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## MACRONUTRIENT DEFICIENCY SYMPTOMS IN *BETULA ALNOIDES* SEEDLINGS

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**CHEN L, ZENG J, XU DP, ZHAO ZG & GUO JJ. 2010. Macronutrient deficiency in symptoms *Betula alnoides* seedlings.** The decline in seedling quality and production of birch tree (*Betula alnoides*) is often associated with nutrient stress. Visual foliar symptoms, growth performance, pigment compositions and nutrient interaction of birch seedlings in response to six macronutrient deficiencies were studied. Visual foliar symptoms were most obvious in no nitrogen (-N), no potassium (-K) and no magnesium (-Mg) seedlings but not apparent in no calcium (-Ca) seedlings. Except for -Mg and no sulphur (-S) treatments, seedlings lacking other nutrients showed decreases in most of the growth measurements but an increase in root/shoot ratio. Phosphorous deficiency had no effect on all fractions of pigments, while N and K deficiencies resulted in reductions in chlorophyll-a (chl<sub>a</sub>), chlorophyll-b (chl<sub>b</sub>), total chlorophyll (chl) and carotenoid (car) but increases in chl<sub>a</sub>/chl<sub>b</sub> and car/chl ratios. Vector analyses showed that N deficiency not only decreased leaf N concentration but also increased leaf P concentration, possibly because of the antagonism between both ions. Similarly, K, Ca and S deficiencies induced a slight decrease in leaf N concentration that could be explained by a synergism between N and these ions. This kind of nutrient interaction also occurred between P and S in -P seedlings or between Mg and Ca in -Mg seedlings. The findings provided a guideline for diagnosing major macronutrient deficiencies of seedlings. The theoretical foundation for silviculture of *B. alnoides* will help improve seedling quality and accelerate the sustainable plantation of this species.

**Keywords:** Foliar symptom, growth performance, nutrient interaction, pigment composition

**CHEN L, ZENG J, XU DP, ZHAO ZG & GUO JJ. 2010. Gejala kekurangan makronutrien dalam anak benih *Betula alnoides*.** Kemerosotan kualiti anak benih pokok birch (*Betula alnoides*) dan penghasilan pokok birch kerap dikaitkan dengan tegasan nutrien. Gejala daun yang dapat dilihat, prestasi pertumbuhan, komposisi pigmen dan interaksi nutrien anak benih pokok birch terhadap kekurangan enam makronutrien dikaji. Gejala daun paling ketara pada anak benih tanpa nitrogen (N), tanpa kalium (K) dan tanpa magnesium (Mg) tetapi tidak jelas pada anak benih tanpa kalsium (Ca). Anak benih yang kekurangan nutrien lain kecuali Mg dan sulfur (S) menunjukkan penurunan dalam kebanyakan parameter pertumbuhan. Namun, nisbah akar/pucuk menunjukkan peningkatan. Kekurangan P tidak mempengaruhi komposisi pigmen. Kekurangan N dan K mengakibatkan penurunan dalam kandungan klorofil a (chl<sub>a</sub>), klorofil b (chl<sub>b</sub>), jumlah klorofil (chl) dan karotenoid (car) tetapi peningkatan dalam nisbah chl<sub>a</sub>/chl<sub>b</sub> serta car/chl. Analisis vektor menunjukkan bahawa kekurangan N bukan sahaja mengakibatkan penurunan kepekatan N daun tetapi juga peningkatan kepekatan P daun. Ini menunjukkan kemungkinan berlakunya antagonisme antara kedua-dua ion. Kekurangan K, Ca and S menyebabkan sedikit penurunan dalam kepekatan N daun yang mungkin diakibatkan oleh synergisme antara N dengan ion-ion tersebut. Interaksi nutrien sedemikian juga berlaku antara P dengan S dalam anak benih tiada P atau antara Mg dengan Ca dalam anak benih tiada Mg. Kajian ini menyediakan garis panduan untuk mengenal pasti kekurangan makronutrien yang utama dalam anak benih. Teori asas tentang silvikultur *B. alnoides* boleh menambah baik kualiti anak benih dan mempercepat penanamannya secara mapan di ladang.

### INTRODUCTION

Soil and water loss has been increasing in most areas of south China over the last two decades due to excessive deforestation and heavy vegetation disturbance. This results in serious nutrient poverty of soils, especially N, P,

K and Mg (Macleod *et al.* 2004). A consequence is a substantial decrease in forest quality and production, particularly for plantation species. While the nutrient availability of sites influences growth of trees in plantations, the nutrient

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status of seedlings is closely related to seedling quality (Elliott & White 1994, Close *et al.* 2005, Birge *et al.* 2006) as it influences the survival rate of young seedlings, initial growth performance and resistance to water stress, low temperature and disease (Van den Driessche 1991, Quoreshi & Timmer 2000, Floistad & Kohmann 2004, Close *et al.* 2005, Hawkins *et al.* 2005). Nutrient deficiency experiments using tree seedlings are often used as a means of estimating the optimisation of nutritional management evaluating the effects of nutrient deficiencies on seedling morphology and physiology (Timmer & Armstrong 1987, Barrett & Fox 1997, Trubat *et al.* 2006, Jeyanny *et al.* 2009).

*Betula alnoides*, also known as birch tree, is a fast-growing indigenous hardwood species in northern India, Myanmar, Indochina and south China. It appears to be the most southerly species of *Betula* in the northern hemisphere. Some studies have shown that *B. alnoides* can grow well on all sorts of soils with pH ranging from 4.2 to 6.5, probably because it is a strong deep-rooted species and able to form ectomycorrhiza or vesicular-arbuscular mycorrhiza (Zeng *et al.* 1999, Gong *et al.* 2000, Zeng *et al.* 2006). Therefore, this species often plays an important role in water conservation, long-term maintenance of land fertility and biodiversity of forest ecosystems. Due to its well-formed stem, moderate density and beautiful texture, the wood of *B. alnoides* is commonly used for high-quality floorboards, furniture and room decorations (Zeng *et al.* 2006). Plantations of *B. alnoides* have been rapidly expanding in south China from 100 ha in the mid-1990s to more than 50 000 ha in 2005 (Zeng *et al.* 2006). Despite its rise as an important economical and environmental species, little is known about the nutritional requirements of *B. alnoides*, which may limit the optimal silviculture of this species.

The objective of this study was to identify the visual symptoms of *B. alnoides* seedlings in response to deficiencies of six major macronutrient elements. The responses of *B. alnoides* seedlings to nutrient deficiencies with regard to growth attributes, pigment composition and mineral element contents were studied as these parameters relate to improving the nutritional management of *B. alnoides* plantations.

## MATERIALS AND METHODS

Healthy tissue-cultured seedlings of *B. alnoides* with almost uniform height of 14.5 cm were used in sand

culture trials and grown in a ventilated greenhouse at the Research Institute of Tropical Forestry, Longdong. Quartz sand of 1–2 mm diameter was washed with water and soaked in 1% HCl for 24 hours to remove impurities, organic matter and nutrient residues. The sand was then leached with running tap water, rinsed with deionised water and finally wind dried. The seedlings were transplanted into 18 × 13 × 16 cm plastic pots filled with equal weights of quartz sand and watered on alternate days. To prevent runoff of water or leaching of nutrients, white plastic bags were placed in the plastic pots. The irrigation regime was measured by an initial watering test in which water was poured slowly into a pot filled with quartz sand using a cylinder. When water was 2 cm from the bottom of the pot, the volume of water added was the irrigation regime. This experiment was replicated four times. The real irrigation regime was influenced by seedling growth demand and weather condition. Fungi diseases were prevented by injecting a 2‰ solution of carbendazin or thiophanate into quartz sand every one or two weeks. The average daytime temperature of the greenhouse ranged from 24 to 29 °C, natural light intensity from 110 to 140 μmol photon m<sup>-2</sup> s<sup>-1</sup> and relative humidity from 48 to 71%. The positions of the pots were rotated every two weeks to minimise edge effects.

The randomised complete block design was arranged with four blocks of seven treatments, i.e.: complete nutrient mix (control), minus nitrogen (-N), minus phosphorus (-P), minus potassium (-K), minus calcium (-Ca), minus magnesium (-Mg) and minus sulphur (-S) nutrient solutions (n = 28). Each experiment unit (plot) consisted of 10 seedlings, totalling 280 seedlings in all treatments. The treatments lasted for 12 weeks and started two weeks after transplanting when all the seedlings were well established. Seedlings were supplied with 80 ml of half-strength nutrient solutions (Table 1) once a week on the first six weeks and full-strength thereafter. The test concluded when all the treatments exhibited foliar symptoms.

The seedlings were observed daily for symptoms of deficiency. The symptoms were recorded. The root collar diameter, height and number of branches of all seedlings were measured every three weeks. At the end of the experiment, three of 10 seedlings per plot were randomly selected and separated from the

**Table 1** Chemical compositions (ml l<sup>-1</sup>) of complete and various macronutrient-lacking nutrient solutions

Source	Control	-N	-P	-K	-Ca	-Mg	-S
1 mol l <sup>-1</sup> KNO <sub>3</sub>	5	–	6	–	5	6	6
1 mol l <sup>-1</sup> Ca(NO <sub>3</sub> ) <sub>2</sub> 4H <sub>2</sub> O	5	–	4	5	–	4	4
1 mol l <sup>-1</sup> MgSO <sub>4</sub>	2	2	2	2	2	–	–
1 mol l <sup>-1</sup> KH <sub>2</sub> PO <sub>4</sub>	1	–	–	–	1	1	1
0.5 mol l <sup>-1</sup> K <sub>2</sub> SO <sub>4</sub>	–	5	–	–	–	3	–
0.5 mol l <sup>-1</sup> CaCl <sub>2</sub>	–	10	–	10	–	–	–
1 mol l <sup>-1</sup> NaH <sub>2</sub> PO <sub>4</sub> 2H <sub>2</sub> O	–	10	–	10	–	–	–
0.01 mol l <sup>-1</sup> CaSO <sub>4</sub> 2H <sub>2</sub> O	–	20	–	–	–	–	–
1 mol l <sup>-1</sup> Mg(NO <sub>3</sub> ) <sub>2</sub> 6H <sub>2</sub> O	–	–	–	–	–	–	2
0.05 mol l <sup>-1</sup> Fe-EDTA	2	2	2	2	2	2	2
Arnon micronutrient solutions	1	1	1	1	1	1	1
0.05 mol l <sup>-1</sup> H <sub>3</sub> BO <sub>3</sub>							
0.01 mol l <sup>-1</sup> MnCl <sub>2</sub> 4H <sub>2</sub> O							
0.77 mmol l <sup>-1</sup> ZnCl <sub>2</sub>							
0.32 mmol l <sup>-1</sup> CuCl <sub>2</sub> 2H <sub>2</sub> O							
0.09 mmol l <sup>-1</sup> H <sub>2</sub> MoO <sub>4</sub> (85%–90%MoO <sub>3</sub> )							

The pH of all nutrient solutions was adjusted to 6 with 1 mol l<sup>-1</sup> NaOH or HCl.

quartz sand by gently washing with deionised water. The seedlings were divided into leaves, stems and roots. The leaf samples were scanned to obtain leaf photographs and leaf pixels were examined using Photoshop Microsoft. Hence, leaf area could be calculated according to the positive relationships between the leaf area and pixel. Different parts of plants were oven dried at 65 °C for 48 hours for dry mass determination. Dried leaf samples were then ground for subsequent chemical analysis. Plant materials were digested in a block digester using a H<sub>2</sub>SO<sub>4</sub> and K<sub>2</sub>SO<sub>4</sub>–CuSO<sub>4</sub> mixture catalyst for total N by diffusion method while HNO<sub>3</sub>–HClO<sub>4</sub> mixture solution for P, K, Ca, Mg and S by inductively coupled plasma optical emission spectrometry. Another three plants in each plot were randomly selected, from which the third or fourth leaf from the apex in each plant were randomly collected at the end of the experiment for pigment composition measurements. The chlorophyll and carotenoid contents of fully expanded leaves were estimated spectrophotometrically in 80% acetone extract. The absorbance was measured at 470, 646 and 663 nm for the estimation of chlorophyll-a (chl<sub>a</sub>), chlorophyll-b (chl<sub>b</sub>), total chlorophyll and carotenoid, and their contents were calculated according to the method by Lichtenthaler and Wellburn (1983).

Analysis of variance (ANOVA) was conducted to study the effects on growth, pigment compositions and leaf nutrient status of the seedlings using SPSS 11.5 (2003). Significant means were separated by Duncan's multiple range test at  $p < 0.05$ . Leaf nutrient conditions between different treatments were characterised by vector analysis following a technique described by Salifu and Timmer (2003), where nutrient content (x), nutrient concentration (y) and dry mass (z) were plotted in a single monogram (Figure 1) satisfying the function  $x = f(y, z)$ . The technique facilitates interpretation of plant nutrient status by simultaneously comparing growth and associated nutrient responses by normalising status of the reference treatment to 100. Diagnosis is based on both vector direction and magnitude in terms of increasing (+), decreasing (-) or unchanging (0) parameter status (Figure 1), identifying cases of possible depletion, dilution, sufficiency, deficiency, luxury consumption and excess of nutrients. The orientation of the major vector commonly signifies an enrichment response, while the magnitude of the major vector reflects the largest relative increase or decrease in nutrient conditions of the reference from the compared point status (Haase & Rose 1995).

**RESULTS**

**Visual deficiency symptoms**

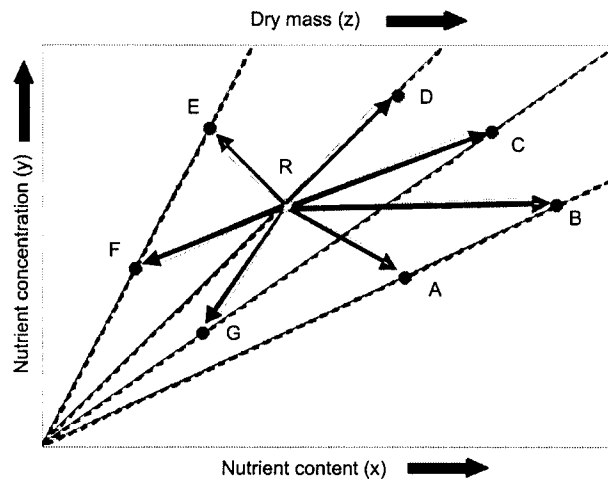
-N seedlings showed deficiency symptoms 21 days after treatment (DAT). At first, middle areas of the relatively old basal leaves turned yellow and spread gradually across the entire leaves. This symptom then extended to the younger apical leaves. The entire seedling grew slowly with fewer leaves, smaller leaf size, lower height and fewer branches compared with the control. Interveneal chlorosis occurred at 45 DAT. As seedlings aged, terminal buds gradually turned brown and died. Irregular brown patches were seen on all leaves at the end of the experiment.

The height growth of -P seedlings was stunted at 28 DAT. As deficiency progressed, leaves and stems gradually exhibited fairly dark green and purplish red coloration respectively. Interveneal

chlorosis was observed on a few old leaves by the end of the experiment.

The symptoms of -K seedlings were first apparent on old basal leaves, which exhibited chlorosis. Seedling height growth was significantly suppressed at 25 DAT. As the seedlings grew, a discolouration similar to iron rust developed, especially along edges of the fourth leaf and gradually extended to the lower leaves. When K deficiency became more severe, the iron-rust discolouration transitioned to reddish-brown necrosis with entire leaves twisting upward. At 45 DAT, interveneal chlorosis was observed and reddish-brown necrotic patches appeared on the upper leaves.

Visible symptoms of Ca deficiency in Ca-deprived seedlings were not apparent. Although seedling height was less than the control at 21 DAT, no other visual symptom was apparent.



Vector shift	Relative change in			Interpretation	Possible diagnosis
	z	y	x		
A	+	-	+	Dilution	Growth dilution
B	+	0	+	Sufficiency	Steady-state
C	+	+	+	Deficiency	Limiting
D	0	+	+	Luxury consumption	Accumulation
E	-	++	±	Excess	Toxic accumulation
F	-	-	-	Excess	Antagonistic
G	0,+	-	-	Depletion	Retranslocation

**Figure 1** Vector interpretation of directional changes in dry mass (z), nutrient concentration (y) and nutrient content (x) of plants. Reference point (R) represents status of seedlings normalised to 100. Diagnosis is based on vector shifts (A to G) which characterise an increase (+), decrease (-) or no change (0) in dry mass and nutrient status relative to the reference status as described in the table (Haase & Rose 1995, Salifu & Timmer 2003)

The seedlings were highly responsive to Mg deficiency. At 21 DAT, an opaque green discolouration appeared between the veins of the fourth or fifth leaf and spread to older basal leaves. The opaque green areas of leaves developed all sizes of bronze patches and the entire leaves curled downwards at severe stage of deficiency. At the end of the experiment, interveinal chlorosis appeared on the leaves of mid-lower part of seedlings and most of the damaged leaves were dropping off.

Seedling growth of the -S treatment was relatively slow with buds and new leaves exhibiting a light green discolouration at 36 DAT. As the severity of deficiency continued, seedling height was increasingly suppressed.

### Seedling growth

There were no significant differences in root collar diameter, height and number of branches

of *B. alnoides* seedlings between treatments at 14 DAT (Table 2). However, profound differences ( $p < 0.05$ ) were evident at each of the three subsequent dates of examination, inferring that nutrient deficiency treatments substantially hindered growth performances of *B. alnoides* seedlings.

Table 2 shows that the growth of *B. alnoides* seedlings is affected by N, P, K and Ca. At the end of the experiment, the root collar diameter, height and number of branches of all the nutrient deficiency treatments were significantly lower than the control except for the number of branches of -Mg treatment and the root collar diameter of -S treatment.

Analysis of variance for leaf area indicated that nutrient deficiency caused great reduction in leaf area (Figure 2). Except for -Mg and -S treatments, the seedling biomasses of the other nutrient deficiency treatments were significantly lower than that of the control. In the case of root/shoot

**Table 2** Effects of different nutrient deficiencies on growth of *B. alnoides* seedlings

Growth index	DAT (days)	Control	-N	-P	-K	-Ca	-Mg	-S
Root collar diameter (mm)	14	1.73 (0.04)	1.78 (0.04)	1.76 (0.03)	1.73 (0.03)	1.80 (0.03)	1.83 (0.04)	1.73 (0.03)
	35	2.15 ab (0.05)	2.14 b (0.04)	2.14 b (0.04)	2.23 ab (0.04)	2.14 b (0.04)	2.28 a (0.05)	2.28 a (0.04)
	56	3.23 a (0.05)	2.36 e (0.05)	2.76 c (0.04)	2.59 d (0.05)	2.83 c (0.06)	3.01 b (0.06)	3.22 a (0.05)
	77	3.72 a (0.06)	2.14 f (0.05)	3.03 d (0.06)	2.63 e (0.05)	3.34 c (0.06)	3.54 b (0.07)	3.76 a (0.07)
Height (cm)	14	14.2 (0.26)	14.2 (0.29)	14.7 (0.22)	14.4 (0.27)	14.9 (0.23)	14.6 (0.27)	14.5 (0.26)
	35	22.1 a (0.45)	16.3 d (0.34)	22.2 a (0.36)	18.8 c (0.36)	20.2 b (0.31)	22.4 a (0.41)	22.5 a (0.36)
	56	37.4 a (0.45)	19.2 f (0.42)	29.9 d (0.55)	25.2 e (0.33)	32.5 c (0.51)	33.2 bc (0.60)	34.1 b (0.49)
	77	48.8 a (0.76)	20.7 e (0.42)	32.8 c (0.57)	27.4 d (0.31)	41.9 b (0.69)	41.8 b (0.69)	41.3 b (0.79)
No. of branches	14	0	0	0	0	0	0	0
	35	10 a (0.23)	5 d (0.30)	9 b (0.22)	9 bc (0.30)	8 c (0.30)	10 a (0.25)	11 a (0.26)
	56	14 a (0.21)	6 d (0.31)	11 c (0.31)	10 c (0.28)	12 b (0.28)	14 a (0.29)	14 a (0.20)
	77	18 a (0.25)	6 d (0.36)	11 c (0.33)	10 c (0.31)	16 b (0.37)	18 a (0.31)	17 b (0.28)

Values followed by the same letter within a row are not significantly different at  $p < 0.05$  according to Duncan's multiple range test. Figures in parentheses are standard errors.

ratio, the nutrient deficiency treatments were all significantly higher than the control except for the -Mg treatment.

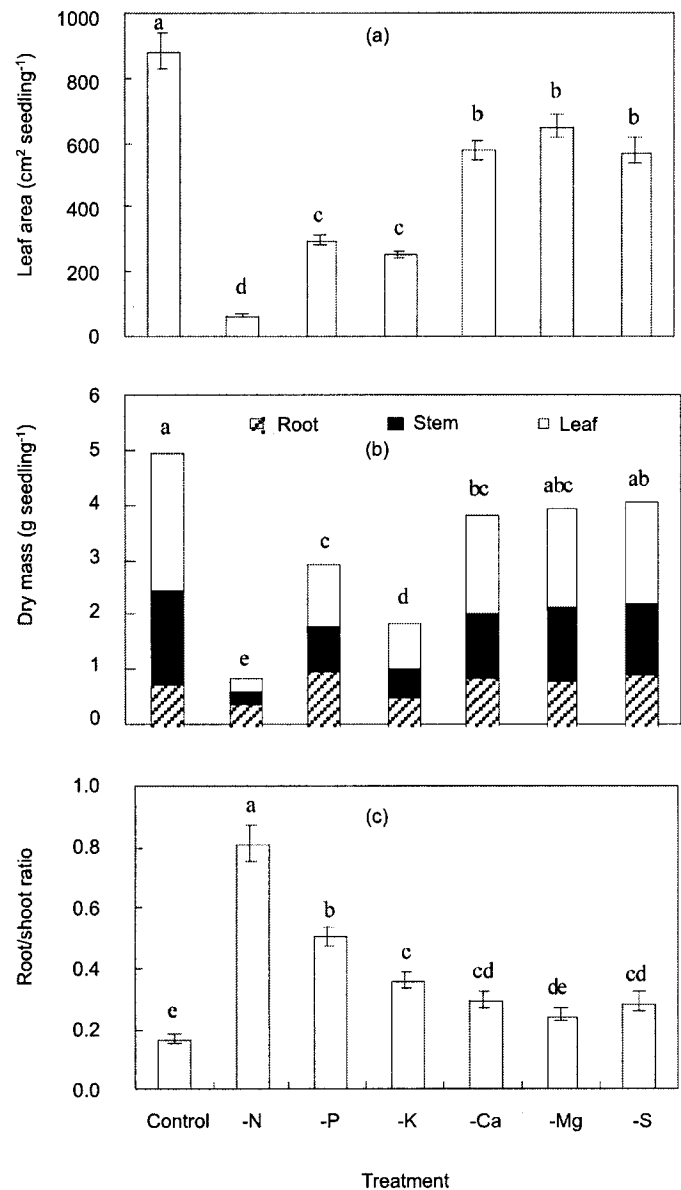
**Pigment compositions**

No difference occurred in the pigment compositions between -P and control treatments (Table 3). Both -N and -K treatments led to significant declines in chlorophyll and carotenoid contents but increases in chl<sub>a</sub>/b and car/chl ratios compared with the

control. As for -Ca, -Mg and -S treatments, the contents of chl<sub>b</sub>, total chlorophyll and carotenoid were lower than the control. There were no profound differences in the chl<sub>a</sub> content, chl<sub>a</sub>/b ratio and car/chl ratio between them and the control.

**Nutrient interaction**

N was the major vector and the leaf N content, leaf N concentration and leaf mass of -N seedlings



**Figure 2** Effects of nutrient deficiencies on leaf area, dry mass and root/shoot (leaf + stem) ratio of *B. alnoides* seedlings. Treatments with the same letter are not significantly different according to Duncan’s multiple range test. Error bars represent standard errors.

were declined by 94.9, 40.9 and 91.4% respectively compared with the control (Figure 3a). Leaf P content and leaf P concentration increased 25.6% and 13.7 times respectively, indicating that P was also important for the growth of -N seedlings. P was, thus, recognised as the secondary vector since its vector magnitude was smaller than the N vector but larger than the other vectors.

While the primary nutrients of the various nutrient deficiency treatments that limit seedling growth were the corresponding deficient elements omitted from the nutrient solutions, the secondary limiting nutrient elements varied between treatments. For example, N was the second limiting nutrient of the -K, -Ca and -S seedlings, while S and Ca were the second limiting nutrient influencing the growth of -P and -Mg seedlings respectively (Figure 3, b–f).

## DISCUSSION

*Betula alnoides* seedlings were most affected by the -N treatment. This is consistent with the fact that N is one of the most important and commonly limiting nutrients for tree growth. Lack of N decreased height, root collar diameter and number of branches of seedlings compared with the control. This is possibly because new cell elongation and division are severely limited by N deficiency (Wang 2000). The root/shoot ratio

is often increased due to N deficiency (Ericsson 1995, Barrett & Fox 1997, Trubat *et al.* 2006). This is observed in the present study and is in line with the nitrogen–carbon balance concept according to Thornley (1972). N is an essential element for the formation of chlorophyll, which is a photosensitive catalyst in the process of photosynthesis (Liu *et al.* 2007). Thus, it appeared that N deficiency led to a reduction in the total chlorophyll content, which caused leaf chlorosis and induced declines of 92.7 and 82.8% of the leaf area and biomass of the seedlings respectively in comparison with the control (Figure 2). The car/chl ratio of the -N seedlings was significantly higher than the control. Thus, N deficiency apparently induced an increase in light protection but a decrease in absorbance ability (Guo *et al.* 2005). We also found that N deficiency induced not only a high reduction of leaf N concentration but also an increase in leaf P concentration (Figure 3a). This interaction indicates a possible antagonism between N and P.

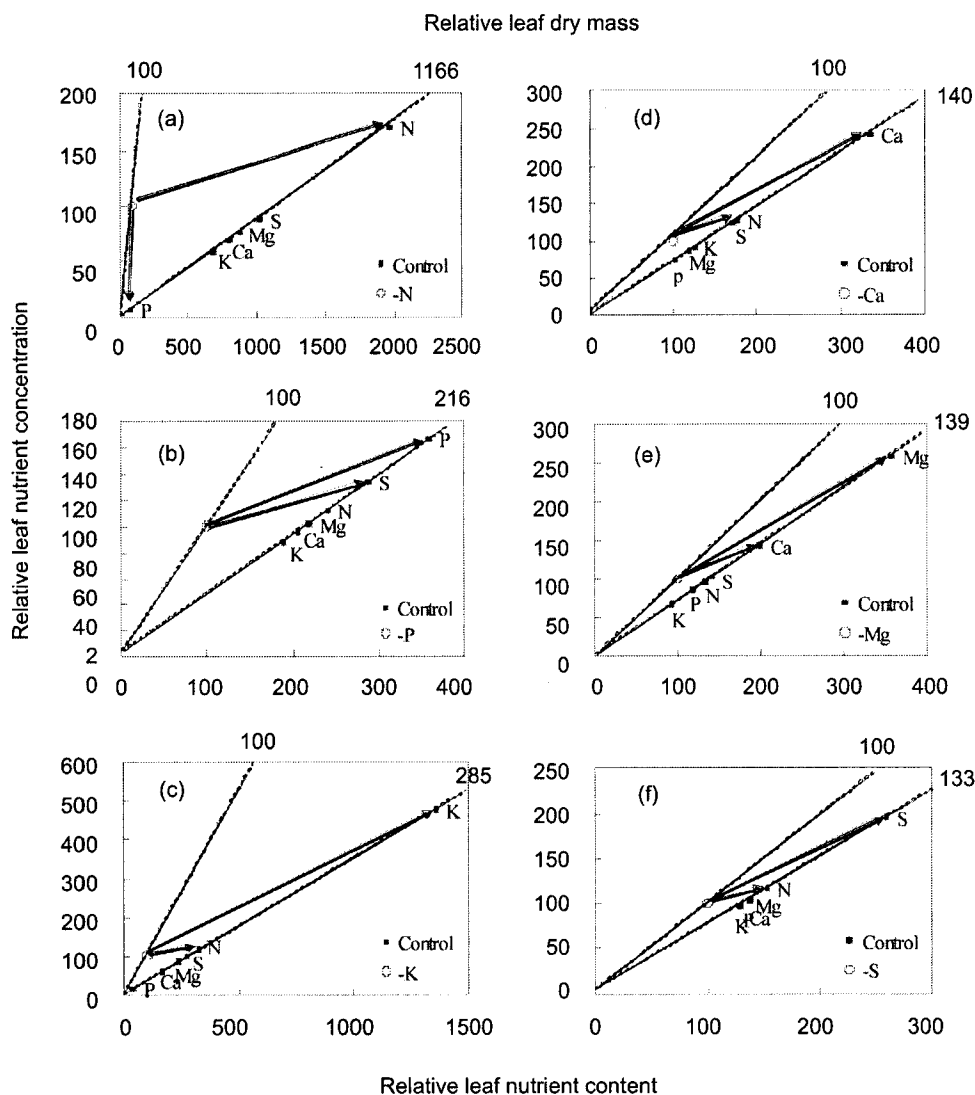
Phosphorus deficiency decreased seedling height, root collar diameter, number of branches, leaf area and biomass. This is related to its central roles in energy metabolism, synthesis of organic compounds such as nucleic acids, phospholipids and nucleotides (Wang 2000). The root/shoot ratio of -P seedlings increased

**Table 3** Effects of different nutrient deficiencies on pigment compositions of *B. alnoides* seedlings

Treatment	Chla (mg g <sup>-1</sup> )	Chlb (mg g <sup>-1</sup> )	Chla/ Chlb	Total chlorophyll, chl (mg g <sup>-1</sup> )	Carotenoid, car (mg g <sup>-1</sup> )	Car/Chl
Control	0.269 a (0.014)	0.537 a (0.013)	0.504 c (0.034)	0.806 ab (0.014)	0.154 ab (0.002)	0.191 c (0.003)
-N	0.147 c (0.003)	0.173 d (0.007)	0.854 a (0.051)	0.320 e (0.005)	0.077 e (0.002)	0.242 a (0.003)
-P	0.270 a (0.013)	0.572 a (0.012)	0.473 c (0.024)	0.842 a (0.019)	0.161 a (0.001)	0.192 c (0.003)
-K	0.214 b (0.009)	0.313 c (0.015)	0.689 b (0.038)	0.527 d (0.019)	0.112 d (0.003)	0.213 b (0.003)
-Ca	0.261 a (0.012)	0.459 b (0.011)	0.569 c (0.028)	0.720 c (0.017)	0.143 c (0.003)	0.199 c (0.004)
-Mg	0.262 a (0.011)	0.483 b (0.015)	0.543 c (0.025)	0.745 c (0.020)	0.145 bc (0.002)	0.196 c (0.004)
-S	0.258 a (0.016)	0.461 b (0.024)	0.561 c (0.015)	0.719 c (0.039)	0.139 c (0.005)	0.193 c (0.004)

Values followed by the same letter within the same columns are not significantly different at  $p < 0.05$  according to Duncan's multiple range test. Figures in parentheses are standard errors.





**Figure 3** Vector nomogram of relative changes in the leaf nutrient concentration, leaf nutrient content and leaf dry mass between *B. alnoides* seedlings supplied with different nutrient deficiency solutions. The leaf N, P, K, Ca, Mg and S contents of the control were 61.11, 5.04, 58.40, 33.84, 11.38 and 7.59 mg respectively. The leaf N, P, K, Ca, Mg and S concentrations of the control were 24.92, 2.06, 23.86, 13.73, 4.65 and 3.09 g kg<sup>-1</sup> respectively. Status of reference treatments (open symbols) at 77 DAT were equalised to 100 to allow nutrient comparison on a common base.

compared with the control as in certain plants, i.e. *Pinus resinosa* (Timmer & Armstrong 1987) and *B. pendula* (Ericsson 1995). However, for *Santalum album* and *Pistacia lentiscus*, P deficiency did not affect root/shoot ratio but affected the root morphology (Barrett & Fox 1997, Trubat *et al.* 2006). It seems that root morphology is more sensitive than biomass allocation to P deficiency. In this study, we found dark green pigment in the leaves of P-deficient seedlings although no significant difference was detected in chl<sub>a</sub> and

chl<sub>b</sub> concentrations between -P treatment and the control (Table 3). Probably cell elongation was more affected than chlorophyll content and this caused an increase in the chlorophyll content per leaf area (Lu 2003). This could be further explained by the great differences of special leaf area (the ratio of leaf area to leaf dry mass) between -P seedlings and control; the special leaf area of the former was only one third that of the latter. This observation was also reported for *Betula papyrifera* (Erdmann *et al.* 1979) and *Khaya*

*ivorensis* (Jeyanny et al. 2009). Of all the nutrient deficiency treatments, only the -P treatment failed to affect leaf pigment compositions. Seedling leaf colour was not obviously distinguished from the control. Their stems were purplish red, apparently due to formation and accumulation of anthocyanin pigments. P deficiency in birch differed from the report by Gopikumar and Varghese (2004) for *Tectona grandis* in which the -P seedlings exhibited purple-bronze patches on older leaves. -P treatment not only led to a decrease in leaf P concentration but also reduced leaf S concentration due to the synergism of both ions.

Foliar symptoms of K deficient seedlings included chlorotic patches appearing on leaves during the initial stage and the entire leaves distorting upwards, as a result of faster growth in the middle of leaves (Wang 2000). Potassium, as an enzyme activator in many important metabolic processes in plants, is important for phloem transport, osmotic balance and photosynthesis (Jeyanny et al. 2009). Hence, K deficiency slowed growth and resulted in dramatic reductions in the root collar diameter, height, number of branches, leaf area and biomass of seedlings. Although Thornley (1972) and Dewar (1993) predicted that K shortage would limit uptake of CO<sub>2</sub> and then reduction of allocation of biomass to the belowground parts, there were contrasting findings suggesting that differences in the root/shoot ratio probably existed between species (Ericsson 1995). In this work, the root/shoot ratio increased significantly for the -K seedlings. In the case of pigment compositions, K deficiency led to significant decreases in the chl<sub>a</sub>, chl<sub>b</sub>, total chlorophyll and carotenoid contents but increases in the chl<sub>a</sub>/chl<sub>b</sub> and car/chl ratios, in the same way as N deficiency. K deficiency also resulted in decreases in leaf K concentration, as well as leaf N concentration, indicating the synergism of both ions.

Discolouration, a symptom of -Ca deficiency, first appeared on young stems and young leaves of *K. ivorensis* because Ca is a relatively phloem immobile element in plants (Jeyanny et al. 2009). Discolouration occurred on all foliage of *B. papyrifera* with basal leaves often paler (Erdmann et al. 1979). However, the foliar symptoms of the -Ca seedlings were not apparent in the present study. This was probably because a small amount of Ca ( $1.6 \times 10^{-5}$  g kg<sup>-1</sup>) was rinsed from the quartz sand when watering or fertilising, which

could satisfy the basic Ca requirements for *B. alnoides*. Alternatively, the -Ca seedlings might need more time to exhibit foliar symptoms. It is known that Ca plays an important role in cell wall and plasma membrane stabilisation (Ericsson 1995). Therefore, Ca deficiency can induce failure in cell extension. In the present study, severe reductions in root collar diameter, height, number of branches, leaf area and biomass as well as an increase in the root/shoot ratio were observed in response to Ca-shortage, which were in accordance with *S. album* (Barrett & Fox 1997). As for pigment compositions, Ca had more effects on the chl<sub>b</sub>, total chlorophyll and carotenoid contents than chl<sub>a</sub> content, chl<sub>a</sub>/chl<sub>b</sub> ratio and car/chl ratio. Moreover, leaf Ca and leaf N concentrations decreased in the -Ca seedlings as a result of the synergism between Ca and N ions. This was also found in other plants such as *K. ivorensis* (Jeyanny et al. 2009).

The characteristic symptom for Mg deficiency was interveinal chlorosis as Erdmann et al. (1979) and Jeyanny et al. (2009) had demonstrated. Mg deficiency also resulted in severe suppression of root collar diameter, height and leaf area. This might be explained by the role of this element in photosynthesis as an activator of a large number of enzymes, including ribulose biphosphate carboxylase and ribulose-5-biphosphate kinase (Ericsson 1995). With regard to the number of branches, biomass and root/shoot ratio, they were relatively less impaired by the -Mg treatment. Mg is a component of the chlorophyll molecule. Mg deficiency led to remarkable reductions of the chl<sub>b</sub> and total chlorophyll contents rather than the chl<sub>a</sub> and carotenoid contents. This indicates that Mg limitation has more effects on the chl<sub>b</sub> content than on the other pigment compositions. Mg deficiency caused a significant decrease in leaf Mg concentration as well as leaf Ca concentration, inferring the synergism between Mg and Ca ions.

Discolouration was also observed on young leaves of the -S seedlings since S is not readily mobile, as with Ca, in plants. Sulphur is a constitution of the amino acids cysteine and methionine (Lu 2003). Thus, S deficiency is likely to inhibit protein synthesis. This led to reductions in the height, number of branches and leaf area of the -S seedlings. However, the root collar diameter and biomass were less influenced by the -S treatment. The S limitation resulted in an increase in the root/shoot ratio of seedlings. This

is in accordance with the findings by Ericsson (1995) for *B. pendula*. However, effects of S deficiency on root morphology rather than on root/shoot ratio were found similar to those of P deficiency in *S. album* (Barrett & Fox 1997). Leaf S concentration decreased as well as leaf N in the -S seedlings. This seems to be related to the synergism of these two ions.

## CONCLUSIONS

The present study indicated that the foliar symptoms of -N, -K and -Mg seedlings were the most apparent. Symptoms included leaf discolouration, bronze patches, leaf twisting, reddish-brown necrosis and interveinal chlorosis. The height, root collar diameter, number of branches, leaf area as well as biomass of *B. alnoides* seedlings significantly decreased and the root/shoot ratio considerably increased with N, P, K or Ca deficiencies. Reductions in the chl<sub>b</sub> and total chl contents were evident under all nutrient deficiencies except for P deficiency. Vector analysis revealed that the synergism and antagonism between foliar nutrient elements commonly existed in nutrient-deficient seedlings. This work provides guidelines for diagnosing major macronutrient deficiencies in *B. alnoides* seedlings. It offers theoretical foundation for improving silviculture and sustainability of *B. alnoides* in the plantation.

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