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Comparative Studies on Seasonal Dynamics of Macronutrient Contents in Different Components of Chinese White Poplar in a Four-year Old Poplar Plantation

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Abstract

In order to attain a better understanding of the seasonal variations of macronutrient contents in different components and identify clones of Chinese white poplar, three representative clones in a four-year old poplar plantation were studied in each season of the year 2008. The plantation was a randomized complete block design. We collected leaves (growing seasons only), bark and xylem of twigs, branches, stem and roots with a tree trimmer knife and an increment borer in the spring (17 April), summer (14 July), autumn (23 September) and winter (9 December). Each sample was a mixture from 3-4 model trees in the block, and then digested by a H₂SO₄-H₂O₂ solution to measure N, P, K, Ca, and Mg contents. Results showed that the order of macronutrient contents in all samples was N> Ca> K> Mg> P. Seasonal changes in N, P and K contents in each component had clearly specific trends through the year, while Ca and Mg contents varied irregularly except in leaves. The P and K contents in twigs were particularly high in spring, and roots always had high K contents through the seasons. The nutrient elements contents in the bark of branches and stems were almost 1.5-4.0 times and 2.5-9.0 times higher than those in the woody tissues, respectively. According to the ANOVA of nutrient contents in each component, especially for the season factor, N, P, Ca and Mg nutrition contents in branch wood varied significantly, and in regard to the clone factor, K, Ca and Mg contents differed significantly in stem bark. Overall, macronutrient elements contents in all parts changed throughout the four seasons. To better manage these fast-growing poplars plantations, it is necessary to retain the foliage, twigs and roots in the cultivated stands, and to remove the bark of stems and branches and leave them in the stands as well.

Key words: Poplar clone, Chinese white poplar (*Populus tomentosa*), nutrient dynamics, macronutrient elements, nutrient cycling

Introduction

Poplar plantations could be managed to produce biomass for timber, bio-energy, pulpwod fiber, agro-forestry, as well as for environmental control (phyto-remediation) (Adler et al. 2008, Swamy et al. 2006, Jiang et al. 2006, Fang et al. 2008). Furthermore, according to Cooke and Weih (2005), poplar researchers are poised to build an integrated understanding of seasonal N cycling that spans genomes to ecosystems. Besides the effects on physiological characters (Hanley et al. 2004, Donaldson et al. 2006, Koike et al. 2007), an understanding of nutrient dynamics is likely to enlighten ecosystem process (Chen et al. 1998), and link natural cycling to nutrient availability (Attiwill and Adams, 1993). On the other hand, a study of nutrient dynamics is a potential method of selecting superior clones or species in terms of plant biomass or nutrient productivity (Karacic and Weih 2006, Karim and Hawkins 1999, Swamy et al. 2006). Therefore, researchers have concluded that there are many implications for forest management practices through studying net primary production and biomass accumulation (Lodhiyal et al. 1995, Son et al. 2007, Wu et al. 2009).

Chinese white poplar (*Populus tomentosa* Carr.) is an indigenous species to northern China, which has been cultivated for more than 2000 years. It is a tall, fast growing, long lived species with excellent quality and grows over a wide area. Chinese white poplar is one of the key tree species for commercial timber production and plays a critical role in ecological and environmental protection along the Yellow River (Zhu 2006).

With developments in biotechnology and genetic modification, a more productive and multi-purpose poplar, triploid *Populus tomentosa*, was created in China. There is a new series of Chinese white poplar clones with integrated genetic improvements, that have the advantages of rapid growth, short-rotation (less than 5 years), long and abundant fibers, high pulp yield, but less lignin. As a result, it is a good species for pulpwod plantations that are need to satisfy demand...
for the fiber production in China (Zhu 2006). In the past few years, scores of different clones of triploid Populus tomentosa have been bred (Zhu et al. 1995; Kang et al. 2000, Kang and Mao 2001, Zhang et al. 2006). Although many studies have been done on genetic improvements to achieve more production for triploid Populus tomentosa plantations as well as on the effects of tree-crop intercropping, different fertilizer-schemes in the seedling period and on leaf nutrient diagnosis (Li et al. 2006, Jiang et al. 2006, Liu et al. 2000, Liu et al. 2009), we still lack studies on nutrient cycling or nutrient dynamics of the plantation.

In addition, studies have never examined whether genotypic variation in nutrient concentrations can be used to identify and select clones (Li et al. 1993, Li et al. 2009, Liu et al. 2003, Wang et al. 1993). In order to extend productivity and distinguish between the qualities of these new poplar clones, it is necessary to conduct research on nutrient contents of the trees, which finally may illustrate the differences between clones (Li et al. 1993, Swamy et al. 2006, Karacic and Wei, 2006).

In our studies of a four-year old poplar plantation, we studied the seasonal dynamics in macronutrient contents of different components of three clones of Chinese white poplar, including leaves, branch wood, branch bark, twig wood, twig bark, stem wood, stem bark and root of trees, mainly to examine the nutrient dynamic in the growing season. Our research objectives were to (1) find if there were any seasonal dynamics and characteristics of nutrient elements contents of Chinese white poplar; (2) distinguish between the clones and try to select the better one on the basis of macronutrient contents of the trees. The data may have useful implications for nutrient management practices and nutrient cycling in ecosystems of Chinese white poplar in the future.

Materials and Methods

Study site

The study site is located in a Chinese white poplar plantation in Zhongmu County (34°26′-34°56′N, 113°46′-114°12′E, 80m a.s.l.), Henan Province of China. The mean annual rainfall is slightly >600 mm, and the mean annual temperature is 14.2ºC. The soil is fluvo-agric soil (typical US Inceptisols) with a clayey loam texture, and with a soil profile of pH 8.0, 9.65 g kg⁻¹ organic matter, 0.62 g kg⁻¹ total nitrogen, 10.8 mg kg⁻¹ available phosphorus and 11.9 m g kg⁻¹ available potassium in 0-20 cm depth.

<table>
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<tr>
<th>Clone</th>
<th>Hybridization</th>
<th>Ploid</th>
<th>Obtained methods</th>
<th>DBH ( cm)</th>
<th>Height ( m)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spring</td>
<td>Summer</td>
</tr>
<tr>
<td>Beilin 1</td>
<td>(P. tomentosa × P. bolliana) × (P. alba × P. glandulos)</td>
<td>2n=3x=57</td>
<td>Triploid obtained by artificially induced chromosome doubling into 2n pollen and pollination, then hybridization and selecting</td>
<td>11.33</td>
<td>13.35</td>
</tr>
<tr>
<td>B305</td>
<td>(P. tomentosa × P. bolliana) × P. tomentos</td>
<td>2n=3x=57</td>
<td>Triploid obtained by natural 2n pollen pollination and hybridization</td>
<td>11.30</td>
<td>12.28</td>
</tr>
<tr>
<td>1319</td>
<td>P. tomentosa Carr.</td>
<td>2n=2x=38</td>
<td>Natively</td>
<td>8.62</td>
<td>9.20</td>
</tr>
</tbody>
</table>

Study material

Three different clones of Chinese white poplar were chosen for the study (Table 1). Clone Beilin 1 and clone B305 are triploid Populus tomentosa (3n=57), while clone 1319 is diploid Populus tomentosa Carr. (2n=38). In general, young native diploid poplar can increase 1.5-3.0 cm in DBH and 1.0-1.5 m in height per year (Shaanxi Institute of Forestry 1981), while the volume of a single 8-year old triploid tree is 3.5 times higher than that of a single native diploid one (Zhu et al. 1995). Huge size is the overall characteristic of a triploid. Namely triploid posses a huge capacity for growth, especially in its volume increment with its tree shape, canopy, leaf, thickness of leaf, floss of leaf back surface, and resistance to plant disease. According to Zhu et al. (2006), the hybridized poplar clones showed a similar foliation period, a similar day of winter bud formation as native poplar, but has a postponed leaf-fall date. This growth rhythm could be attributed to the fast growth trait in the triploid poplar.

Table 1 lists the descriptions of clones and the growth increment in the studied year. As to the growth increment in 2008, clone Beilin 1 had the largest increment in DBH growth (2.43 cm). Yet clone B305 had the greatest height increment (1.9 m). According to the observations on the phenological phases of Chinese white poplar, the flowering period is between late January and the middle of February, which lasts for about 1 month. Leaves are unfolded from late March to the middle of April. Then the fruits ripen in late April. Leaves turn yellow in late September, and the tree will be completely bare a month later. The growth period is about 160-180 d for the observation period (Shaanxi Institute of Forestry 1981).

Experimental design

About 1 ha area trial plantation of Chinese white poplar was established in Henan Province in 2005, which was divided into plot I and plot II (Figure 1). Both were a randomized complete block design, with four and three times replicates, respectively. Then, one-year old seedlings of 20 different clones of Chinese white poplar were planted with a 4 × 3m row spacing that spring. Among them, 8 different clones were planted at plot I and replicated four times. Other 11 clones were cultivated in plot II and replicated three times. And the last clone, clone 1319, was planted in both plots as control. Finally, every plot has an area of 72 m² or 144 m², with 6 or 12 trees, respectively. The average survival rate of the poplar trees was 91.1% in the fourth year of the plantation in 2008. Taking into
consideration the difficulty of the project, just three representative clones, clone Beilin 1 (in plot I), B305 and 1319 (both in plot II) were studied.

Plant sampling
We analyzed the bark of poplar because many results suggest that the bark is very important in nutrient cycling of trees (Shen et al. 1992, Liu et al. 2001, Liu et al. 2003, Ericsson 1994, von Fircks et al. 2001). About 50% N nutrient and 40% P nutrient accumulations are stored in bark (Shen et al. 1992, Swamy et al. 2006) of a poplar tree. Many nutrient elements in leaves are transferred to other organs in the third growing season. It is recognized as a critical mechanism for plants to adjust to poor nutrient conditions in infertile soil. It can improve the nutrient efficiency in the body of plants and reduce the dependency of plants on the nutrient supply of the external environment (Li et al. 1993, Li et al. 2003, Xue et al. 2005).

Leaves, branch wood, branch bark, twig wood, twig bark, stem wood, stem bark and roots were collected in the spring (17 April), summer (14 July), autumn (23 September) and winter (9 December; except leaves), respectively in 2008. We avoided collecting samples in the same trees in which we had taken samples the previous season. Every sample of different components was mixed with samples from 3-4 model trees (Fang et al. 2008, Liu et al. 2001) in the plots under study. At the time of sampling, many mature leaves near the top 1/3 of the canopies in the east, south, west and north directions were cut with a tree trimmer and combined as leaf samples (Xue et al. 2005).

In this study, we sampled 2-year-old branches and 1-year-old twigs. A few branches with different diameters in all four directions were cut with a tree trimmer and broken into many small parts as branch samples. Twigs were also sub sampled from these parts. At last, both branch bark and twig bark were removed to be used as samples: branch wood (=xylem), branch bark, twig wood, and twig bark were separated as in earlier studies (Li et al. 2006, Liu et al. 2003, Lodhiyal et al. 1995). Similarly, a 5×5 cm area of stem bark was removed with a knife at the 1.3 m height (DBH) of trees in four directions; then stem wood was collected with an increment borer at these areas (Li et al. 2006, Lodhiyal et al. 1995). An area of 50×50 cm was excavated to a depth of 30 cm under the sampled trees and both the thick and fine roots were gathered and immediately stored (Coyle and Coleman 2005).

Elemental analyses
Plant samples were oven-dried at 90°C for 10 min and then at 70°C for at least 48h to a constant mass...
before they were ground with a plant-sample mill into uniformly fine powder. The powder was sieved with a 0.5 mm-mesh screen. As to chemical analysis, first all plant samples were digested in a solution of H$_2$SO$_4$-H$_2$O$_2$, and then N contents were determined by the Kjeldahl analyzer (KDY-9830, Beijing) (Fang et al. 2008). Finally, P, K, Ca and Mg contents were examined by ICP of Leeman Lab, US (Wu and Ge 1999, Wang 2005).

**Statistical analyses**

Variance analyses of effects of the season and clone on nutrient contents in each organ were performed by SPSS 11.

**Results**

In general, N, P and K contents in each component varied with seasonal changes, but the seasonal dynamics of Ca and Mg contents were only clearly seen in the leaves. All three poplar clones had the same seasonal macronutrient contents dynamics.

**Seasonal dynamics of N contents in each component**

Figure 2 shows the seasonal dynamics of N contents in different components of three clones of Chinese white poplar. N contents in leaves (growing season only, the descriptions below are the same), twig wood and twig bark were highest in spring and lowest in summer, and a bit higher in autumn and winter. N contents in branch wood and branch bark, stem wood and stem bark increased with time. As to N contents in roots, increased minutely at the end of the growing season.

What’s more, comparing the nutrient contents in wood and bark of all three clones, N contents in twig bark were slightly more than those in twig wood, while the contents in branch bark were about 1.5 times as high as those in branch wood, and the contents in stem bark were about 3 times higher than those in stem wood. In addition, if nutrient contents in twigs, branches and stems were calculated by nutrient contents values in the wood plus those in the bark (the calculations below are the same), N contents in twigs or leaves of all three clones were highest in all four seasons. The order of N contents in the rest of the components was branches > roots > stems in spring, summer and autumn. But in winter, the order was branches > stems > roots.

**Seasonal dynamics of P contents in each component**

Figure 3 illustrates the seasonal dynamics of P contents in each component of the three clones. Dynamics of P contents in leaves, twig wood and twig bark were the same as those of N contents, which were highest in spring and lowest in summer, with the values continuing to increase minutely in autumn and winter. At the same time, the P contents in branch xylem and bark decreased in summer, but accumulated in autumn until the highest point of the whole year in winter. P contents in stem wood and stem branch increased with time. And the P contents in roots also rose in autumn. Both the results in stems and roots were the same pattern as found in N nutrient dynamics.

In addition, comparing nutrient contents in wood and bark of the three clones, P content in twig wood was higher than that in twig bark in every season, which was just the opposite to the results of the other nutrient contents. In perennial organs, P contents in branch bark were about 1.5 times greater than those in branch wood, the same as the results of N contents, with stem bark about 2.5 times higher than those in stem wood. As to P contents in different components, the values in twigs of all the three clones were highest in all four seasons. The order of the other components was leaves > branches > roots > stems in spring, summer and autumn. But in winter, the order was basically branches > stems > roots.

**Seasonal dynamics of K contents in each component**

Figure 4 indicates the seasonal K contents trends in
Variations of macronutrient contents

different components of three poplar clones. As for leaves of all three clones, the seasonal dynamics of K contents were similar to N and P. As regards to K contents in twig wood, twig bark, branch wood and branch bark, the results generally were greatest in the spring, and smallest in the autumn, with values in winter higher than those in summer. Stem wood, stem bark and roots showed a higher concentration in autumn. As to the results of comparisons to wood with its corresponding bark, K contents in twig wood differed little from those in twig bark, but the values in branch bark were 2.5 times as high as those in branch wood, and K contents in stem bark 9 times greater than those in stem wood. In sum, K contents in twigs or leaves of all three clones of Chinese white polar were highest in all four seasons. The order of K contents for the rest of the components was roots > branches > stems generally.

Seasonal dynamics of Ca and Mg contents in leaves, twigs, branches, stems and roots

Figure 5 and Figure 6 note the seasonal dynamics of Ca and Mg contents in each part of three Chinese white poplar clones. Of all three clones, Ca and Mg contents in leaves were highest in summer, lower in autumn and lowest in spring, just opposite of those of N, P and K. Since there was no clear tendency on the seasonal changes in the separate wood and bark of all three clones, we made use of the total of contents in wood and its bark to observe seasonal trends in twigs, branches and stems.

Ca contents in twigs were relatively low in spring and autumn and rose in summer and winter, respectively. In general, Ca contents in branches decreased in autumn, but increased in winter. As regards seasonal variations of Mg contents, in twigs, the values showed the accumulation in winter; in branches, Mg contents continued to increase from spring to winter; and in stems, the results showed Mg contents were lowest in autumn. At the same time, Figure 5 and Figure 6 show Ca contents in roots of all three clones were lowest in autumn. No clear dynamics
were observed in Ca contents in stems or Mg contents in roots.

Moreover, according to results shown in the Figure 5 and Figure 6, Ca contents in branch bark were nearly 4 times greater than those in branch wood, with the contents in stem bark about 8 times greater than those in stem wood. Similarly, the Mg contents in branch bark were about 2 times higher than those in branch wood, with those in stem bark roughly 9 times higher than those in stem wood.

Finally, Ca contents in twigs or in stems of all three clones of Chinese white poplar were highest in all four seasons. The order of Ca contents in other organs was irregular. In addition, Mg contents in twigs or in leaves of all three clones of Chinese white poplar were highest in all four seasons. The order of Mg contents of the other parts was branches > roots > stems basically throughout the year.

**Analysis of variance of N, P, K, Ca and Mg contents in each component**

Table 2 shows the variance analysis of the season and clone effects on nutrition contents in each component. The criteria for significant models were set at a probability level of $p < 0.05$, $n=9$ for leaves and $n=12$ for the others. With regard to variance sources from the season factor, significant variance was shown in the N, P, Ca and Mg nutrition contents in branch wood. We also found remarkable variation in N, P and K contents in twig wood and bark, N, K and Ca contents in stem bark, N and K contents in stem wood and P contents in leaves. In regard to the clone factor, K, Ca and Mg contents in the stem bark of the three clones differed significantly. Clonal variation was found in N and Mg contents in twig bark and K contents in roots.
Discussions

The three clones had the same seasonal dynamics of N, P, and K contents. Dynamics of N, P, and K contents in leaves were found in combination with physiological characteristics of leaves and macronutrient elements (Li et al. 2006, Xue et al. 2005, Li et al. 2009, Wu et al. 2009). Generally, in spring, leaves are small, so nutrient contents are high. With leaf development, nutrient contents are apparently lower in summer when the trees grow rapidly. It may be due to “dilution effect” that indicates the rate of the nutrient accumulation in leaves does not keep pace with the growth of leaves, therefore the nutrient contents decrease (Figures 2–6). Then, in autumn, because the leaves are fully mature and their relative mass declines, nutrient contents rise a little again; at the same time, the macronutrient elements are transferred to the other components, such as branches and stems, etc. (Xue et al. 2005, Wu et al. 2009).

Likewise, N, P, and K content dynamics in twigs are also related to physiological characteristics of the plant. Twigs or shoots are the most active part in spring flush, these three essential elements transfer into these vegetative points since they generally have key physiological effects on plant growth. Thus nutrient elements contents in twigs are highest in spring (Shaanxi Institute of Forestry 1981). In summer, the whole tree grows quickly to get more biomass with more nutrients in twigs moving into leaves, branches and stems, leading to a reduction in the contents in twigs. Then, twigs will save enough nutrients to go through the winter and germinate in the coming spring, thus N, P, and K contents in twigs rebound in winter (Shaanxi Institute of Forestry 1981, Shen et al.1992, Liu et al. 2003).

In perennial organs, i.e., branches, stems and roots, N, P, and K contents increase again with nutrient remobilization from the falling leaves (Shen et al. 1992, Ericsson 1994, Kitaoka and Koike 2004, 2005, Koike et al. 2007, Liu et al. 2001, Liu et al. 2003). These plant-internal processes are helpful in avoiding nutrient loss to some extent (Swamy et al. 2006, Attiwill and Adams 1993). However, unlike N and P contents dynamics, the K contents in leaves and twigs in autumn were lowest among the three seasons which suggests that the K element possesses better mobility as a kind of ion nutrient in the body of plants (Lu 2003); what’s more, K content was much greater in roots, which may reveal the K element’s excellent mobility so that more K nutrients are stored in woody tissues (Wu et al. 2001, Yu et al. 2004, Lu 2003).

Moreover, P and K contents in twigs of the three poplar clones were higher than those in leaves in spring. There are three possible reasons for these results: (i) in plants, the transportation of P and K shows obvious apical dominance, they strongly tend to move where newer tissues are forming (Lu 2003), plus the storage of P and K contents in winter (Shaanxi Institute of Forestry 1981), there are more P and K contents in twigs finally; (ii) the twig tissues are more sensitive to the abundance or deficiency of P and K nutrients than leaves in spring; (iii) finally, differences between species of the different kinds of trees might account for the results. Poplars might show specific growth characteristic in spring (Zhang et al. 2001, Shen et al. 1992, Guan et al. 2006). Since there is a lack of data for biomass of twigs and biomass distributions of whole trees in this paper, more research on the twigs effects on the internal nutrient cycling of poplars is required.

The results (Figure 3 and 4) showed that the P and K contents in twigs decreased greatly in summer. The photosynthesis and photosynthetic production accumulation may be more significant in the summer flush. Twigs are growing quickly and mature in that phase (Table 1), so P and K elements are remobilized to other parts and those contents in the twigs are reduced substantially (Shaanxi Institute of Forestry 1981). Besides, the twig wood of all three clones had less P contents than twig bark in spring, which was different from the other nutrient elements and the other three seasons. It is possible that the newly formed twigs had difficulty in debarking and were not well divided into phloem and xylem in the beginning of spring and the abundance of P element in woody tissues increases its efficiency (Lu 2003, Yu and Chen 1998).

Only Ca and Mg contents in leaves displayed seasonal dynamics clearly (Figure 5 and 6). Indeed, dynamics in the other components were not very clear, and there needs to be more research done in the future. These results suggested that in leaves they might display their ionic elementary forms throughout plant’s growth and development to secure a steady increase in plant water content. It’s associated with strong
transpiration in summer and other effects of Ca$^{2+}$ in the plant physiological processes (Amtmann and Blatt 2009), which leads to Ca contents in leaves reaching a peak. Furthermore, since Ca element has poor mobility in plants, the contents are higher in autumn than those in spring. Mg element effects on photosynthesis (Amtmann and Blatt 2009) and the available mobility characteristic could explain the previously mentioned dynamics (Liu et al. 2001).

Nutrient elements contents in branch bark (1.5-4.0 times) and stem bark (2.5-9.0 times) were higher in woody tissues. Therefore, as in other studies, this paper also demonstrated that bark has a very important role in nutrient storage and cycling in plantations. In practice, if the debarking of stems and branches is done before the removal of timber from the plantation area, it will help minimize “nutrient drain” in poplar plantations (Swamy et al. 2006, von Fircks et al. 2001, Ericsson 1994, Liu et al. 2003, Liu et al. 2001).

Analysis of variance from the season factor might imply that there is a large transfer of these nutrient elements between organs. For instance, the N nutrition contents in branch wood varied significantly, in combination with the seasonal dynamics of N contents in branch wood, which might mean that more N nutrients move to branch wood when leaves were falling. Likewise, P, Ca and Mg showed significant variations and also imply that there was more nutrient remobilization in branch wood in the entire year. The same reasons are applied for other nutrient elements having significant variations in the parts of plants. If K content variations are studied, it is better that twigs or stems rather than leaves and other organs be collected as plant samples (Wu 1980) for nutrient management in plantations.

In regard to the clone factor in variance sources of each part, there exists remarkable variance of nutrient contents in the same parts between the three clones, and the results might be used to identify different poplar clones. For instance, according to the results of seasonal K, Ca and Mg contents in stem bark of different poplar clones with significant variances, it is possible that different poplar clones can be distinguished in actual practice by using K determinations in stems throughout the year (Li et al. 1993, Wang et al. 1993, Liu et al. 2003, Swamy et al. 2006). However, for the factors of [season] × [clone], it was not possible to see the results of ANOVA because of the limited sampling numbers. We just ensure that the hybridized clones had more nutrient contents and volume increments in contrast to the native species. To identify a superior clone (Karim and Hawkins 1999), more studies are needed on nutrient dynamics (Shen et al. 1992, Swamy 2006) combining biomass and volumes with nutrient contents for each plant part in the future.

In conclusion, N, P and K contents in leaves and twigs were highest in spring and lowest in summer, while those of Ca and Mg were highest in summer. N, P and K contents in aged branches and stems were higher in autumn and winter. And, these nutrients contents in roots all increased minutely in autumn. The three clones showed the same dynamics generally. The order of macronutrient contents in different components of Chinese white poplar in four seasons was N> Ca> K> Mg>P. At the same time, the order of N, P and Mg contents in all components was twigs or leaves > branches > roots > stems in spring, summer and autumn; but in winter, the order was twigs > branches > stems > roots generally. However, the order of K contents in the different parts was twigs or leaves > roots > branches > stems in four seasons. Ca contents in twigs or in stems of all three clones of Chinese white polar were nearly highest through the year. Mg contents showed twigs or leaves > branches > roots > stems in all four seasons.

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