



Research article

Characteristics of chemical components in the trunk xylem sap of pine trees by means of a centrifugation collection method

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ABSTRACT

Knowledge of the characteristics of chemical components transported in the xylem sap of trunks remains deficient and limited because no appropriate method exists to extract the xylem sap from this part of the tree. We thus explored the differences in xylem sap components extracted by means of centrifugation and water displacement methods and depicted the level and behavior of chemical components in the xylem sap of trunks and branches of different aged trees from a pine forest in northern China. There were no significant differences between the two methods with respect to nitrogen (N) compounds and inorganic ions in the xylem sap. Potassium concentrations obtained by the methods were similar and consistent with the values obtained from earlier publications on woody species. This suggests that contamination of the xylem sap by the centrifugation method is negligible, and this method would be a reliable and robust tool for collection of the trunk xylem sap. Dissolved organic N was the dominant component of total N followed by nitrate (NO_3^-) and ammonium (NH_4^+). Potassium and chloride were the predominant cation and anion, respectively, of the xylem sap. The NO_3^- concentration basically did not change, whereas the NH_4^+ concentration was larger transported from the trunk to branches for the large tree class during foliage senescence. More inorganic N components (mainly NO_3^-) were found in young trees than in old trees. Our study contributes to improve the diagnostic assessments of tree physiological processes and growth in mature forest trees under environmental changes.

1. Introduction

Trunk xylem is the major conduit for the transport of water, nutrients, metabolic products and signals from the root to the shoot of forest trees (Kant, 2017; Diaz-Espejo and Hernandez-Santana, 2017; Pratt and Jacobsen, 2018). These components are transported by gradients in hydrostatic pressure and water potential by mass flow, which provides a rapid connection over long-distance transport systems in a matter of hours (Benkert et al., 1995). The xylem sap components from root uptake and assimilation, which are transported to photosynthetically active leaves, mainly consist of nitrogen (N) compounds and inorganic ions and are fundamental nutrients and limiting factors for forest growth and productivity (Gessler et al., 1998; Peuke and Rennenberg, 2011; Tegeder and Masclaux-Daubresse, 2018). Along the long-distance pathway in the trunk xylem, some components (e.g., N compounds) may decrease because of removal from the trunk xylem for

metabolism or N storage (Glavac and Jochheim, 1993). Additionally, the behavior of trunk xylem sap components varies considerably with plant growth stages, plant species and environmental conditions (Suzuki and Kohno, 1983; Glavac and Jochheim, 1993; Dambrine et al., 1995). Hence, studying variations in the chemical components of xylem sap is of basic interest, as it reflects the internal mineral uptake and translocation of trees (Pate and Jeschke, 1993; Rennenberg et al., 1996; Siebrecht et al., 2003; Tegeder and Masclaux-Daubresse, 2018), and also provides insight into the diagnostic assessments of tree physiological processes and growth in a changing environment (Kant, 2017; Peuke and Merchant, 2018).

The characterization of xylem sap components and their responses to various nutritional conditions (e.g., soil N fertilization/deficiency, foliar application of nitrate/ammonium, and salt stress), as well as different climatic effects (e.g., cold, flooding and water deficit), have been examined, mainly in herbaceous species or tree saplings within the

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last three decades (e.g., Gessler et al., 1998; Peuke et al., 2001; Peuke and Rennenberg, 2011; Liu et al., 2013; Macduff and Bakken, 2003; Gloser et al., 2016; Li et al., 2018). These studies provided interesting findings on the behavior and flow profiles of xylem sap components, in addition to the partitioning of nutrients within the plant. However, these results were reported based on culture experiments and were only valid for a particular set of nutritional and climatic conditions, which may not be appropriate for depicting the nature and components transported in the xylem sap of adult trees in natural forests exposed to complex climatic conditions and elevated atmospheric N deposition.

So far, knowledge of the characteristics of xylem sap components in the trunks of forest trees has been deficient (Glavac and Jochheim, 1993; Dambrine et al., 1995). The lack of trunk xylem sap analysis can be attributed to difficulties and inability of extracting the xylem sap from this part of the tree (Rennenberg et al., 1996). Despite a varied array of methods that have been devised in an attempt to extract xylem sap, many of them, such as root pressure (Goodger et al., 2005), the Scholander pressure chamber (Scholander et al., 1965), vacuum extraction (Bollard, 1953; Furukawa et al., 2011), and xylem-feeding insects (Zimmermann and Balling, 1990), have been conducted on branches of adult trees or trunks of herbaceous species/tree saplings because the collection of xylem sap samples from the organs of these species is relatively easy. However, for collecting xylem sap from the trunk of adult trees, these methods are not suitable and are restricted due to their sampling level (e.g., small organs) and the limited amount of sap. In addition, the water displacement method developed by Bennett et al. (1927) and improved by Glavac et al. (1990), based on the flow characteristics of the xylem catheter, has proved to be reliable for extracting xylem sap from large organs (e.g., trunks) with negligible contamination by the impaired cells, and also allows analysis of the changes of xylem sap components in an axial distribution along the trunk of adult trees. For example, Dambrine et al. (1995) felled and cut the trunk of Norway spruce adult trees into segments (0.6–1.2 m) to collect the trunk xylem sap. They reported that nutrient concentrations of xylem sap in the trunk were lower compared to the twigs, and xylem sap concentrations in the trunk and twigs were higher at nutrient-rich sites than at poor sites. Nevertheless, this method requires the tree to be felled and the trunk to be cut into segments, which is destructive and not practical for field studies.

The centrifugation method that separates xylem sap from the trunk is based on the lower axial resistance in the xylem vessels and the lower mobilization force of the xylem sap than cellular constituents of trunk tissues (Dixoh, 1915; Schurr, 1998). This method needs a small number of xylem samples and has a low level of destruction. Cellular constituents from surrounding tissues during the collection procedure and the damage of cells from sample tissues during centrifugal processes may potentially affect the components and concentration of the extracted xylem sap (Schurr, 1998). The centrifugation method has been used previously to examine xylem cavitation (Alder et al., 1997) and phloem sap components (Hijaz and Kiliny, 2014). However, little is known about how and to what extent the centrifugation method alters the components and concentrations of xylem sap. If this method is reliable for the collection of trunk xylem sap from adult trees, it would be a widely applicable approach for studying the nutrient characteristics transported in the xylem sap, particularly the trunk of mature trees, and for further elucidating mineral cycling in forest ecosystems.

Therefore, the aims of this study were to (1) examine how and to what extent the centrifugation method alters the components and concentrations of trunk xylem sap and (2) characterize the chemical components of trunk xylem sap in a temperate coniferous forest. We analyzed the differences in xylem sap components obtained by the centrifugation method by comparison with those obtained by the water displacement method from a pine (*Pinus tabulaeformis* Carr.) forest plantation in northern China. Trunks and branches based on different tree characteristics (i.e., diameter at breast height (DBH), tree age and height) were chosen to collect xylem sap using both collection methods.

A comprehensive analysis of the xylem sap components was then conducted and discussed.

2. Materials and methods

2.1. Study site

The study site is located at Baxian Mountain, Jizhou district, Tianjin city, Northern China (40°10'N, 117°33'E; 820 m a.s.l.). The region has a warm temperate, monsoon continental climate, with a mean annual temperature of 9 °C and a mean annual precipitation of 960 mm. The rainy season is from July to August, with intensive abundant precipitation. The soil is a brown cambisol with a mainly gravel texture. The dominant coniferous species in the forest is pine, and the dominant broad-leaved species are *Quercus mongolica*, *Tilia amurensis* and *Fraxinus chinensis*, and *Quercus aliena*. The understory shrub species are dominated by *Spiraea trilobata*, *Deutzia grandiflora*, *Deutzia parviflora*, and *Vitex negundo* var. *heterophylla*, and the herbaceous species are dominated by *Carex humilis* var. *nana*, *Carex lanceolata*, *Phlomis umbrosa*, and *Clematis heracleifolia*.

2.2. Experimental design

Our study was carried out in a pine forest stand. Six trees were selected for the extraction of xylem sap by means of the centrifugation and water displacement methods in October 2018. Based on tree height and DBH, the sampled trees were divided into two classes, small and large. The small class had a mean tree height of 1.9 ± 0.5 m and a mean DBH of 2.0 ± 0.8 cm. The large class had a mean tree height of 8.0 ± 1.0 m and a mean DBH of 14.2 ± 2.4 cm. The trees in the small class were collected as a whole with root and soil, were transported to the laboratory from the field site and were subsequently prepared and processed for extracting the xylem sap of both the trunk and branches using the centrifugation and water displacement methods. For the trees in the large class, the xylem sap of the trunks was extracted only by the centrifugation method because of the restriction of felling trees in this national nature forest reserve. The xylem sap of the branches was collected by both the centrifugation and water displacement methods.

2.3. Sampling of xylem sap by the centrifugation method

Each sampled tree in the small class was cut into four segments: three segments (30–40 cm in length) of the trunk and one segment of the branches. Three branches were cut to about 40 cm in length. Subsequently, the trunk segments and selected branches were cut approximately in half, and then the bark was removed, after which the samples were rinsed with deionized water and blotted with absorbent tissue to remove contaminants from the phloem sap. The remaining trunk segments and selected branches were treated for extracting xylem sap by the water displacement method as described in the next section. In addition, for the large tree class, three branches per tree were cut off at a height of about 10 m above the ground using a pair of high-branch scissors in the field and immediately shortened to about 40 cm in length. Half of these branches were treated by the aforementioned procedure and were refrigerated in a container in the field. The remaining branches were stored in the container for the collection of xylem sap by the water displacement method. Approximately 4 cm² bark was removed using a firmer chisel at a height of 1.3 m at the four azimuthal aspect points. The exposed xylem was sprayed with deionized water and blotted with absorbent tissue to remove contaminants from the phloem sap. Sapwood samples were extracted with a 5-mm increment borer and stored in the container in the field. All the samples were kept below 4 °C in a refrigerator in the laboratory. These peeled tissues (i.e., trunks and branches) and sapwood samples were cut into about pieces about 1 cm in length using a sterile razor blade, and four or five of these pieces were then vertically placed in a 50-ml centrifuge

tube with a strainer; the centrifugation speed was 11,000 rpm for 15 min for xylem sap collection. Xylem sap (0.58–0.97 ml) was obtained from the samples and was stored at -40°C for further chemical analysis.

2.4. Sampling of xylem sap by the water displacement method

The remaining trunk and branch segments for both the small and large classes were recorded for fresh weigh and were then placed vertically after removing 5-cm-wide bark strips around the lower cut surfaces. The cut surfaces of these tissue samples were rinsed with deionized water and blotted with absorbent tissue. A water-tight transparent catheter was connected to the upper end of each segment and protruded beyond the cut end. Deionized water (30 ml) was injected into the catheter. The lower cut surface was connected via a tube to a sample collection container. The container was kept at -0.08 MPa with a vacuum pump. The first fraction of about 5 ml was discarded because of the contaminants from the destroyed living cells, while the second fraction was collected, about 25 ml (Glavac et al., 1990). The displacement process required approximately 30 min, and the xylem sap was then stored at -40°C until further analysis. The tissue samples after water displacement were dried at 60°C for 12 h, and the dry weight was recorded to calculate the water volume and the xylem sap concentration.

2.5. Chemical and statistical analyses of xylem sap

Xylem sap was pressure-filtered through a $0.45\text{-}\mu\text{m}$ non-phosphorus filter and diluted if required. Total N (TN) was analyzed with an automated Skalar continuous flow analyzer (CFA) (Skalar Analytical B.V., Netherlands). Inorganic N components (e.g., nitrate (NO_3^-) and ammonium (NH_4^+)), in addition to anions and cations in the xylem sap, were determined by ion chromatography (ICS-5000+, Dionex, Sunnyvale, CA, USA). Anions were determined on a Dionex IonPac AS11-HC analytical column ($4 \times 250\text{ mm}$, 30 mM KOH eluent), and cations were determined on a Dionex IonPac CS12A analytical column ($4 \times 250\text{ mm}$, 20 mM methanesulphonic acid eluent). Dissolved organic nitrogen (DON) was derived from the difference between TN and inorganic N.

To examine the influence among tree tissues (branch and trunk), and tree classes (small and large) on the variance of xylem sap components collected by the centrifugation and water displacement methods, one-way analysis of variance (ANOVA) with the least significant difference (LSD) post hoc test was conducted. All statistical analyses were carried out using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). A significance level of $P < 0.05$ was used for all analyses.

3. Results

3.1. N components in xylem sap based on the centrifugation and water displacement methods

The concentrations of TN, NO_3^- and NH_4^+ were identified in the xylem sap of the trunk and branches for the small and large tree classes using the two collection methods (Table 1). The TN concentrations based on the centrifugation method were very close to those obtained by the water displacement method (Fig. 1a and b). The concentrations of NO_3^- and NH_4^+ based on the centrifugation method were slightly higher or lower than those obtained by the water displacement method (Fig. 1c–f). However, no significant differences were found between the two methods in the analysis of TN, NO_3^- and NH_4^+ ($P > 0.05$) (Fig. 1; Table 1).

The TN concentration of trunk xylem sap ($4.08 \pm 0.46\text{ mM}$) in the large tree class was somewhat high in comparison with the branch xylem sap ($3.72 \pm 0.41\text{ mM}$), whereas it was higher than that in the

Table 1
Chemical components and concentrations of xylem sap extracted using the centrifugation and water displacement methods for the branch and trunk of pine forest plantations divided into two classes expressed as small and large. Average values of sample number (n) are presented with the associated standard error ($3 < n < 12$). Note that only one sample was obtained for the xylem sap extracted using water displacement method for the branches of the large tree class due to improper operation of the sap extraction process.

Tree class	Sample level	Collection method	TN (mM)	NO_3^- (mM)	NH_4^+ (mM)	K^+ (mM)	Ca^{2+} (mM)	Mg^{2+} (mM)	Na^+ (mM)	Cl^- (mM)	SO_4^{2-} (mM)	PO_4^{2-} (mM)
Small	Branch	Water displacement	3.56 ± 0.14	0.14 ± 0.08	0.07 ± 0.03	1.69 ± 0.27	1.14 ± 0.34	0.49 ± 0.16	0.25 ± 0.12	0.74 ± 0.22	0.23 ± 0.05	0.06 ± 0.05
		Centrifugation	3.89 ± 1.12	0.24 ± 0.08	0.07 ± 0.04	2.11 ± 0.06	1.00 ± 0.18	0.60 ± 0.15	0.12 ± 0.02	1.46 ± 0.09	0.34 ± 0.07	0.14 ± 0.07
Large	Trunk	Water displacement	2.95 ± 0.22	0.14 ± 0.02	0.03 ± 0.01	1.54 ± 0.18	0.88 ± 0.18	0.30 ± 0.06	0.18 ± 0.08	1.00 ± 0.17	0.25 ± 0.06	0.14 ± 0.07
		Centrifugation	2.87 ± 0.41	0.18 ± 0.03	0.05 ± 0.03	2.75 ± 0.26	1.17 ± 0.15	0.68 ± 0.06	0.18 ± 0.02	1.96 ± 0.16	0.48 ± 0.07	0.25 ± 0.07
	Branch	Water displacement	2.9	0.11	0.04	1.03	0.35	0.22	0.24	0.67	0.25	0.08
		Centrifugation	3.72 ± 0.41	0.09 ± 0.06	0.12 ± 0.05	2.01 ± 0.64	0.59 ± 0.20	0.41 ± 0.15	0.14 ± 0.05	1.17 ± 0.39	0.76 ± 0.35	0.20 ± 0.10
Trunk	Centrifugation		4.08 ± 0.46	0.09 ± 0.02	0.02 ± 0.01	2.04 ± 0.12	1.23 ± 0.11	0.55 ± 0.07	0.28 ± 0.09	1.44 ± 0.13	0.53 ± 0.05	0.32 ± 0.05

Note: Differences of values between the two collection methods were not significant ($P > 0.05$).

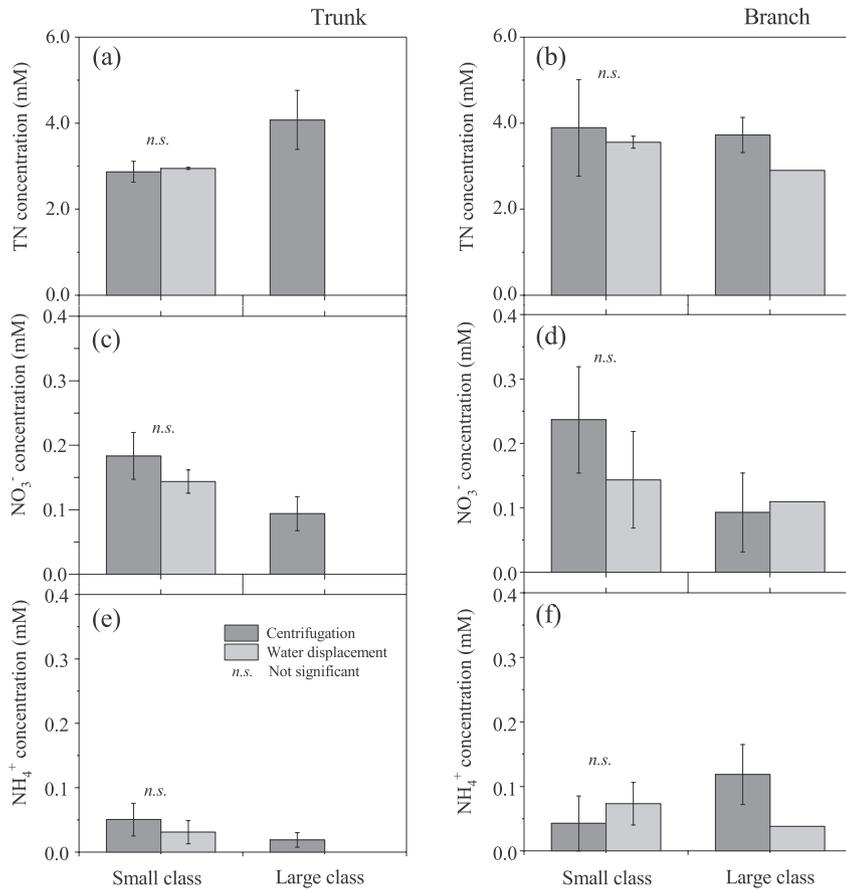


Fig. 1. Concentrations of nitrogen (N) components (total N, TN; nitrate, NO₃⁻; and ammonium, NH₄⁺) in the xylem sap of trunks (a, c, e) and branches (b, d, f) extracted using the centrifugation and water displacement methods for small and large tree classes. Average values of the sample number (*n*) are presented along with the associated standard error ($3 < n < 12$). Note that there was only one sample of xylem sap extracted from the branches of the large tree class using the water displacement method due to improper operation of the sap extraction process. A significance level of $P < 0.05$ was used.

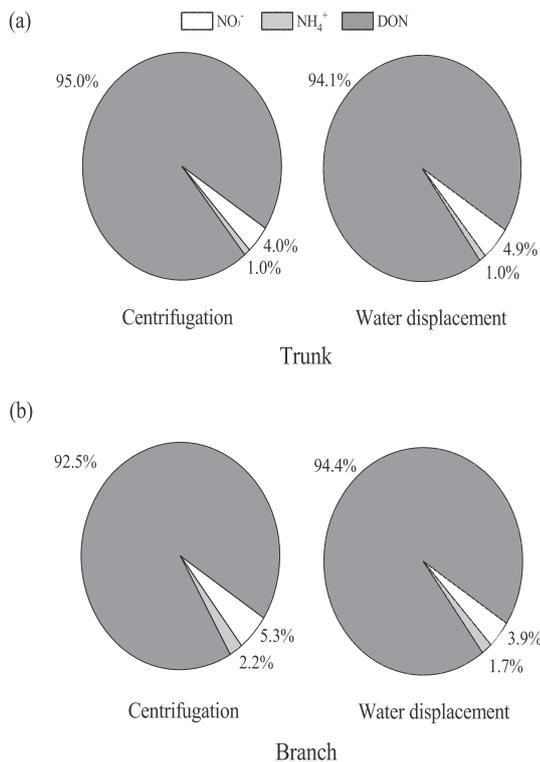


Fig. 2. Percentage composition of nitrogen (N) compounds (dissolved organic N, DON; nitrate, NO₃⁻; and ammonium, NH₄⁺) in the xylem sap of trunks (a) and branches (b) extracted using the centrifugation and water displacement methods.

small class (2.87 ± 0.41 mM) (Fig. 1a and b; Table 1). The NO₃⁻ concentration of the trunk xylem sap (0.09 ± 0.02 mM) was also similar to that of the branch xylem sap (0.09 ± 0.06 mM) in the large tree class, whereas it was lower than that of the trunk xylem sap in the small tree class (0.18 ± 0.03 mM) (Fig. 1c and d; Table 1). Unlike the patterns of NO₃⁻ concentration, the NH₄⁺ concentration of trunk xylem sap (0.02 ± 0.01 mM) was lower than that of the branch xylem sap (0.12 ± 0.05 mM) in the large tree class, whereas it was comparable with that of the trunk xylem sap in the small tree class (0.05 ± 0.03 mM) (Fig. 1e and f; Table 1). Overall, the NO₃⁻ and NH₄⁺ concentrations of xylem sap in the trunk and branches for the small and large tree classes were very low, within 0.3 mM (Fig. 1c–f; Table 1), and accounted for less than 8% (NO₃⁻: ~5.3%; NH₄⁺: ~2.2%) of TN in both methods (Fig. 2a and b). These results indicate that DON was the predominant N component in the xylem sap at the end of the growing season (Fig. 2). In addition, based on the centrifugation method, the ratio of NH₄⁺ to NO₃⁻ (NH₄⁺/NO₃⁻) in the xylem sap of the respective trunk and branches was 27.8% and 29.2% for the small tree class, and 22.2% and 133.3% for the large tree class.

3.2. Inorganic ion components in xylem sap based on the centrifugation and water displacement methods

Four cations (potassium, K⁺; calcium, Ca²⁺; magnesium, Mg²⁺; and sodium, Na⁺) and three anions (chloride, Cl⁻; sulfate, SO₄²⁻; and phosphate, PO₄³⁻) detected by the centrifugation method were also found by the water displacement method in the xylem sap of trunk and branch. The concentrations of these cations and anions by the centrifugation method tended to be higher than those based on the water displacement method, whereas the differences among them were not significant ($P > 0.05$; Figs. 3 and 4; Table 1).

In the xylem sap of the trunk and branches of both tree classes, K⁺

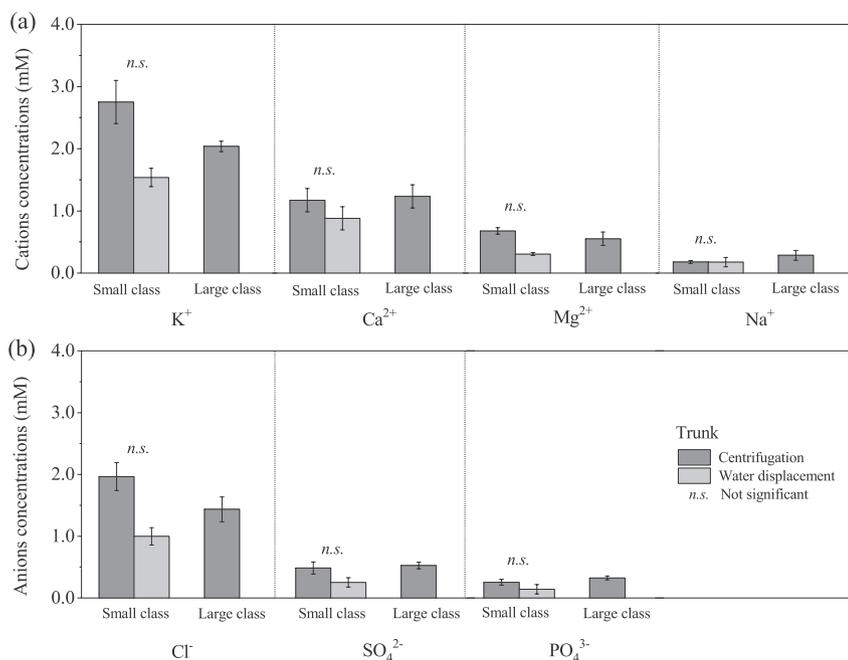


Fig. 3. Concentrations of (a) cations (potassium, K⁺; calcium, Ca²⁺; magnesium, Mg²⁺; and sodium, Na⁺) and (b) anions (chloride, Cl⁻; sulfate, SO₄²⁻; and phosphate, PO₄³⁻) in the xylem sap of trunks extracted using the centrifugation and water displacement methods for the small (sample number, *n* = 9) and large (*n* = 12) tree classes. Values are presented as means with the associated standard error. A significance level of *P* < 0.05 was used.

was the predominant cation, ranging from 0.73 to 3.32 mM, followed by Ca²⁺, and Cl⁻ was the predominant anion, ranging from 0.40 to 2.39 mM, followed by SO₄²⁻ (Table 1). Unlike the patterns of inorganic N components (Fig. 1), the cations and anions were relatively comparable among tree tissues (branch and trunk) and tree classes (small and large) (Figs. 3 and 4).

4. Discussion

4.1. Performance of xylem sap extraction by the centrifugation method

The chemical components and concentrations of xylem sap in the trunk and branches of trees extracted by the centrifugation method were very similar to those obtained by the water displacement method. We found no significant differences among these components between the two methods (Figs. 1, 3 and 4; Table 1), and TN was the

predominant component of xylem sap in both the trunk and branch (Table 1). The TN concentration based on the centrifugation method (2.87–4.08 mM) was comparable with that of the water displacement method (2.90–3.56 mM) (Table 1) and agrees with previous studies on woody species, ranging from 0.32 to 11.73 mM, obtained by other collection methods (Table 2). The detected NO₃⁻ (0.09–0.24 mM) and NH₄⁺ (0.02–0.12 mM) concentrations were also in the range of 0.01–0.75 mM and 0.01–3.01 mM, respectively, as reported in previous studies (Table 2).

The concentrations of N compounds and inorganic ions by the centrifugation method were slightly higher than those based on the water displacement method (Figs. 1, 3 and 4). This indicates that the centrifugation method may damage living cells (e.g., ray parenchyma cells) during sample procedures and centrifugal processes, releasing the solutions in the protoplast into the apoplast and thus probably causing the concentrations of xylem sap obtained by the centrifugation method

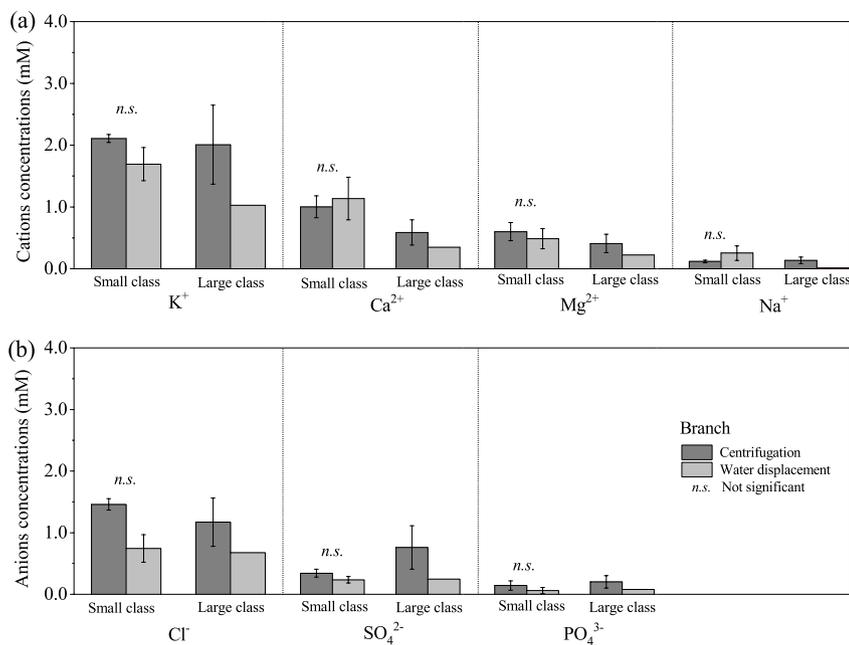


Fig. 4. Concentrations of (a) cations (potassium, K⁺; calcium, Ca²⁺; magnesium, Mg²⁺; and sodium, Na⁺) and (b) anions (chloride, Cl⁻; sulfate, SO₄²⁻; and phosphate, PO₄³⁻) in the xylem sap of branches extracted using the centrifugation and water displacement methods for the small and large tree classes. Values of the sample number (*n*) are presented with the associated standard error (*n* = 3). Note that only one sample was obtained for the xylem sap extracted using the water displacement method for the branch of the large tree class due to improper operation of the sap extraction process. A significance level of *P* < 0.05 was used.

Table 2

Summary of the concentrations of nitrogen (N) components (total N, TN; nitrate, NO_3^- ; and ammonium, NH_4^+) in xylem sap extracted using different collection methods in woody species from earlier publications.

Species	Collection method	Sampling tissue	Sampling season	TN (mM)	NO_3^- (mM)	NH_4^+ (mM)	Reference
<i>Actinidia chinensis</i> P.	Vacuum extraction	Branch	Spring	–	0.30	–	Ferguson et al. (1983)
<i>Vitis vinifera</i> L.	Root pressure	Branch	Spring	–	0.75	0.10	Prima-putra and Botton (1998)
<i>Ribes nigrum</i> L.	Root pressure	Branch	Spring	–	0.44	0.03	Prima-putra and Botton (1998)
<i>Rubus idaeus</i> L.	Root pressure	Branch	Spring	–	–	0.01	Prima-putra and Botton (1998)
<i>Sorbus aucuparia</i> L.	Root pressure	Branch	Spring	–	0.39	0.10	Prima-putra and Botton (1998)
<i>Hippophae rhamnoides</i>	Pressure Vessel	Branch	Summer	–	–	1.67	Liu et al. (2013)
<i>Citrus sinensis</i> L.	Water displacement	Branch	Spring	2.13	0.10	–	Moreno and García-Martínez, 1983
<i>Citrus sinensis</i> L.	Water displacement	Branch	Summer	0.99	0.06	–	Moreno and García-Martínez, 1983
<i>Citrus sinensis</i> L.	Water displacement	Branch	Autumn	1.35	0.05	–	Moreno and García-Martínez, 1983
<i>Citrus sinensis</i> L.	Water displacement	Branch	Winter	1.48	0.03	–	Moreno and García-Martínez, 1983
<i>Picea abies</i> L.	Vacuum extraction	Branch	Autumn	5.51	–	–	Osonubi et al. (1988)
<i>Picea abies</i> L.	Scholander chamber	Branch	Summer	2.37	–	–	Smith and Shortle (2001)
<i>Picea abies</i> L.	Scholander chamber	Branch	Autumn	1.86	–	–	Smith and Shortle (2001)
<i>Sorbus aucuparia</i> L.	Root pressure	Branch	Spring	–	0.39	0.10	Prima-putra and Botton (1998)
<i>Juglans regia</i> L.	Root pressure	Branch	Spring	–	0.38	0.06	Prima-putra and Botton (1998)
<i>Populus × euramericana</i>	Vacuum extraction	Branch	Spring	11.73	–	0.20	Escher et al. (2004)
<i>Populus × euramericana</i>	Vacuum extraction	Branch	Summer	2.33	–	0.07	Escher et al. (2004)
<i>Populus × euramericana</i>	Vacuum extraction	Branch	Autumn	0.617	–	0.02	Escher et al. (2004)
<i>Abies alba</i> Mill.	Vacuum extraction	Branch	Spring	6.58	–	0.31	Escher et al. (2004)
<i>Abies alba</i> Mill.	Vacuum extraction	Branch	Summer	1.76	–	0.07	Escher et al. (2004)
<i>Abies alba</i> Mill.	Vacuum extraction	Branch	Autumn	4.57	–	0.08	Escher et al. (2004)
<i>Populus nigra</i>	Vacuum extraction	Branch	Spring	–	–	0.05	Furukawa et al. (2011)
<i>Populus nigra</i>	Vacuum extraction	Branch	Summer	–	–	0.01	Furukawa et al. (2011)
<i>Populus nigra</i>	Vacuum extraction	Branch	Autumn	–	–	0.25	Furukawa et al. (2011)
<i>Populus nigra</i>	Vacuum extraction	Branch	Winter	–	–	0.21	Furukawa et al. (2011)
<i>Robinia pseudoacacia</i>	Pressure Vessel	Branch	Summer	–	–	3.01	Liu et al. (2013)
<i>Eucalyptus, Corymbia</i>	Vacuum extraction	Branch	Spring	2.55	0.02	–	Soper et al. (2015)
<i>Hakea; Grevillea;</i>	Vacuum extraction	Branch	Spring	5.55	0.03	–	Soper et al. (2015)
<i>Acacia; Eucalyptus; Corymbia; Hakea</i>	Vacuum extraction	Branch	Spring	2.60	0.10	–	Soper et al. (2015)
<i>Morus alba</i> L.	Root pressure	Trunk	Summer	2.93	0.71	0.55	Suzuki and Kohno (1983)
<i>Morus alba</i> L.	Root pressure	Trunk	Autumn	2.68	0.54	0.41	Suzuki and Kohno (1983)
<i>Morus alba</i> L.	Root pressure	Trunk	Winter	1.75	0.39	0.36	Suzuki and Kohno (1983)
<i>Fagus sylvatica</i> L.	Water displacement	Trunk	Spring	6.10	0.01	–	Glavac and Jochheim (1993)
<i>Fagus sylvatica</i> L.	Water displacement	Trunk	Summer	1.13	0.01	–	Glavac and Jochheim (1993)
<i>Fagus sylvatica</i> L.	Water displacement	Trunk	Autumn	0.61	0.03	–	Glavac and Jochheim (1993)
<i>Fagus sylvatica</i> L.	Water displacement	Trunk	Winter	0.32	0.03	–	Glavac and Jochheim (1993)
<i>Picea abies</i> L.	Water displacement	Trunk	Summer	0.88	–	–	Dambrine et al. (1995)

Entries with dashes indicate that data could not be found in the paper.

to be higher than those based on the water displacement method. To what extent the centrifugation method affects the chemical components and concentrations of xylem sap needs to be addressed. K^+ is essential for a wide range of cellular functions and is particularly important in the osmotic potential of plants (Britto and Kronzucker, 2008). If the solutions of both protoplast and apoplast compartments were mixed, this would yield a high K^+ concentration, assumed to be approximately 20–23 mM (Schill et al., 1996; Britto and Kronzucker, 2008). Hartung et al. (1988) took K^+ concentration as the indicator for cytoplasmic contamination in the apoplastic solution of *Gossypium hirsutum* L. leaves. Schill et al. (1996) also used the K^+ concentration to determine the purity of the xylem sap, that is, whether the living wood cells released K^+ into the apoplast and contaminated the xylem sap by a pressure/decompression method in *Acer platanoides* trees. In the present study, the K^+ concentration of xylem sap based on the centrifugation method was slightly higher than that obtained by the water displacement method, whereas there was no significant difference in K^+ concentration ($P > 0.05$; Fig. 5). Moreover, the K^+ concentrations obtained by both methods were consistent with the values (0.20–6.20 mM) obtained by means of other collection methods in different woody species summarized from earlier publications (Fig. 5). These results illustrate that the contamination of xylem sap by the centrifugation method can be negligible, and the quality of the extracted xylem sap by this method is reliable and robust according to a comparison with the water displacement method and the K^+ concentration of xylem sap.

In addition to checking the K^+ concentration to determine contamination with cellular components, scanning electron micrographs of

xylem wood samples after centrifugation treatment and measuring luminometrically ATP concentration of xylem sap are feasible methods to evaluate the quality of the extracted xylem sap (Schill et al., 1996; Gessler et al., 1998). Furthermore, the contamination of extracted xylem sap may depend on the sampled tissues, the sampling time and season, soil water potential and nutrient availability, and tree species (Dambrine et al., 1995; Rennenberg et al., 1996; Peuke and Rennenberg, 2011). The xylem sap obtained by the centrifugation method should be tested to determine the actual contamination in different tree species under different environmental conditions.

The characteristics of N compounds and inorganic ions transported in the xylem sap of trunks have rarely been reported for forest trees in the field because there is no appropriate method to extract xylem sap from this part of the tree. Previous studies have developed the water displacement method to examine the variations of xylem sap components in the trunk of adult trees (Bennett et al., 1927; Glavac et al., 1990). However, this method requires felled trees and cannot be extensively carried out in natural forest stands. Therefore, the centrifugation method presented in this study, with little destruction and no felling of trees, would be a useful and practical tool to collect xylem sap for the analysis of mineral nutrients and their transport, particularly in the trunk of mature trees.

4.2. Characteristics of chemical components in the xylem sap of trunks

The characteristics of xylem sap components obtained by the centrifugation method were determined for the temperate pine forest stand at the end of the growing season. We observed the differences in the

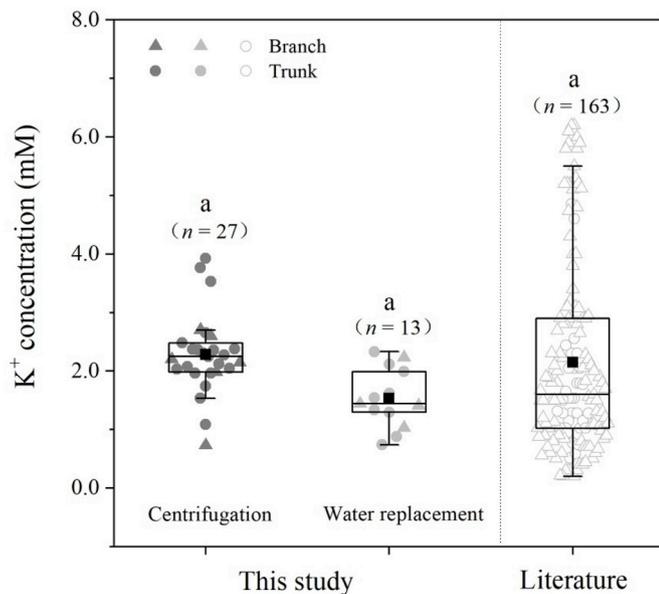


Fig. 5. Potassium (K^+) concentration in the xylem sap of trunks and branches extracted by the centrifugation and water displacement methods, obtained this study, compared with that in different woody species summarized from the literature (Kazda and Weilgony, 1988; Osonubi et al., 1988; Glavac et al., 1990; Dambrine et al., 1992, 1995; Jeschke and Pate, 1995; Prima-Putra and Botton, 1998; Siebrecht and Tischner, 1999; Smith and Shortle, 2001; Merchant et al., 2010; Furukawa et al., 2011; Losso et al., 2018). The horizontal line and black square in each box denote the median and mean values, respectively. The depth of the box equals the interquartile range (25–75th percentile). Unique letters above the boxes indicate significant differences at the level of $P < 0.05$. Values in parentheses are sample number (n).

behavior of N compounds between the trunk and the branch. A decrease in the TN concentration between the lower part of the trunk and the branch for the large tree class (Fig. 1a and b) suggests that N compounds are probably withdrawn from the xylem and stored in older trunk tissues over long-distance transport. The NO_3^- concentration showed little change (Fig. 1c and d), whereas the NH_4^+ concentration was larger (Fig. 1e and f). This is unlike the findings of Glavac and Jochheim (1993). They reported that the NO_3^- concentration increased with increasing trunk height, and the NH_4^+ concentration could barely be traced in autumn in a beech forest stand. The variations in N compounds of xylem sap are determined not only by tree species but also sampling season (Moreno and García-Martínez, 1983; Glavac and Jochheim, 1993; Escher et al., 2004; Furukawa et al., 2011; Losso et al., 2018). Since the period of main mineral demand for tree growth (i.e., the start of the growing season) and the formation of new canopy extensions (i.e., during the growing season) is out of phase with nutrient uptake at the end of growing season (Glavac and Jochheim, 1993; Gerant et al., 2017), mobilization of stored organs is required from old to young shoot tissues through the xylem in perennial plants. The ratio of NH_4^+/NO_3^- in xylem sap can also provide some indication as to the NO_3^- reduction of the site as well as the mineral form of N uptake (Glavac and Jochheim, 1993). In the present study, the higher ratio of NH_4^+/NO_3^- in the branch than in the trunk for the large tree class indicates that NO_3^- may be reduced to NH_4^+ when transported to the branch, or NH_4^+ originates from internal N recirculation processes of the tree during the foliage senescence process.

The percentage composition of N compounds was quantified and showed that DON was the dominant component of TN followed by NO_3^- and NH_4^+ in the xylem sap of both the trunk and branch (Fig. 2). Our data agree with previous results that DON is the main component of TN in the woody species *Citrus sinensis* L. (Moreno and García-Martínez, 1983), *Fagus sylvatica* L. (Glavac and Jochheim, 1993),

Populus nigra (Furukawa et al., 2011), *Populus × euramericana* and *Abies alba* (Escher et al., 2004), but are in contrast to previous findings for herbaceous plants that inorganic N compounds (mainly NO_3^- and NH_4^+) are the dominant component transported in the xylem (Peuke et al., 2001; Macduff and Bakken, 2003). The contrasting findings observed between the two species may be attributed to the higher assimilation in the roots of woody species than in herbaceous plants (Dambrine et al., 1995; Miller and Cramer, 2005), resulting in the absorbed inorganic N almost reduced to DON in the roots and then transported by xylem sap flow to the canopy of woody species.

In addition, the NO_3^- concentration in the xylem sap of both the trunk and branch for the large tree class was lower than that for the small tree class (Fig. 1c and d), whereas the NH_4^+ concentration was relatively low for both tree classes (Fig. 1e and f). This indicates that tree age can probably affect the xylem sap composition, and young trees may take up more inorganic N, mainly as NO_3^- , than old trees. This may be explained by the following reasons: (1) the length of the tree root is proportional to age and can lead to differences in the ability to absorb NO_3^- (Thorup-Kristensen, 2001), (2) the demand of inorganic N is high for young trees because of their development and growth (Pate, 1973; Siebrecht et al., 2003), and (3) the assimilation of NO_3^- uptake in the root is weak in young trees (Andrews, 1986). Moreover, the variations in the cations and anions in the xylem sap of the trunk and branch were also demonstrated in this study. These ions can also provide some evidence on the origin, uptake, metabolism and cycling of mineral substances within trees (Dambrine et al., 1995; Jeschke and Pate, 1995; Peuke and Merchant, 2018), but need further investigations for expanding the understanding of the species-specific internal circulation of nutrients of forest trees under environmental changes.

5. Conclusion

This study proposed a centrifugation method for the collection of trunk xylem sap and showed that the quality of the extracted xylem sap by this method is reliable and robust by comparison with the water displacement method and examination of the K^+ concentrations of xylem sap summarized from the literature. This method would be a useful and practical tool to collect xylem sap for the analysis of mineral uptake and translocation, particularly in the trunk of mature trees, which is still deficient to our knowledge. DON was the dominant component of TN followed by NO_3^- and NH_4^+ in the xylem sap in this temperate pine forest at the end of the growing season. The NO_3^- concentration showed little change, whereas the NH_4^+ concentration was larger when transported from the trunk to branches in the large tree class. Moreover, the level and behavior of the cations and anions in xylem sap of the trunk and branches were demonstrated in this study. K^+ and Cl^- were the predominant cation and anion, respectively, of xylem sap. Such examinations provide insight into the diagnostic assessments of tree physiological processes and growth, in addition to the species-specific internal mineral cycling of forest trees in a changing environment.

Conflicts of interest

The authors confirm that this article content has no conflict of interest.

Authors' contributions

X. Sun designed and performed the experiments. L. Chen analyzed the data, interpreted the results, and drafted the manuscript. L. Chen, Z. Zhang, Y. Cao and X. Zhang performed the experiments. X. Liu and M.T. Teramage revised the manuscript.

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