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# Leaf and whole tree adaptations to mild salinity in field grown *Populus euphratica*

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**Summary** *Populus euphratica* Oliv. is a highly salt tolerant tree species, and this study represents the first comprehensive investigation of salt tolerance mechanisms of mature trees of *P. euphratica* in the field. We measured NaCl concentration in xylem sap, NaCl accumulation in leaves, the effect of NaCl on leaf physiological parameters and osmotic adjustment and the allocation and distribution of NaCl between different plant organs on a whole plant level in trees exposed to mild saline groundwater (around 30 mM) in China. *Populus euphratica* showed three key mechanisms of salt tolerance. The primary mechanism had a strong control over $Na^+$ and $Cl^-$ uptake with effective exclusion mechanisms for $Cl^-$ with up to 99% of the external NaCl being excluded from the xylem. Secondly, the trees allocated large proportions of NaCl into the leaves, which served as a salt elimination mechanism as the leaves are ultimately shed at the end of the growing season. Thirdly, the trees tolerated high foliar $Na^+$ concentrations through a combination of osmotic adjustment using sucrose and probable sequestering of $Na^+$ in the apoplast. Our results indicate that the control of $Na^+$ and $Cl^-$ uptake and the regulation of $Na^+$ and $Cl^-$ delivery to the shoot are key to salt tolerance of *P. euphratica* in the field with tolerance of high $Na^+$ concentrations in leaves being a critical component.

*Keywords: apoplast, biomass allocation, compatible solutes, osmotic adjustment, salt tolerance.*

## Introduction

Salinity is a major environmental stressor that can have a significant impact on vegetation in semiarid and arid landscapes. In general, salt tolerant plants express two different types of responses to salinity: (a) mechanisms that minimize the entry of the salt into the plant and (b) mechanisms that partition salt at the tissue and cellular level so that it does not build up to toxic concentrations in the cytosol of the shoot and transpiring leaves (Munns 2002). While significant progress has been made in the past decade to decipher the physiological and molecular basis of both types of mechanisms in agricultural species and in herbaceous plants (summarized by Tester and Davenport 2003, Munns 2005, Munns et al. 2006), little progress has been made in identifying the mechanisms that allow trees to adapt to high levels of salinity in the soil.

*Populus euphratica* Oliv. is a salt tolerant poplar species that naturally occurs in the desert and semiarid regions of Central Asia. *Populus euphratica* grows in highly saline soils despite being a non-halophyte (Wang et al. 1996). The capability of *P. euphratica* to tolerate high salt concentrations has been ascribed to root-born processes such as limited ion loading into the xylem (Chen et al. 2002, 2003) and high capacity to exclude $Na^+$ and $Cl^-$ ions at the root level (Sun et al. 2009). *Populus euphratica* can tolerate large amounts of salt in its leaves (Arndt et al. 2004), and this has been attributed to the compartmentalization of $Na^+$ into the apoplast (Ottow et al. 2005). *Populus euphratica* also exhibits a high capacity for maintaining anti-oxidant enzyme activity and for restricting salt accumulation in the chloroplasts, which in turn enables high photosynthetic rates to be maintained (Wang et al. 2007). Despite the present knowledge of the salt tolerance mechanisms of *P. euphratica*, we still have a limited understanding of how these mechanisms allow the species to adapt to changes in salinity within its natural environment. Critically, almost all studies on salt tolerance have been performed in the glasshouse using juvenile seedlings. Glasshouse studies have the advantage of controlled environmental conditions. However, they have the disadvantage of usually short periods of stress, difficulties in controlling the amount of salt and often unrealistic stress conditions and salt shocks. Moreover, there can be large differences in the salt tolerance of species at the juvenile stage and at maturity (Niknam and McComb 2000). Hence, it is currently unknown if *P. euphratica* shows similar mechanisms of salt tolerance in mature trees in the field, where

the environmental conditions are different and the persistence of salt stress over decades compounds stress as compared to glasshouse experiments.

Recent studies on *P. euphratica*'s native habitat in the Taklamakan desert of China showed that it exhibits both deciduous and phreatophytic nature (Arndt et al. 2004). The constant contact to groundwater enables a high water use and transpiration throughout the growing season in *P. euphratica* (Thomas et al. 2006), despite a low drought tolerance (Hukin et al. 2005). This form of drought avoidance enables a substantial accumulation of biomass (3–6 Mg $ha^{-1}$) during the region's 6-month growing season (Gries et al. 2005). The groundwater in the Taklamakan, however, is mildly saline (around 50 mM NaCl), and the continuously high transpiration rates suggest that a substantial accumulation of harmful $Na^+$ and $Cl^-$ is likely, even if uptake of these ions is restricted. The degree of a seasonal accumulation of salt and the subsequent physiological consequences are currently unknown, and it is unclear what mechanisms *P. euphratica* employ to withstand seasonally increasing salt levels.

The aim of this study was to investigate the mechanisms that mature *P. euphratica* trees employ in their natural environment to withstand the adverse effects of a mildly saline groundwater. More specifically, we attempted to investigate (1) the intra-seasonal variation of NaCl accumulation in leaves; (2) its effect on leaf physiological parameters, ion balance and osmotic adjustment; and (3) the plant-level allocation and distribution of $Na^+$ and $Cl^-$ between different organs.

## Materials and methods

### *Site description*

We studied *P. euphratica* near the Cele Research Station of the Chinese Academy of Sciences in the foreland of the river Cele oasis (1365 m a.s.l.). The oasis is located on the southern fringe of the Taklamakan desert and is sheltered from sand drift and shifting dunes by a sparse belt of vegetation. The Taklamakan desert in Western China has an extreme environment due to its inland location and proximity to the mountain ranges of the Kunlun and Tian Shan. The climate is extremely dry with an annual precipitation of 33 mm (maximum in May and July) and with an annual potential evaporation of about 2600 mm (Xia et al. 1993). Maximum temperatures reach 42 °C in summer and minimum temperatures are as low as −24 °C in winter. The vegetation is dominated by perennial species, and the aboveground biomass is produced in the growing season that lasts from April to October. Herbaceous aboveground components die back in winter. The perennial species depend on groundwater to meet their water and nutrient demands (Zeng et al. 2006). Soils in the study area are aeolian loose sediments with non-discernible horizons. Soil texture is very homogeneous, with silt being the predominant fraction (88%), and sand and clay contributing < 5% to the soil texture.

We selected three sites where *P. euphratica* grows naturally in the foreland of Cele oasis. Near-surface groundwater (1–15 m) at the study sites is mildly saline (around 30 mM NaCl), which is typical throughout the Taklamakan desert. The salinity of groundwater varies slightly across the study region; 'control' sites with non-saline groundwater are non-existent. Due to the lack of control sites, we chose to replicate sites at multiple locations to evaluate the response of trees to mildly saline groundwater across the study area and to avoid pseudo-replication using trees in only one location.

All three study sites were located within 10 km of Cele oasis. Site A was located near a dry river bed of the Qira river, 5 km west of the oasis (37°00.662′ N and 080°39.966′ E). Adjacent to the southern side of the river bed was a stand of 5–7-m tall *P. euphratica* trees, growing scattered on sand dunes (Gries et al. 2003). Site B was located 3 km north-west of the oasis (37°04.548′ N and 080°44.890′ E) and consisted of 4–6-m high trees (Thomas et al. 2000). Gas exchange, water relations, water use and biomass production of the trees at this stand were investigated previously (Gries et al. 2005, Thomas et al. 2006). Site C was located 10 km north of the oasis (37°05.778′ N and 080°50.444′ E) and consisted of several groups of 4–6-m tall *P. euphratica* trees along an ephemeral creek.

### *Tree harvests*

Two destructive tree harvests were carried out to study the variation in the concentrations of NaCl and solutes in the leaves, branches, stems and roots of *P. euphratica* at the beginning and at the end of the growing season. Three 2-m tall trees were harvested at site B on April 24 after full leaf emergence (typically occurs from late March to early April) and on October 5, 2006 before leaf discoloration set in (typically begins in mid-October). The aboveground biomass was harvested between 9:00 and 13:00 h and separated in leaves, branches and stems. Roots were excavated during the day from a 2 × 2 m quadrant to a depth of 1.5 m. Fresh weight of each tissue fraction was determined in the field. Harvested samples from each tissue fraction, for each individual tree, were thoroughly mixed, and subsamples were selected for solute analyses. The subsamples were rapidly heated to 100 °C in a microwave oven in the field (Popp et al. 1996) to ensure minimal change in solute composition and then dried to constant weight at 80 °C in a drying oven in the laboratory. Total dry weights for each tree were calculated from the fresh:dry weight ratios of the subsamples and from the total fresh weight of each tree fraction.

### *Leaf samples*

Between May and October 2006, leaf samples were collected at the end of each month and used to determine

solute concentrations. A single sample was obtained by collecting 20–30 fully expanded outer canopy leaves per tree, four trees at each site. Trees were selected at random at each site, and mid-canopy outer branches were sampled. Half of the samples were heated in a microwave oven in the field (see above) and used for solute analysis. The other half of the samples were used to determine the fresh weight, leaf area and dry weight of each leaf sample to calculate specific leaf area (SLA, leaf area to dry weight ratio, $cm^2$ LA $g^{-1}$ DM) and leaf succulence (leaf water content to leaf area ratio, g $H_2O$ $m^{-2}$ LA).

### *Gas exchange and water relation measurements*

Net rate of carbon assimilation and stomatal conductance of sunlit and fully expanded leaves were measured with a Li-Cor 6400 portable infrared gas analyzer (IRGA) (Li-Cor, Lincoln, NE). The IRGA was equipped with an automatic $CO_2$ control ($CO_2$ injector) and a standard broadleaf cuvette that measured gas exchange by an illuminated leaf area of 2.5 $cm^2$. The external concentration of $CO_2$ was controlled by the Li-Cor at about 360 ppm. The measurements were taken between 11:00 and 14:00 h under ambient light levels, temperature and relative humidity. We measured five fully expanded outer canopy leaves per tree, six trees at each site and ensured maximum light exposure during the measurement. Climatic parameters (air T, RH and PAR) were recorded during the gas exchange measurements. Predawn leaf water potential was measured in a pressure chamber (PMS Instrument Co., Corvallis, OR) on fully expanded exposed leaves of outer canopy shoots harvested from six trees at each location.

Leaf relative water content (RWC) of leaves sampled at predawn was determined gravimetrically. Three leaves from each tree were weighed and then rehydrated through the petiole for 4 h, and preliminary tests indicated that this period was sufficient to ensure complete rehydration. The leaf samples were then weighed (saturated weight), dried at 80 °C for 48 h and weighed again (dry weight).

### *Groundwater and xylem sap samples*

Groundwater samples were obtained by drilling permanent sampling wells with an auger. Plastic pipes were inserted into the bore and capped between sampling times. At site A, groundwater was detected at a depth of 7.5 m, 5.0 m at site B and 2.3 m at site C. Groundwater was sampled four times during 2006 (May, August, September and October). One sample per well was collected at each sampling time and kept frozen until solute analyses were conducted.

Xylem water from six trees at each site was collected in the morning of the same day that leaf physiological measurements were taken (see below). Xylem sap was collected from the lower branches (15–20-mm diameter) from six sample trees using a mild vacuum extraction technique (Jeschke and Pate 1995). In brief, the cut end of a branch was freed from its bark and leaves, and inserted tightly into a 50-ml centrifugation tube using a polymer sealant (Terostat). Xylem sap was collected by creating a mild vacuum (pressure 25–60 kPa) using a vacuum pump and by successive cutting of 5–10-cm segments from the proximal end of the branch to facilitate sap displacement from conducting xylem vessels. Extracted xylem fluid (0.5–2 ml) was collected in 2-ml safe lock vials and kept frozen until solute analyses were conducted.

### *Solute analyses*

We determined anions, organic acids, cations and soluble carbohydrate concentrations in all plant samples and anions and cations in xylem sap and groundwater samples. Dried leaf, branch, stem and root samples were ground to a fine powder in a ball mill (Retsch MM2, Vienna, Austria) and extracted with hot water (4:1, w/v, 95 °C, 1 h). Anions (chloride, nitrate, sulfate and phosphate) and organic acids (malate, oxalate and citrate) were quantified on the Dionex HPLC system using an anion-exchange column (AS11 HC 250 × 2 mm) with guard column (AS11 HC 50 × 2 mm). Solutes were isocratically eluted at 25 °C using a flowrate of 0.38 ml $min^{-1}$ 30 mM KOH and then quantified using an ED40 electrochemical detector. Cations (potassium, magnesium, sodium and calcium) were determined by ICP analysis (Vista Pro-Axial, Varian Instruments, Palo Alto, CA). Water-soluble carbohydrates and inositols (myo-inositol, glucose, fructose and sucrose) were measured by high-performance anion-exchange chromatography (HPAEC-PAD; DX 500 and ED 40, Dionex; Arndt et al. 2000). Proline was quantified in aliquots of the hot water extracts based on the method of Troll and Lindsey (1955).

### *Statistical analyses*

Statistical analysis was performed using the Statgraphics 4.0 analytical software (Statpoint Technologies Inc., Warrenton, VA). One-factor ANOVA was performed to identify statistically significant differences between different sampling times within each site. Post hoc tests were made using Tukey HSD. Noted differences between values are at the 0.05 level of significance. A Student's *t* test was performed to evaluate significant differences in solute concentrations in leaves between May and October (Table 1).

## Results

In the Taklamakan desert, *P. euphratica* meets its demands for water and nutrient supply by maintaining permanent contact with groundwater. We detected near-surface groundwater (between 2.3 and 7.5 m) that was moderately saline at all three sites (Figure 1). The groundwater level did not change, but the salinity of groundwater showed small variations during the growing season. Salinity increased by about 20 mM during the growing season at sites A and C, and was variable at site B (Figure 1).

Table 1. Concentration of solutes (mmol $kg^{-1}$ dry matter) in leaves of *P. euphratica* grown at three different sites in the Taklamakan desert near Cele oasis in May and October 2006; values are mean of $n = 6$ trees per site; asterisks represent significance of the difference between May and October harvest (Student's $t$ test; $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.001$).

| | Site A | | Site B | | Site C | |
|---|---|---|---|---|---|---|
| | May | October | May | October | May | October |
| $Na^+$ | 70.6 | 256.6 * | 71.6 | 351.8 *** | 380.6 | 757.2 ** |
| $K^+$ | 411.2 | 255.6 * | 390.9 | 161.8 *** | 348.1 | 227.1 |
| $Ca^{2+}$ | 13.0 | 225.3 ** | 46.7 | 278.9 *** | 29.9 | 154.1 ** |
| $Mg^{2+}$ | 64.2 | 135.6 * | 207.5 | 307.9 * | 127.9 | 143.4 |
| $Cl^-$ | 57.0 | 155.3 ** | 93.0 | 127.9 * | 76.1 | 120.1 * |
| $SO_4^{2-}$ | 23.9 | 163.0 ** | 94.5 | 118.7 | 73.3 | 205.6 ** |
| $PO_4^{2-}$ | 6.0 | 16.7 | 7.6 | 3.5 ** | 5.8 | 2.6 *** |
| Malate | 34.0 | 56.9 | 24.4 | 133.9 ** | 59.7 | 61.1 |
| Oxalate | 4.3 | 2.0 | 2.4 | 2.1 | 7.3 | 3.4 ** |
| Citrate | 13.8 | 58.5 * | 29.2 | 86.1 * | 52.7 | 99.8 * |
| Myo-inositol | 21.2 | 58.8 * | 56.8 | 61.5 | 61.4 | 42.8 ** |
| Glucose | 2.9 | 9.7 * | 26.3 | 21.7 | 25.5 | 12.7 ** |
| Fructose | 9.0 | 4.5 ** | 12.1 | 18.5 * | 11.7 | 8.2 |
| Sucrose | 57.5 | 174.9 ** | 58.9 | 152.8 * | 125.5 | 126.3 |
| Proline | < 2 | < 2 | < 2 | < 2 | < 2 | < 2 |
| Sum solutes | 779 | 1573 ** | 1083 | 1827 *** | 1386 | 1964 ** |

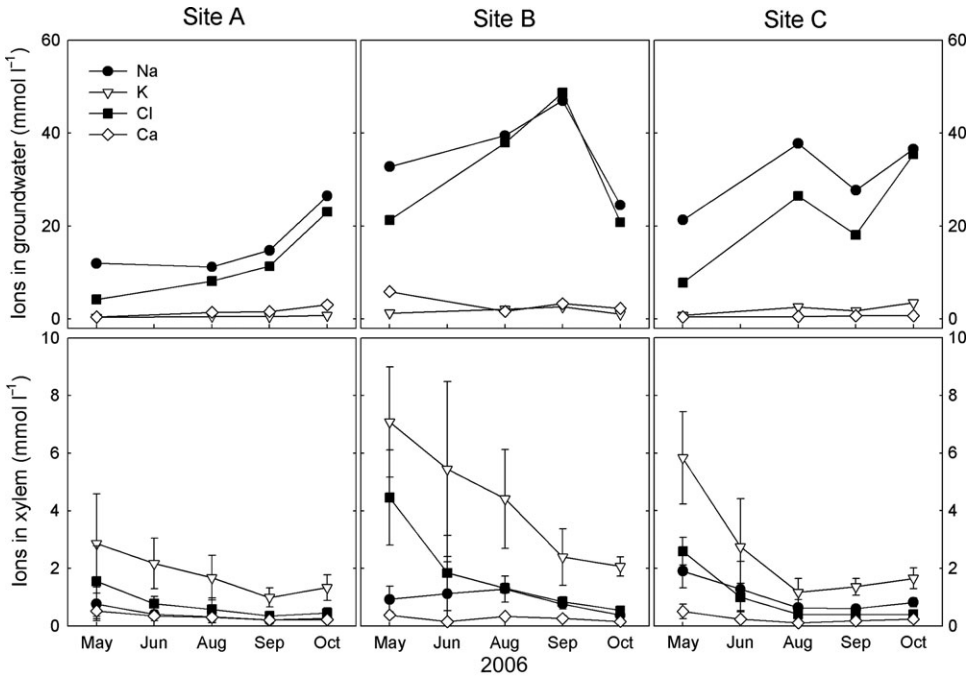


Figure 1. Seasonal variation in the concentrations of $Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$ (mmol $l^{-1}$) in groundwater and xylem sap of *P. euphratica* at three different locations in the Taklamakan desert near Cele oasis, China. Error bars are standard deviation of xylem sap samples ($n = 6$) per sampling event, only one groundwater sample was collected per sampling event and site.

Xylem sap analysis confirmed that trees at all three sites were efficient in excluding $Na^+$ and $Cl^-$ from xylem sap. Trees at all sites had concentrations of < 2 mM $Na^+$ and $Cl^-$ in their xylem sap, despite the greater NaCl concentrations in groundwater. Conversely, trees had greater $K^+$ concentrations in the xylem sap than in groundwater indicating the presence of effective $K^+$ uptake mechanisms. The concentrations of both $Na^+$ and $Cl^-$ in the xylem sap decreased toward the end of the growing season, which coincided with the decreases in transpiration (Figure 3D).

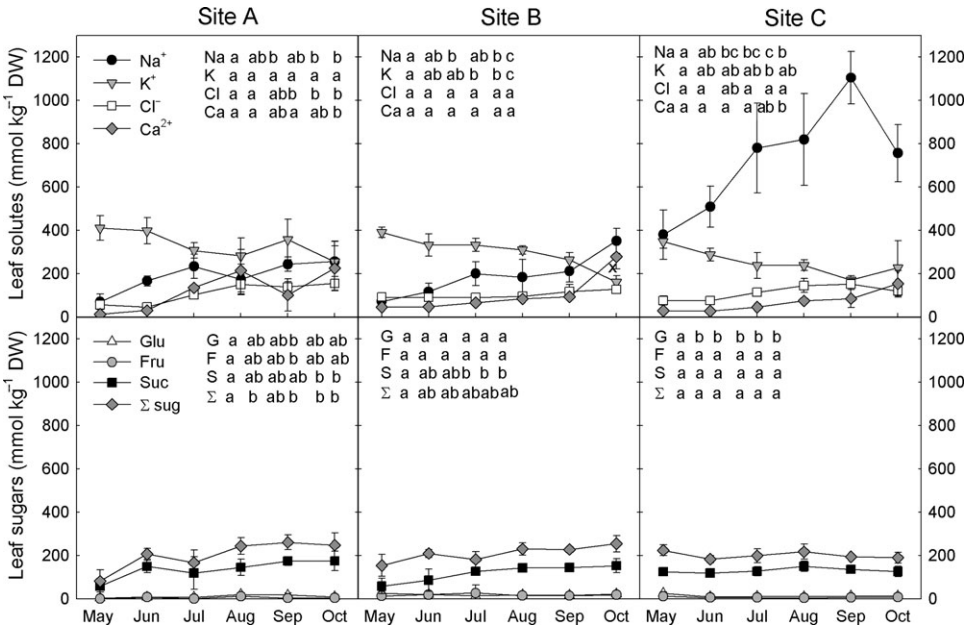


Figure 2. Seasonal variation in concentrations of $Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$ (upper panels) and glucose, fructose, sucrose and sum of carbohydrates (lower panels) in the leaves of *P. euphratica* grown at three different sites in the Taklamakan desert. Error bars are standard deviation of $n = 4$ trees; different letters indicate statistically significant differences between the different sampling events (ANOVA, Tukey HSD, $P < 0.05$) in the table above the figure.

There were also differences between the three sites, with trees at site A having consistently lower NaCl concentrations in the xylem sap and with trees at site C having greater $Na^+$ concentrations early in the growing season.

The constant exposure to saline groundwater led to an increase in osmotically active substances by 580–800 mmol kg$^{-1}$ DW in the leaves of *P. euphratica* between May and October (Table 1). $Na^+$ and $Cl^-$ increased significantly from May to October in the leaves of trees from all sites. By May, trees at site C had much greater foliar $Na^+$ concentrations (380 mmol kg$^{-1}$ DW), which doubled in concentration by October (Figure 2; Table 1). Other ions also showed significant increases in concentration between May and October, with $Ca^{2+}$ and $SO_4^{2-}$ showing the largest increases; conversely, $K^+$ concentrations decreased over the growing season (Figure 2; Table 1).

Among the organic acids, citrate increased significantly in trees at all sites (Table 1). Sucrose, which was the dominant carbohydrate, also increased in concentration over the course of the year, but only at sites A and B. The marked increase in inorganic osmotica was not accompanied by a similar increase in compatible solutes. This was particularly evident in the leaves at site C, where substantial increases in $Na^+$ concentrations were not accompanied by increases in either carbohydrates or cyclitols (Figure 2; Table 1). Proline concentrations in leaves were also negligible (< 2 mmol kg DW).

The increase in foliar inorganic ions had no detrimental effect on water relations or gas exchange parameters of *P. euphratica*. Initially, trees at all sites had similar predawn leaf water potentials; however, from July onward trees at site C had higher predawn water potentials (Figure 3A). The RWC in leaves ranged between 85% and 95% during the year indicating a good water status (Figure 3B). The absence of significant drought stress was confirmed by high gas exchange rates all year round, with *P. euphratica* leaves having $CO_2$ assimilation rates of around 10–18 μmol m$^{-2}$ s$^{-1}$ and transpiration rates between 7 and 18 mmol m$^{-2}$ s$^{-1}$ (Figure 3C and D). One exception was trees at site C, which showed a distinct drop in $CO_2$ assimilation rates and transpiration rates in September/October. Leaf morphological parameters showed some changes during the year with SLA decreasing from values of > 100 cm$^2$ g$^{-1}$ DW in May to around 70 cm$^2$ g$^{-1}$ DW for the rest of the year (Figure 3E), with no differences being detected between sites. Leaf succulence was more or less stable during the year (Figure 3F). Climatic conditions during the growing season were very similar across measurement dates (Figure 3G).

The total tree biomass harvests in April and October at site B revealed that concentrations of $Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$ remained stable in branches, stems and roots, and that the total amounts of these ions did not change significantly during the year (Figure 4). One notable exception was leaves. We observed significant increases in concentrations and total amounts of $Na^+$, $Ca^{2+}$ and $Cl^-$ between April and October, whereas $K^+$ concentrations decreased at the same time, confirming the results from foliar analysis

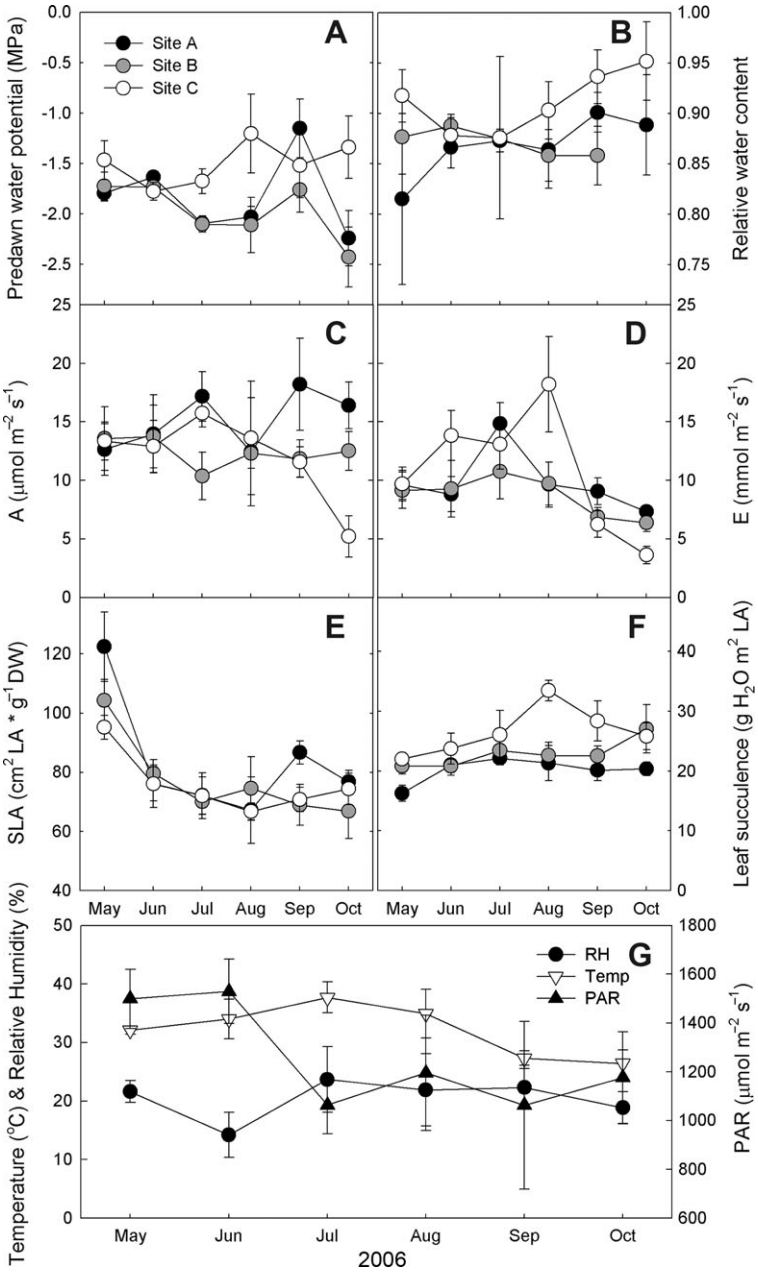


Figure 3. Seasonal variation in (A) predawn water potential, (B) relative water content, (C) photosynthesis, (D) stomatal conductance, (E) specific leaf area and (F) leaf succulence of leaves of *P. euphratica* grown at three different sites in the Taklamakan desert, China. Error bars are standard deviation of $n = 6$ trees. Panel (G) shows the average climatic conditions during the measurement period of gas exchange, mean of the three sites.

at the other sites. At the total tree level, around one-fifth of $Na^+$ and $Ca^{2+}$ and one-third of $Cl^-$ were stored in leaves in October, which represented only 4% of the total tree biomass (Table 2). The concentration of $Na^+$ and $Cl^-$ in leaves represents an effective mechanism for *P. euphratica* to eliminate NaCl from the tree, as NaCl-concentrated leaves are shed by this deciduous species in November. The stem wood in *P. euphratica* contained about one-third of all $Na^+$ and just under 20% of $Cl^-$, while branches are an important storage organ for $Na^+$ and roots are for $Cl^-$ (Table 2).

## Discussion

Our results give important insights into whole tree adaptive mechanisms of salt tolerance of *P. euphratica*, and the data indicate three key findings: (i) *P. euphratica* exhibits

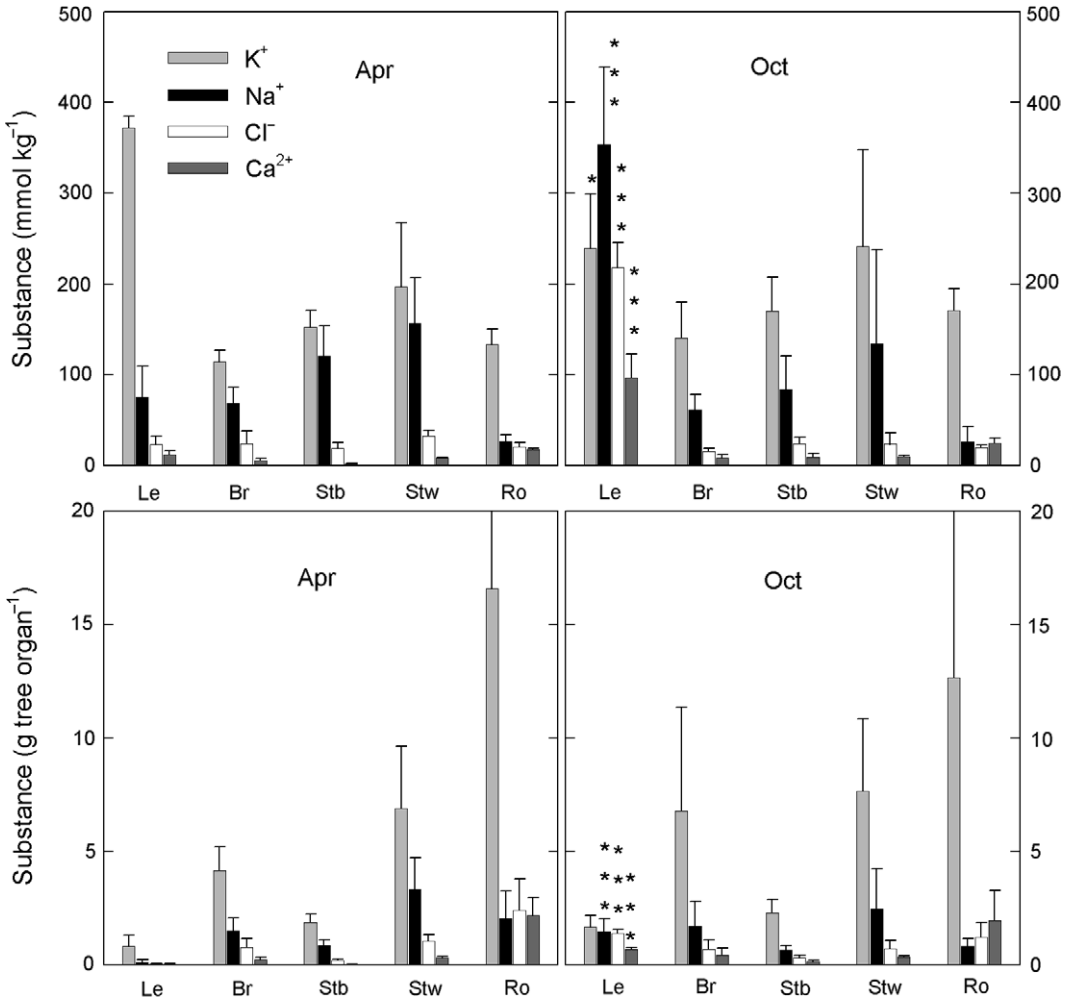


Figure 4. Concentration (upper panels) and total amount (lower panels) of $Na^+$ (solid bars), $K^+$ (gray bars), $Cl^-$ (open bars) and $Ca^{2+}$ (dark gray bars) in leaves (Le), branches (Br), stem bark (Stb), stem wood (Stw) and roots (Ro) of *P. euphratica* trees in April (left panels) and October (right panels). Error bars are standard deviation of $n = 3$ trees; asterisks represent significance of the difference between April and October harvest (Student's $t$ test; $^*P < 0.05$ and $^{***}P < 0.001$).

Table 2. Percentage of biomass and content of $Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$ in different organs of *P. euphratica* as a fraction of total tree biomass or $Na^+$, $K^+$, $Ca^{2+}$ and $Cl^-$ content; data are mean values of three individual trees harvested in April and October 2006.

| | April | | | | | October | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Biomass (%) | $Na^+$ (%) | $Cl^-$ (%) | $K^+$ (%) | $Ca^{2+}$ (%) | Biomass (%) | $Na^+$ (%) | $Cl^-$ (%) | $K^+$ (%) | $Ca^{2+}$ (%) |
| Leaves | 1.0 | 1.4 | 0.9 | 2.7 | 1.0 | 4.2 | 20.9 | 32.3 | 5.4 | 19.1 |
| Branches | 17.4 | 19.0 | 17.2 | 13.7 | 8.2 | 26.3 | 23.9 | 15.8 | 21.8 | 12.1 |
| Stem bark | 5.8 | 10.9 | 4.5 | 3.1 | 0.9 | 8.1 | 9.1 | 7.1 | 7.4 | 3.8 |
| Stem wood | 17.0 | 42.5 | 23.5 | 22.8 | 11.2 | 19.2 | 34.8 | 16.2 | 24.7 | 9.4 |
| Root | 58.7 | 26.1 | 53.9 | 54.8 | 78.7 | 42.2 | 11.3 | 28.5 | 40.7 | 55.6 |

a strong control over $Na^+$ and $Cl^-$ uptake with effective exclusion mechanisms for $Cl^-$; (ii) the trees allocate large proportions of $Na^+$ and $Cl^-$ into the leaves, which serves as a salt elimination mechanism as the leaves are ultimately shed at the end of the growing season; and (iii) the trees tolerate the large concentrations of $Na^+$ in leaves by a combination of osmotic adjustment and probable sequestering of $Na^+$ in the apoplast.

### *Control of $Na^+$ and $Cl^-$ uptake*

Our results identify that salt exclusion is the primary mechanism of salt tolerance in *P. euphratica* in its natural environment. The species is able to control $Na^+$ and $Cl^-$ transport in the xylem and to maintain salt concentrations in every part of the tree throughout the growing season, except for the leaves. *Populus euphratica* maintained the capacity to exclude $Na^+$ and $Cl^-$ from the xylem. This indicates that the observed increases of $Na^+$ and $Cl^-$ in leaves are *not* caused by deficiencies in the control of $Na^+$ and $Cl^-$ via xylem loading. At the end of the growing season, *P. euphratica* was still able to exclude 98–99% of $Na^+$ and $Cl^-$ in the groundwater. Although we did not measure $Na^+$ and $Cl^-$ uptake directly, our results are in agreement with laboratory based studies using young seedlings (Sun et al. 2009) that *P. euphratica* has a high capacity to extrude $Na^+$ and $Cl^-$ in roots (Chen et al. 2002, 2003).

At the whole plant level, there were marked differences in the capacity to exclude $Na^+$ and $Cl^-$. Despite similar concentrations in the groundwater, *P. euphratica* was more effective in excluding $Cl^-$ than $Na^+$. $Cl^-$ concentrations were much lower particularly in the aboveground organs at the end of the season. Our results suggest that the lower $Cl^-$ concentrations are not only the consequence of tight transport control in the xylem (Chen et al. 2002, 2003) but also possibly by the retranslocation of $Cl^-$ in the phloem and by the subsequent effective exclusion/exudation of $Cl^-$ by the roots. The $Cl^-$ concentrations in the xylem were of similar magnitude compared to $Na^+$ concentrations, which means that similar amounts of $Na^+$ and $Cl^-$ were transported to the aboveground organs, yet $Na^+$ concentrations in the leaves were almost double that of $Cl^-$. $Cl^-$ concentrations in the other aboveground plant tissues were also substantially lower compared to $Na^+$ (Figure 4). It is, therefore, possible that a proportion of $Cl^-$ is being retranslocated into the roots via the phloem where it is then excluded/exuded. Within the phloem, $Cl^-$ is known to be relatively mobile, and the recirculation of $Cl^-$ has been reported for a number of plant species (White and Broadley 2001).

### *Osmotic adjustment and $Na^+$ sequestration in the apoplast*

The substantial increase of osmotically active substances between May and October in leaves of *P. euphratica* was driven by the accumulation of ions, in particular, $Na^+$ (Table 1). The significant increase in $Na^+$ did not result in any detrimental effect on the gas exchange parameters of the trees as leaves maintained substantial $CO_2$ assimilation rates, regardless of the foliar $Na^+$ and $Cl^-$ concentrations (Figures 2 and 3). This means that *P. euphratica* has effective mechanisms for tolerating high $Na^+$ concentrations in its leaves. Non-halophytes typically sequester excess amounts of $Na^+$ and many other ions in the vacuole to keep cytosolic $Na^+$ concentrations at a minimum to protect biosynthetic processes (Tester and Davenport 2003, Munns 2005). Typically, an increase of inorganic ions in the vacuole is accompanied by an increase of compatible (non-toxic) solutes in the cytoplasm, which allows osmotic adjustment between the two cellular compartments. Previous glasshouse studies on *P. euphratica* concluded that osmoprotectants, such as sugar alcohols and amino compounds (e.g., proline), do not play a key role in cell pressure adjustment (Ottow et al. 2005), and our results confirm this. The only compatible solute that increased in response to increasing foliar $Na^+$ and $Cl^-$ concentrations was sucrose. It is, therefore, possible that sucrose acts as a compatible solute in the cytoplasm balancing high ion concentrations in the vacuole when $Na^+$ concentrations are at low to moderate levels. The lack of an increase in compatible solutes in the high $Na^+$ leaves at site C, however, indicates that $Na^+$ is most likely located in both the apoplast and the vacuole. If $Na^+$ was only stored in the vacuole, there should have been a concurrent increase of compatible solutes in the cytoplasm to counterbalance the increased osmotic pressure of the vacuole, but this increase was not observed. Apoplastic storage of $Na^+$ in *P. euphratica* was observed in glasshouse experiments in high salt treatments (Ottow et al. 2005). The authors concluded that the apoplastic $Na^+$ replaces cell wall-bound cations, thereby making it inactive as an osmolyte. Our results also suggest that at high foliar $Na^+$ levels the apoplastic storage of $Na^+$ is deactivating the ion as an osmolyte in the leaves of *P. euphratica*. The dilution of foliar salt by an increase in leaf water content or leaf succulence is not a major mechanism in mature field grown trees (Figure 3F).

### *NaCl allocation into leaves*

The allocation of $Na^+$ and $Cl^-$ into the leaves is a favorable strategy for *P. euphratica* as the leaves are shed at the end of the growing season, thereby eliminating salt from the tree on an annual basis. Large foliar concentrations of $Na^+$ in *P. euphratica* had been reported previously in the glasshouse studies (e.g., Chen et al. 2001, Ottow et al. 2005); however, these were short-term salt stress experiments using seedlings. In the desert environment of the Taklamakan, *P. euphratica* is exposed to very different levels of stress compared to those grown in glasshouse experiments. Observed increases in inorganic ion concentrations in the leaves of naturally occurring trees were found to be driven mainly by consistently high transpiration rates at all three sites. Previous measurements by Thomas et al. (2006), in 1999 and 2000, had confirmed the high water use of *P. euphratica*. Trees at site B used up to 400 kg $H_2O$ $m^{-2}$ $a^{-1}$ at a tree density of around 2000 trees per hectare; this is equivalent to the use of 2000 l of groundwater per tree per year. The phreatophytic nature and the high water use of this species highlight that even with efficient NaCl exclusion mechanisms these trees will still take up substantial amounts of NaCl. Our results demonstrate that most of the salt that is taken up is allocated to the leaves. Some salt is also distributed to the woody tissues with increases in

biomass associated with the allocation of $Na^+$ and $Cl^-$ to these tissues. However, as there was no net increase of $Na^+$ and $Cl^-$ in stems, branches or roots, it can be concluded that these tissues have only a limited capacity to act as storage compartments for $Na^+$ and $Cl^-$.

## Conclusion

Plant adaptations to salinity involve a wide range of mechanisms that generally involve the control of uptake of $Na^+$ and $Cl^-$ and their distribution within the plant (Tester and Davenport 2003): (1) regulation of $Na^+$ and $Cl^-$ delivery to the shoot, (2) retranslocation from shoots via the phloem for exclusion through roots, (3) allocation to parts of the shoot which are insensitive to $Na^+$ and $Cl^-$, (4) secretion onto the surface of the leaf and (5) control of transpiration. Our results demonstrate that *P. euphratica* uses a combination of the first three mechanisms, while the latter two do not seem to play a role under the mild saline conditions. The field-grown trees showed a tight control over $Na^+$ and $Cl^-$ uptake, allocation of excess $Na^+$ and $Cl^-$ to leaves for subsequent elimination and possibly the retranslocation of $Cl^-$ in the phloem and apoplastic/vacuolar storage of large amounts of $Na^+$ in leaves. The trees in this study were exposed only to mild salinity levels, but even under these conditions they accumulated large amounts of salt in their leaves with distinct differences in foliar salt accumulation between the different sites.

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