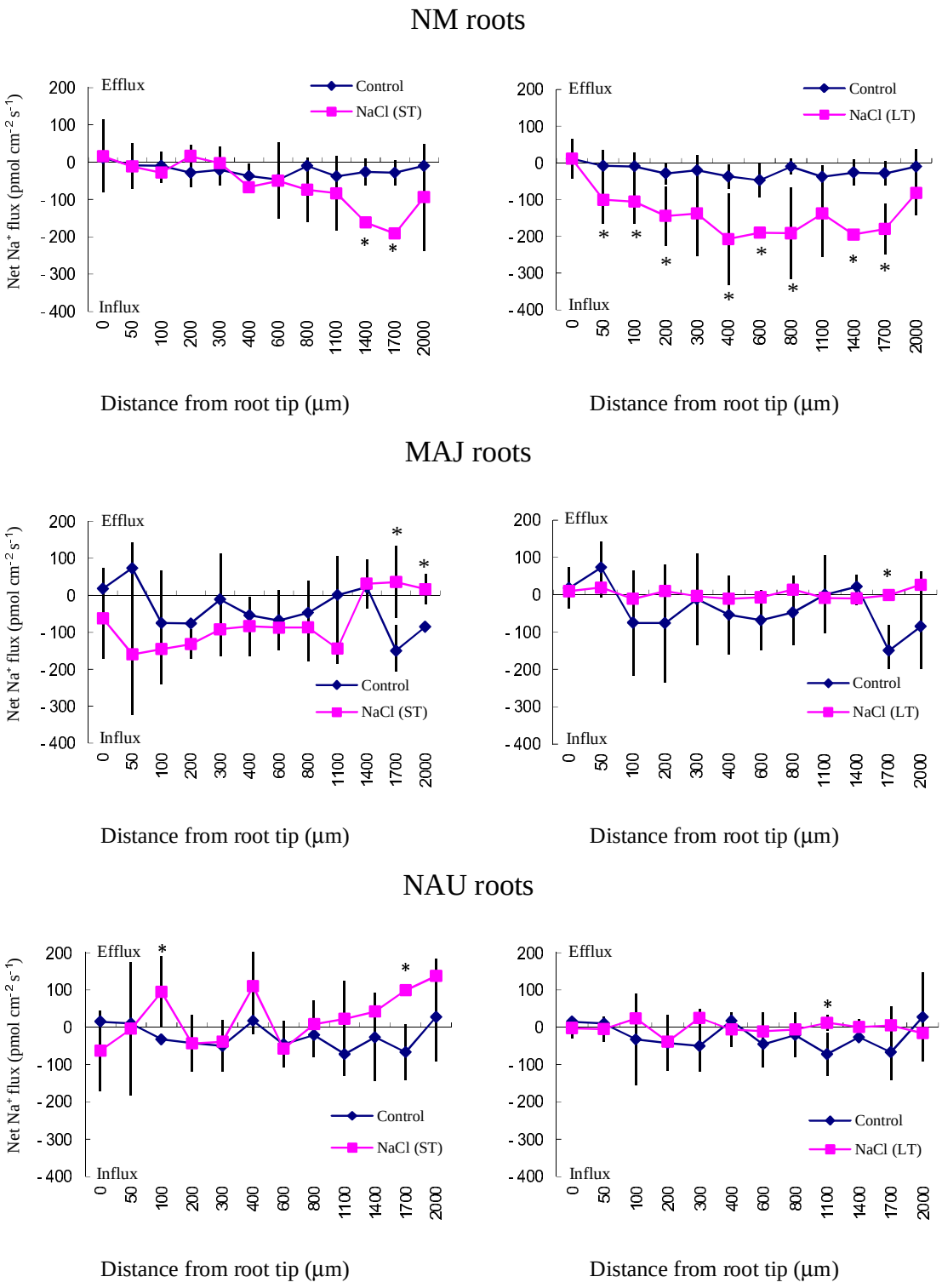

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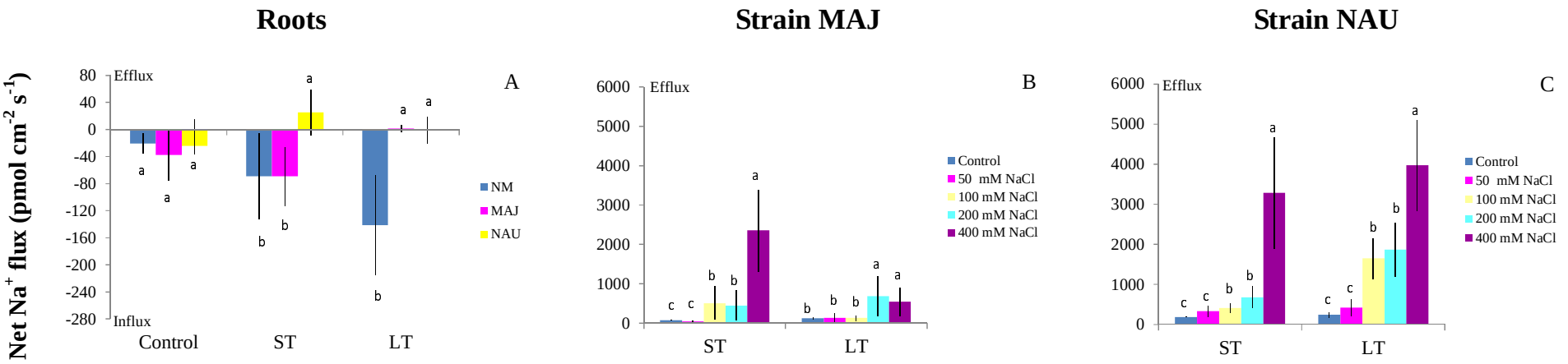

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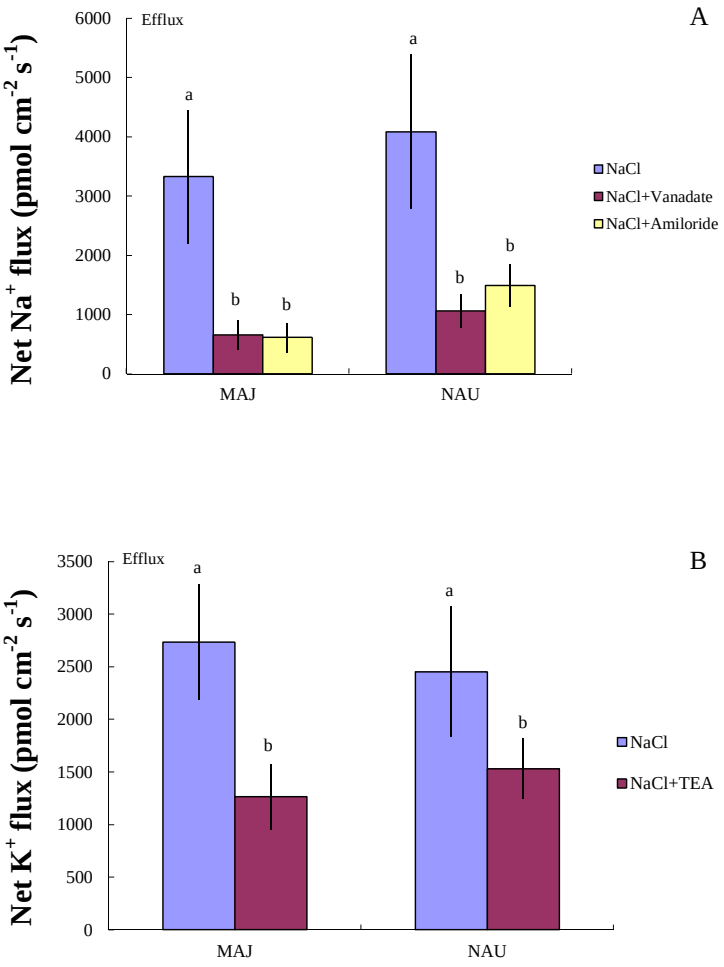

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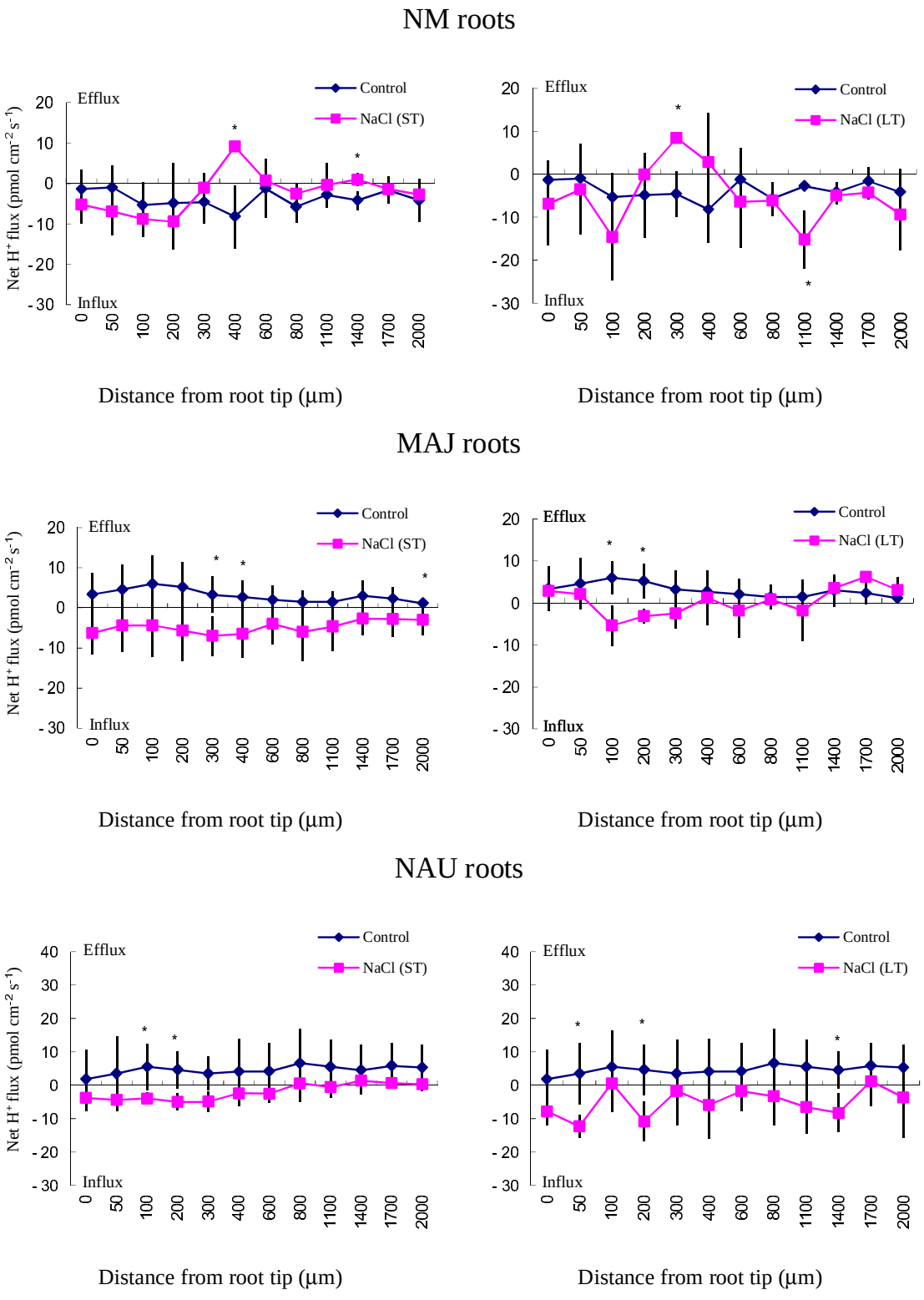

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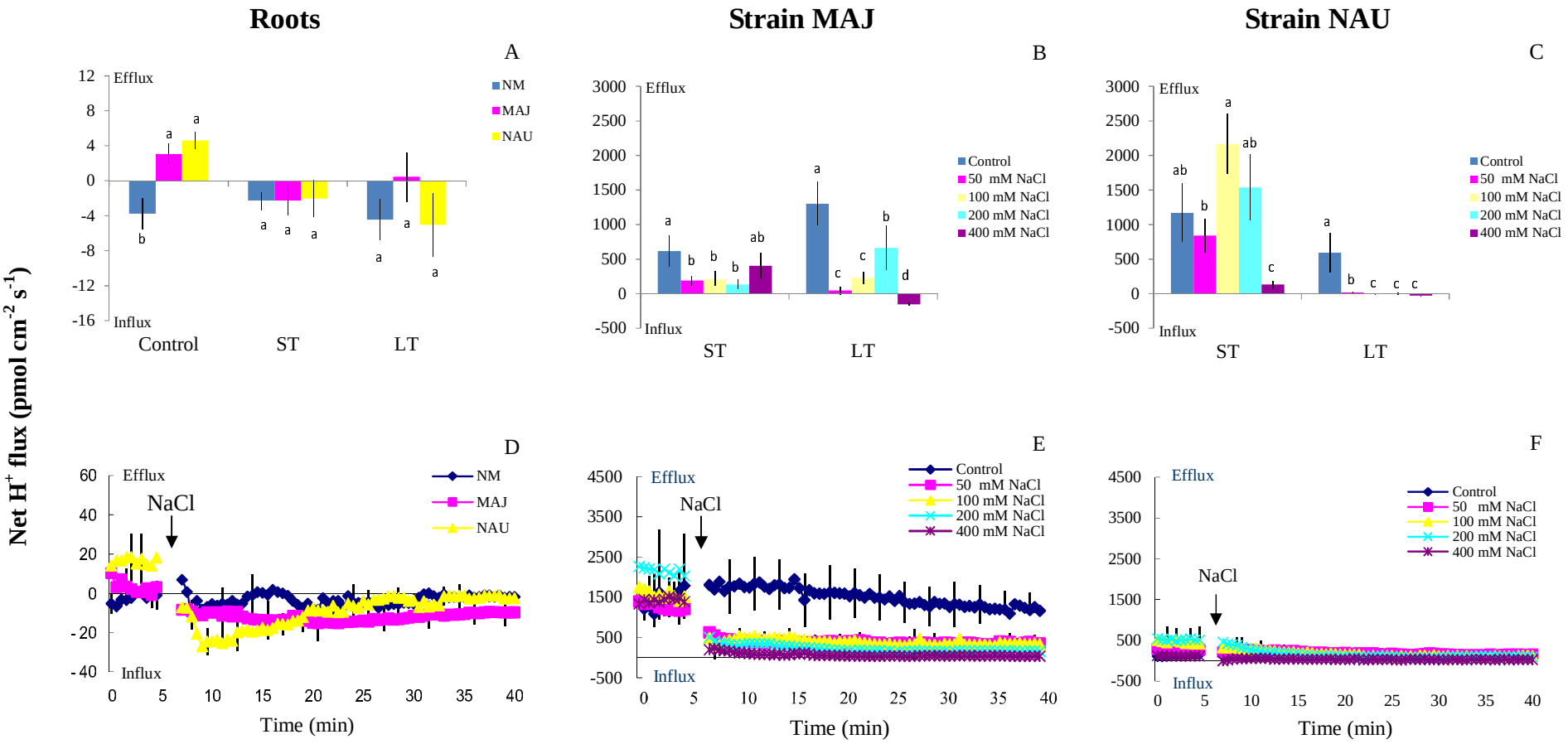

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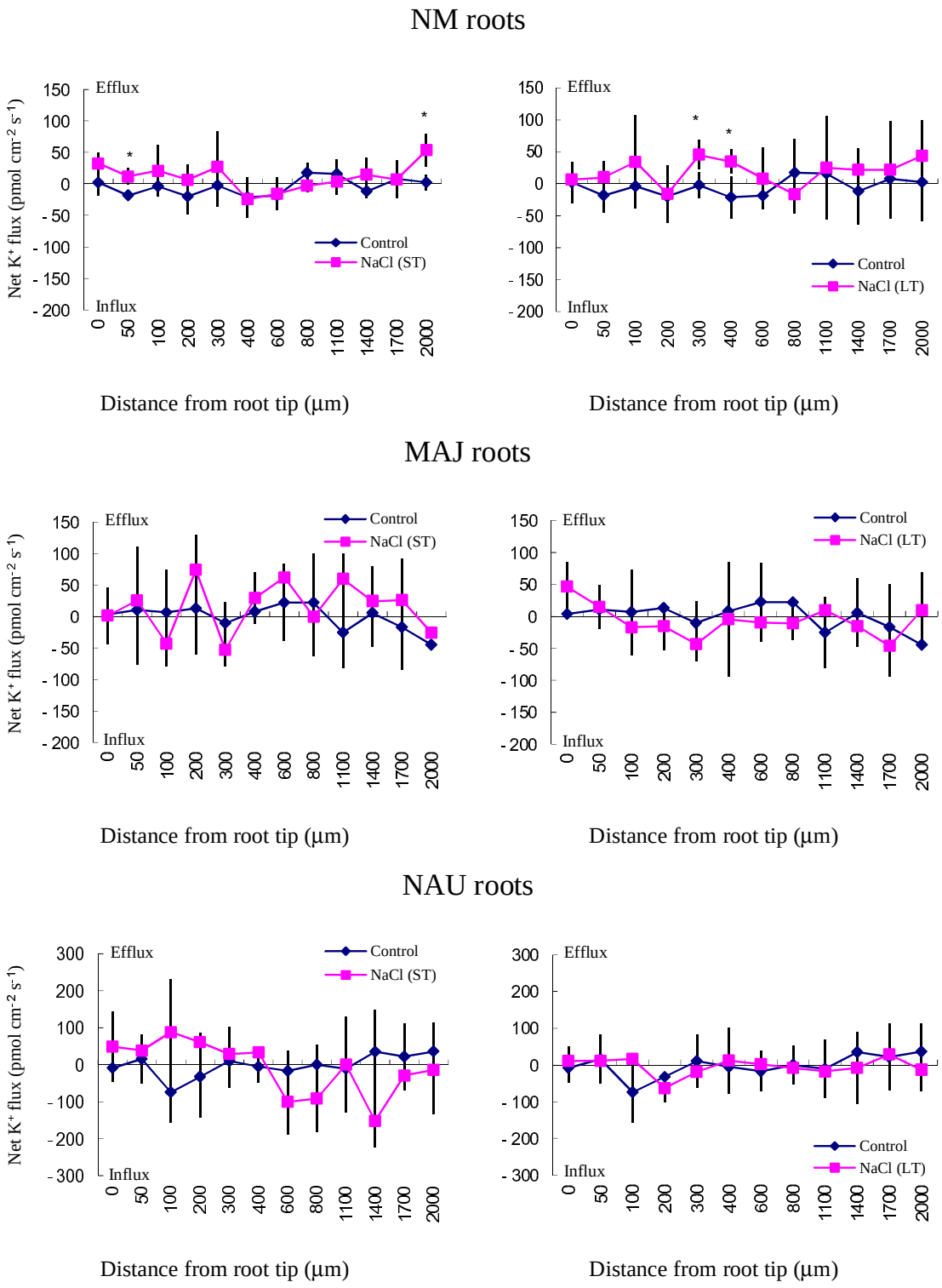

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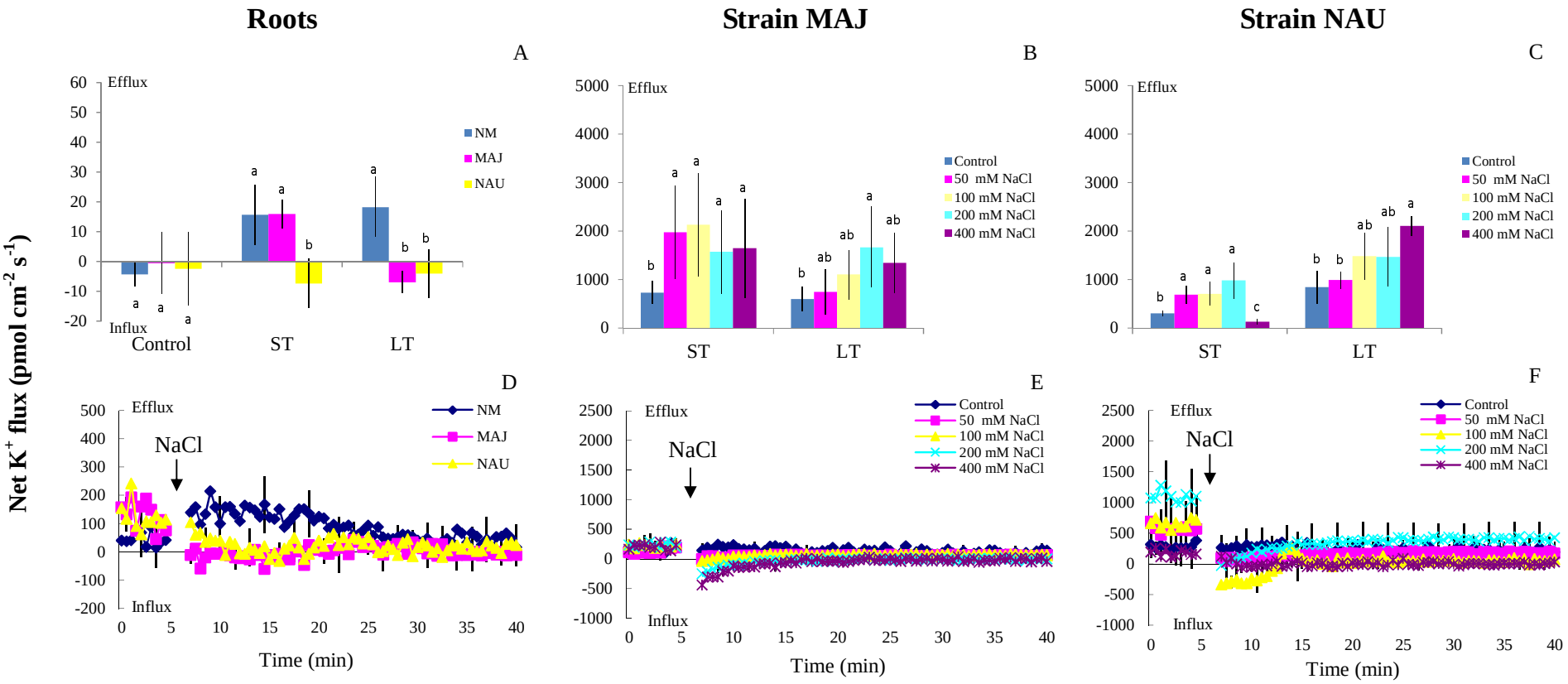

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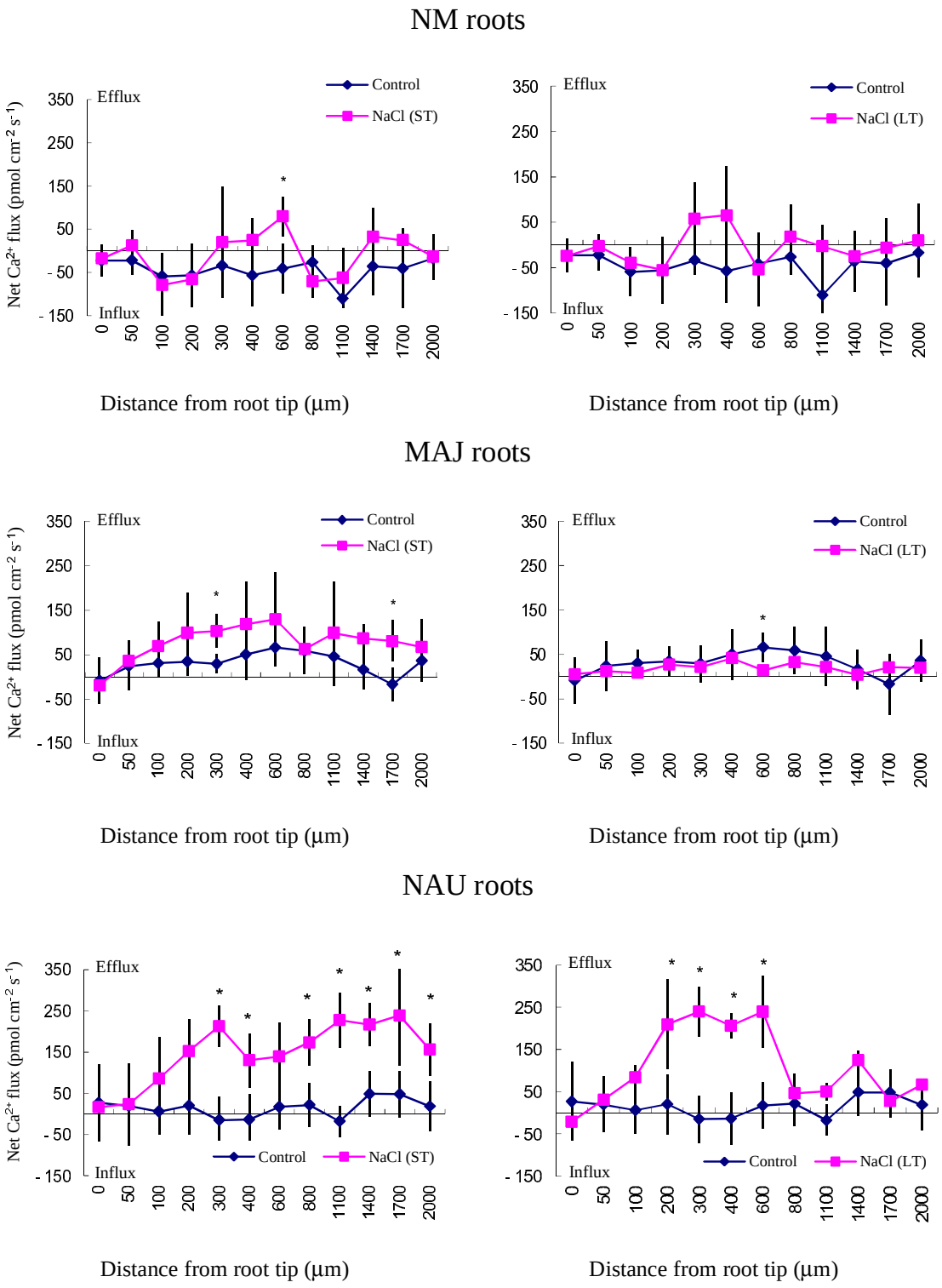

Figure 9. Effects of short-term (ST) salinity (50 mM NaCl for 24 h) and long-term (LT) salinity (50 mM NaCl for 7 d) on the net Ca^{2+} flux in the roots of mycorrhizal (MAJ and NAU)

and non-mycorrhizal (NM) *Populus* × *canescens* plants. Control roots were treated without NaCl. The Ca^{2+} flux was measured along root axes (0–2,000 μm from the apex) at intervals of 50 to 300 μm. Each point is the mean of five to six individual plants and bars represent the standard error of the mean. *$P < 0.05$ between treatments.

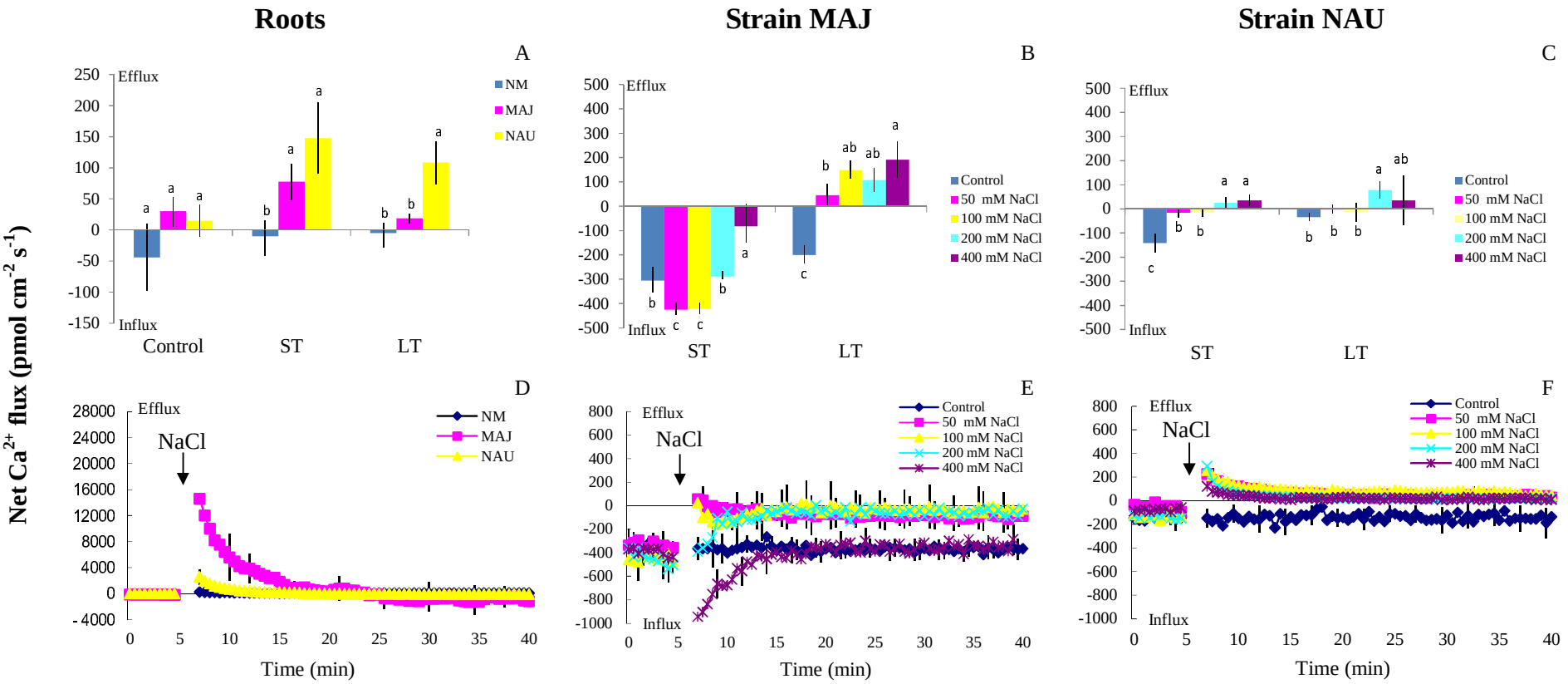

Figure 10. Effects of NaCl on steady and transient Ca^{2+} fluxes in *Populus* × *canescens* roots (NM, MAJ, NAU) and *P. involutus* strains MAJ and NAU. A, Mycorrhizal (MAJ and NAU) and non-mycorrhizal (NM) *Populus* × *canescens* plants were subjected to short-term (ST) salinity (50 mM NaCl for 24 h) and long-term (LT) salinity (50 mM NaCl for 7 d), respectively. Control roots were treated without NaCl. For each plant, Ca^{2+} fluxes were measured along root

axes (0–2,000 μm from the apex at intervals of 50 to 300 μm) and a mean value is given. Each column is the mean of five to six individual plants and bars represent the standard error of the mean. Columns labeled with different letters, a, and b, indicate significant difference at $P < 0.05$ between NM and EM roots. B and C, *P. involutus* isolates MAJ and NAU were subjected to ST salinity (50, 100, 200, and 400 mM NaCl for 24 h) and LT salinity (50, 100, 200, and 400 mM NaCl for 7 d), respectively. Control axenic mycelia were treated without NaCl. Ca^{2+} fluxes of fungus hyphae were measured over a recording period of 30-40 min and a mean value is given. Each column is the mean of five to six axenic EM cultures (pelleted hyphae) and bars represent the standard error of the mean. Columns labeled with different letters, a, b, c, indicate significant difference at $P < 0.05$ between salinity levels. D, Mycorrhizal (MAJ and NAU) and non-mycorrhizal (NM) *Populus* × *canescens* plants were subjected to salt shock with 50 mM NaCl. Ca^{2+} kinetics were recorded at the apex (measuring site was ca. 500 μm from the root tip) after the required amount of 200 mM NaCl stock was introduced into the measuring chamber. E and F, *P. involutus* isolates MAJ and NAU were subjected to salt shock with 50 mM NaCl. Ca^{2+} kinetics of axenic mycelia were recorded after the required amount of 200 mM NaCl stock was introduced into the measuring chamber. Before the salt shock, steady Ca^{2+} fluxes were monitored for ca. 5 min. In D-F, each point is the mean of four individual plants or axenic EM cultures (pelleted hyphae) and bars represent the standard error.

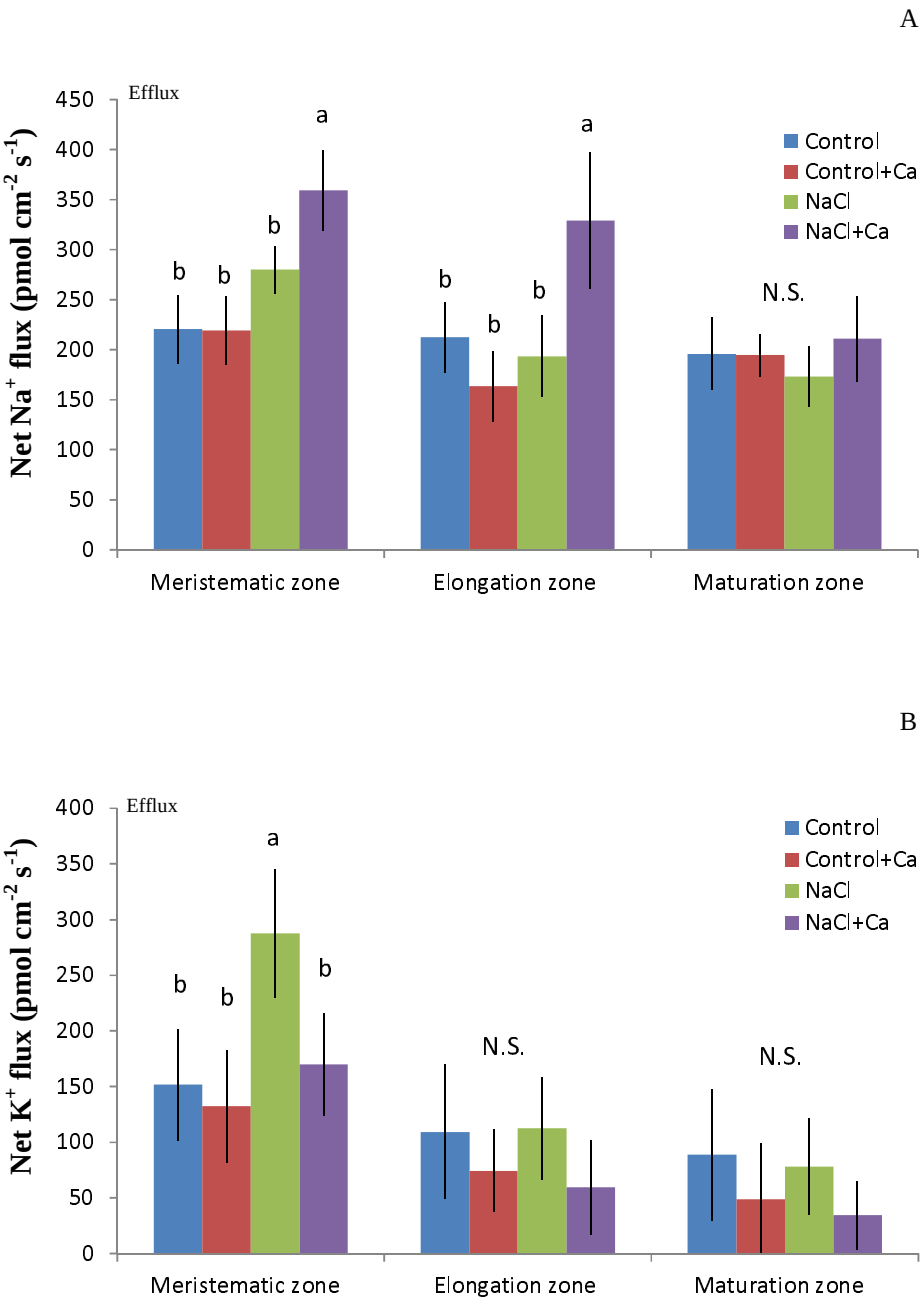

Figure 11. Effects of NaCl and Ca^{2+} supplementation on Na^+ (A) and K^+ (B) flux at the meristematic, elongation, and maturation zones of *Populus* × *canescens* roots. Plants were subjected to one week of NaCl stress (50 mM) supplemented with or without 10 mM $CaCl_2$. Control plants were well fertilized but treated without additional NaCl. Roots were sampled at 24 h and 7 d, and mean flux values are given. Steady fluxes at the meristematic, elongation,

and maturation regions were measured along the root axes at an interval of 30-50 μm. Each column is the mean of six to eight individual plants and bars represent the standard error of the mean (for each plant, the average fluxes at the meristematic, elongation, and maturation zones were calibrated from measuring points in the measured region). Columns labeled with different letters, a, b, indicate significant difference at $P < 0.05$ between treatments. N.S., no significant difference.