

Nutrient, chlorophyll and caloric dynamics of *Phyllostachys pubescens* leaves in Yongchun County, Fujian, China

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Abstract—Nutrient, chlorophyll and caloric dynamics of the mature and senescent leaves of *Phyllostachys pubescens* were studied in Yongchun County, Fujian, China, from April 2002 to March 2003. There were notable seasonal variations in nutrient concentrations in the mature and senescent leaves. Mature leaves had significantly higher N, P and K concentrations than senescent leaves, while senescent leaves had higher Ca concentration and Mg concentration changed little during senescence. Resorption efficiencies on leaf area basis of N, P and K were positive, and those of Ca and Mg were negative, suggesting that with leaf senescence, N, P and K were translocated out of senescing leaves to other parts of plant, while Ca and Mg accumulated in senescing leaves. Seasonal changes in Chl *a*, Chl *b* and total chlorophyll were curvilinear in style, with Chl *a* making up the major portion of total chlorophyll. Chl *a* increased substantially in winter in mature leaves only, reflecting that the photosynthetic apparatus of mature leaves adapted to changes in the environment (i.e., temperature), whilst senescent leaves were less sensitive to environmental changes. Chlorophyll content decreased but the Chl *a*/Chl *b* ratio increased during leaf senescence. *Ph. pubescens* leaves had relatively high ash contents, with $10.29 \pm 2.59\%$ for mature leaves and $10.82 \pm 2.63\%$ for senescent leaves respectively. There was an increasing trend ($P = 0.0199$) in the ash content during leaf senescence, showing that *Ph. pubescens* leaves possibly possessed the mechanism to maintain the nutrition balance. Gross caloric value and ash free caloric value remained almost constant during leaf senescence ($P > 0.05$), demonstrating that energy was not lost with leaf senescence.

Key words: *Phyllostachys pubescens*; nutrient; chlorophyll; caloric value; season change; mature leaf; senescent leaf.

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INTRODUCTION

Nitrogen and phosphorus are essential elements that limit not only plant growth but growth of microbial populations in soil systems. Potassium, calcium and magnesium are also macronutrients necessary for plant growth. But leaf nutrient concentrations varied with species, age of the tissue, climate, soil and other factors [1, 2]. In particular, seasonal changes in leaf nutrients occur in response to resorption or retranslocation before senescence [3]. Retranslocation from senescing leaves is the process by which plants withdraw nutrients from these leaves, making them available for later investment in new structure [4, 5]. This increases the use of absorbed nutrients and reduces plant dependence on soil supply [6]. This process plays an important role in nutrient conservation [3]. Obviously, species and seasonal pattern of nutrients strongly influence the nutrient resorption [2, 5, 7–10].

The chlorophylls, Chl *a* and Chl *b*, are the most important of photosynthetic pigments, and are thus virtually essential for the oxygenic conversion of light energy to the stored chemical energy that powers the biosphere. From an applied perspective, leaf pigmentation is important to both land managers and ecophysiologicalists [11]. First, the amount of solar radiation absorbed by a leaf is largely a function of the concentrations of leaf photosynthetic pigments and, therefore, low concentrations of chlorophyll can directly limit photosynthetic potential and hence primary production [12, 13]. Second, much of leaf nitrogen is incorporated in chlorophyll, so quantifying chlorophyll content gives an indirect measure of nutrient status [13, 14]. Third, pigmentation can be directly related to stress physiology, as concentrations of chlorophylls generally decrease under stress and during senescence [15]. Fourth, the relative concentrations of pigments are known to change with abiotic factors such as light [16], so quantifying these proportions can provide important information about relationships between plants and their environment.

In comparison to foliar nutrients and chlorophyll, the seasonal dynamics in caloric values of the leaves is less intensively studied. Leaf senescence is not a single process of element retranslocation, but a process accompanied by respiration, hydrolysis of carbohydrate, protein and the translocation of hydrolyzates such as soluble sugars or amino acids out of the senescing leaves [17]. Changes in caloric value may indicate changes in the chemical composition of leaves. Sun *et al.* [18] pointed out that the most importance on the caloric value measurements was that it could reflect changes of physiological activities and plant growth. The variation in caloric equivalents for plants was a possible response to environment and caloric value was an evaluation index of plant growth. However, many aspects with respect to seasonal caloric dynamics and changing trend during leaf senescence remain unclear.

Phyllostachys pubescens, a species native to China and called the giant woody bamboo, has been widely used for food, handicraft, furniture and construction materials in many parts of the world. However, very little is known about ecophysiology of this species and bamboo in general. Considering bamboo is an important natural resource, the efficiency of bamboo production can be maximized

by understanding aspects of their structure–function relationship. Leaf functional and physiological traits are key factors in determining plant photosynthetic rates and play a role in ecosystem nutrient cycling.

The objective of the present study was to test the validity of the following hypotheses: (1) the concentrations of nutrients follow a seasonal pattern both in mature and senesced leaves; (2) mature leaves have a higher concentration of nutrients than senesced leaves; (3) leaf caloric values decrease with senescence.

MATERIALS AND METHODS

Site

Research was conducted in a natural *Ph. pubescens* forest at Yidu Town, Yongchun County (25°21'33"–25°31'33"N, 117°40'40"E), Fujian, China. The climate is subtropical monsoon, the average annual temperature is 18.3°C and the average annual precipitation is 1724 mm. The frost-free season lasts for 310 days in this region. The mountain red soil is about 90 cm in depth, with 3–5 cm humus layer, pH 5.0–5.1, with total nitrogen and phosphorus contents of 0.102–0.293% and 0.042–0.075%, respectively, and organic C content of 3.45–3.65% in the upper 30 cm. The characteristic of *Ph. pubescens* forest was described in a previous report [19]. The coverage of dense forest was 0.8, tree density was 26.8 trees/100 m², and mean height and base diameter were 12 m and 8 cm, respectively. The understory consisted of *Engelhardtia fenzelii* and *Ilex pubescens* with *Pteridium aquilinum* var. *latiusculum* and *Paris polyphylla* in herb layer.

Materials

Fifty 2-year-old individuals were chosen and labelled. The height and living conditions of the chosen trees were similar. Ten pieces of mature leaves and ten pieces of senescent leaves from the same shoot on the upper canopy of each labelled tree were collected every month from April 2002 to March 2003. 'Mature leaf' is a leaf that is fully expanded, just prior to the onset of the leaf senescence (determined by appearance). 'Senescent leaf' is a leaf that is ready to abscise. Leaves collected from an individual were pooled, resulting in one sample for mature leaves and one sample for senescent leaves each month. Leaves damaged by insects or by mechanical factors were avoided.

Chemical analysis

The collected leaves were taken to the laboratory and the leaf area was measured by LI-3000A portal leaf area instrument (LI-COR). The leaves were washed with distilled water, some of them (about 1–2 g) were used in determining contents of photosynthetic pigments, the others were dried at 80°C, ground in a mill to

pass through a 1-mm sieve and stored for chemical analyses and caloric value determination.

Concentrations of chlorophyll *a* and *b* were determined according to Lichtenthaler and Wellburn's method [20], which involved preparation of leaf extract by immersing the leaves in a solution of water/acetone/alcohol (1 : 4.5 : 4.5, v/v).

Subsamples of leaves were digested in sulfuric acid/hydrogen peroxide, and N was determined using the micro-Kjeldahl method [21], P was determined by ascorbic acid/antimony reducing phosphate colorimetric method [22]. Samples were digested in nitric acid/perchloric acid, and K, Ca and Mg determinations were made with a Model WFX-IB Atomic Absorption Spectrophotometer (Beijing Analytical Instrument). Two or three replicate determinations were done for each sample. The coefficients of variation between replicates were less than 5% for K, Ca and Mg, and less than 2% for N and P.

Caloric value determination

The caloric value per gram dry weight (referred to below as gross caloric value) was determined by burning samples in a GR-3500 Oxygen Bomb Calorimeter. The measurement was done at a laboratory temperature of 20°C. Three sets of duplicate samples were tested for each component [23]. Caloric value per gram ash-free dry weight (referred to below as ash-free caloric value, AFCV) was used for the comparison. The quantitative relationship between these two kinds of caloric values was $AFCV = GCV / (1 - \text{Ash}\%)$ [24].

The ash content was determined by the ashing method at 550°C for 5 h.

The coefficients of variation between replicates were less than 2% for gross caloric value and ash content.

Calculation

Expression of nutrient resorption efficiency (RE) on leaf area basis is as follows [25]:

$$RE = (1 - A_2/A_1 \times S_1/S_2) \times 100\%,$$

where A_1 is the mean element content (mg) per mature leaf; A_2 is the mean element content (mg) per senescent leaf; S_1 is the mean area (cm²) per mature leaf; S_2 is the mean area (cm²) per senescent leaf. A negative RE value indicated that the element accumulated in senescing leaves.

Statistics analysis

Analysis of variance (ANOVA) was employed to test for differences among components. Computer programs: Excel and SPSS 11.0 for Windows.

RESULTS AND DISCUSSION

Element mean concentrations and seasonal changes in element concentrations of mature and senescent leaves

The element concentrations in mature leaves of *Ph. pubescens* displayed monthly changes. N concentration was higher in the winter months compared to the summer. Nitrogen concentration in February 2003 was 59.75% higher than in April 2002; P concentration remained relatively stable, ranging from 1.93 mg/g (in July 2003) to 2.65 mg/g (in January 2004); K concentration displayed V-shaped changes, with decreasing trends from April to October, and then increasing; Ca concentration was high in summer (from June to August) while Mg concentration remained relatively stable ranging from 1.20 mg/g to 1.89 mg/g. Similarly, there was a notable seasonal variation of element concentrations in the senescent leaves (Fig. 1).

Leaf element concentrations reflect the element supply status of *Ph. pubescens*. For example, N concentration of mature leaves was high in winter and low in summer. The higher N concentration in winter can be attributed to the greater demand for nutrients by plants for production of new leaves and bamboo shoots in next spring.

The rank order of the mean element concentration in mature leaves was $N > Ca > K > P > Mg$ ($P < 0.05$), and was maintained in the senescent leaves as well (Table 1). The result was different from the observations of Lin *et al.* [26] ($N > K > Ca > Mg > P$) on *Castanopsis eyrei* and *Pinus taiwanensis* leaves in Wuyi mountains. The characteristics of element accumulation were different with species.

As far as element mean concentrations of mature and senescent leaves are concerned,

N: mature leaves (6.42–15.95 mg/g) > senescent leaves (4.61–12.76 mg/g)
($P = 0.0056$);

P: mature leaves (1.93–2.65 mg/g) > senescent leaves (1.27–2.14 mg/g)
($P = 0.000367$);

K: mature leaves (2.92–6.58 mg/g) > senescent leaves (2.61–5.90 mg/g)
($P = 4.59 \times 10^{-6}$);

Ca: mature leaves (3.89–7.21 mg/g) < senescent leaves (6.01–7.67 mg/g)
($P = 0.000253$);

Mg: mature leaves (1.20–1.89 mg/g) \approx senescent leaves (1.21–1.74 mg/g)
($P = 0.653$).

Mature leaves had significantly higher N, P and K concentrations than senescent leaves, while senescent leaves had higher Ca concentration, and Mg concentration changed little during senescence. The ratio of N:P in present study ranged from 3.13 to 6.57 for mature leaves, and 2.39 to 7.60 for senescent leaves, respectively. A ratio of N:P higher than 15:1 indicates a P-limited growth in vegetation [27]. We observed that P concentrations in the soils and mature leaves were 0.042–0.075% and 0.193–0.265%, respectively, which indicated that *Ph. pubescens* had high P-use

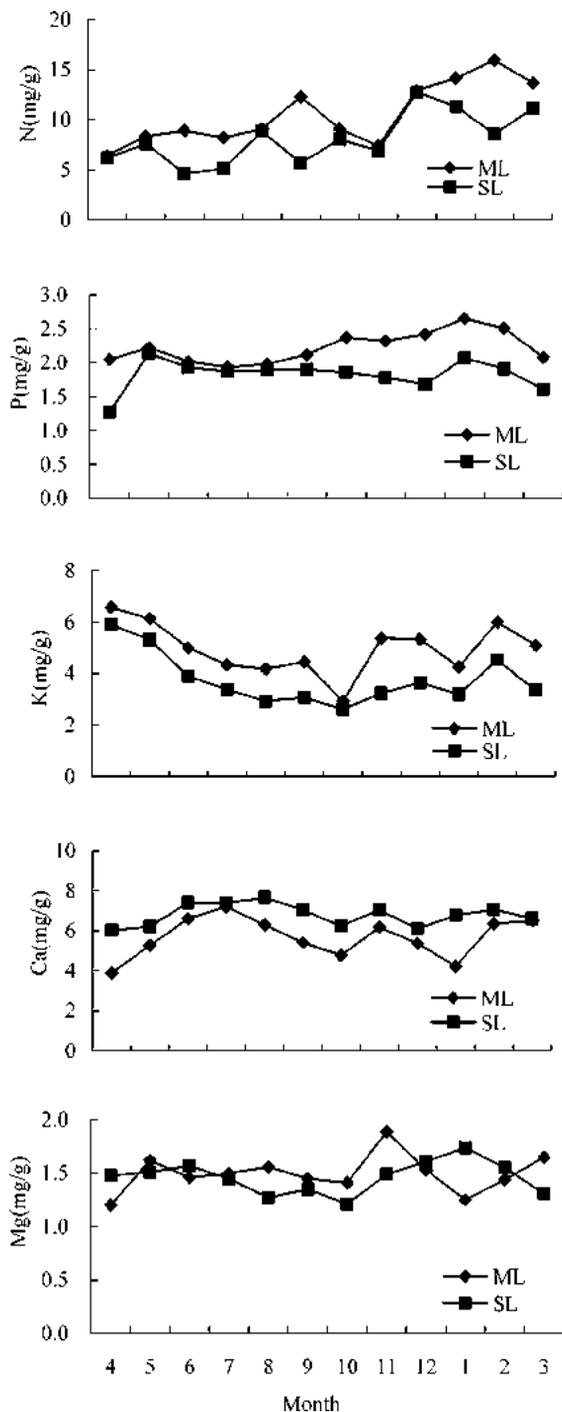


Figure 1. Seasonal changes in element concentrations of mature leaves (ML) and senescent leaves (SL) of *Phyllostachys pubescens* from April 2002 to March 2003.

Table 1.

Element mean concentrations (mg/g) in the mature leaves and senescent leaves of *Phyllostachys pubescens*

		N (mg/g)	P (mg/g)	K (mg/g)	Ca (mg/g)	Mg (mg/g)
Mature leaf	Mean	10.54	2.22	4.98	5.68	1.50
	SD	3.09	0.23	1.02	1.02	0.18
Senescent leaf	Mean	8.06	1.83	3.75	6.80	1.46
	SD	2.61	0.23	0.96	0.56	0.15

efficiency and great demands on phosphorus. Vitousek found that adding P to P-limited sites increased foliar P concentrations 3-fold and litter P concentrations up to 10-fold [28].

There were significant correlations between N and P, Ca and Mg, and K and Ca in the mature leaves with the correlation equations as follows:

$$N/P : y = 0.043x + 1.7692, r = 0.577, P < 0.05;$$

$$Ca/Mg: y = 0.1059x + 0.8956, r = 0.596, P < 0.05;$$

$$K/Ca: y = -0.4878x^2 + 4.6497x - 4.9197, r = 0.596, P < 0.05.$$

This result reflected that the uptake and allocation of nutrients between N and P, Ca and Mg, and K and Ca by *Ph. pubescens* might be dependent on each other.

Nutrient resorption efficiency during leaf senescence

Retranslocation from senescing leaves is the process by which plants withdraw elements from these leaves, making them available for later investment in new structure [4, 5]. This increases the use of absorbed elements and reduces plant dependence on soil supply [6]. The process of retranslocation is closely associated with leaf senescence and conservation of elements, and is an important mechanism enabling plants to maintain growth in element-poor sites [3, 25, 29, 30].

Resorption efficiencies (RE) on leaf area basis of N, P, K, Ca and Mg were 25.70%, 2.04%, 10.68%, -45.88% and -18.26%, respectively; it was found that RE of N, P and K were positive, and those of Ca and Mg were negative, suggesting that with leaf senescence, N, P and K were translocated out of senescing leaves to other parts of plant, while Ca and Mg accumulated in senescing leaves. Nevertheless, these relationships were no tenable for the RE of each month (Table 2). Ca and Mg increased with leaf age. The result accorded with the observations of Lin and Wang [25], and Ralhan and Singh [31]. Increases in Ca concentration with leaf age result from secondary thickening, including calcium pectate deposition in cell walls, and from increasing storage of calcium oxalate in cell vacuoles. The accumulation of Ca and Mg during senescence is related to the fact that they are immobile nutrients and cannot be readily loaded by transfer cells into phloem for retranslocation [32, 33]. Compared with some tree leaves (Table 3), RE of N of *Ph. pubescens* leaves was similar

Table 2.

Changes in nutrient resorption efficiency (%) during leaf senescence

Time	N	P	K	Ca	Mg
Apr. 2002	2.92	18.14	-18.48	-104.15	-62.97
May 2002	13.11	-3.21	7.23	-26.37	0.20
Jun. 2002	48.38	-10.97	9.41	-30.18	-24.89
Jul. 2002	38.77	-16.95	6.00	-23.72	-16.68
Aug. 2002	8.95	-18.07	13.30	-50.59	-0.70
Sep. 2002	58.52	-9.47	16.20	-58.94	-13.72
Oct. 2002	17.32	17.73	6.30	-37.13	10.04
Nov. 2002	10.97	8.93	28.73	-35.61	6.42
Dec. 2002	11.37	30.42	31.49	-13.64	-5.47
Jan. 2003	23.08	1.75	5.81	-101.61	-75.09
Feb. 2003	49.85	3.52	4.43	-40.95	-36.48
Mar. 2003	25.17	2.69	17.75	-27.65	0.19
Mean \pm SD	25.70 \pm 18.59	2.04 \pm 14.94	10.68 \pm 12.84	-45.88 \pm 29.22	-18.26 \pm 27.29

Table 3.

Nutrient resorption efficiencies (%) of some tree leaves

Species	N	P	K	Ca	Mg	Reference
<i>Shorea robusta</i>	38.6	40.9	31.6	-14.8	ND	[31]
<i>Mallotus philippensis</i>	34.3	30.1	26.1	-6.6	ND	[31]
<i>Murraya peniculata</i>	33.8	40.4	26.9	-12.8	ND	[31]
<i>Pinus roxburghii</i>	53.5	49.2	44.3	-9.7	ND	[31]
<i>Quercus leucotrichophora</i>	25.2	24.8	29.6	-6.3	ND	[31]
<i>Quercus floribunda</i>	21.5	23.1	27.3	-10.4	ND	[31]
<i>Quercus lanuginosa</i>	23.9	25.8	20.3	-6.6	ND	[31]
<i>Myrsine semiserrata</i>	30.6	27.0	24.2	-7.8	ND	[31]
<i>Myrica esculenta</i>	25.7	36.4	25.8	-9.5	ND	[31]
<i>Quercus kelloggii</i>	40.0	7.0	36.0	ND	ND	[7]
<i>Phyllostachys pubescens</i>	25.70	2.04	10.68	-45.88	-18.26	This study

to that of *Myrica esculenta* leaves [31], but RE of P, K was relatively low. However, Teklay [2] found that K accumulated in senescing leaves of *Albizia gummifera*, *Milletia ferruginea* and *Cordia africana* during dry season in southern Ethiopia. The low RE of P (7.0%) was reported in *Quercus kelloggii* leaves [7]. Similar P concentrations in the mature and senescent leaves were found in the *Fraxinus americana* (0.18 \pm 0.04% and 0.17 \pm 0.09%, respectively) and *Acer pensylvanicum* (0.06 \pm 0.01% and 0.07 \pm 0.03%, respectively), indicating these two species had the very low RE of P [34]. The RE of *Ph. pubescens* leaves may be underestimated because of different calculation method. Noteworthy, RE changed significantly with month. For example, RE of N, P varied from 2.92% to 58.52% and -18.07% to 30.42% respectively. RE of P was negative in some months, Schlesinger *et al.* [35] also found that RE of P in *Arctostaphylos patula*

was negative. These situations were impossible physiologically [25]. Aerts [5] suggested that high nutrient resorption efficiency is clearly not characteristic of evergreen species of low-nutrient environments. Escudero *et al.* [9] showed that leaf longevity was far more important as a nutrient conservation mechanism than high resorption efficiency. Reich *et al.* [36] arrived at a similar conclusion in a study with evergreen and deciduous woody species of an oligotrophic Amazonian forest. Low resorption efficiency in species from infertile sites may partly be explained as a consequence of an anti-herbivory mechanism [37]. Herbivorism, which would increase nutrient losses and would reduce photosynthetic carbon gain, is reduced in plants growing at infertile sites because they generally contain higher amounts of phenolic compounds than do plants from more fertile habitats [38]. While *Ph. pubescens* is a high-Si species, silica deposition is one of the important characteristics, Si afforded *Ph. pubescens* against the detrimental effects of abiotic and biotic stresses. The leaves of the low-Si plants were prone to wilting, and senesced earlier than those of high-Si plants [39]. This Si reinforcement of cell walls, and further effects elicited by Si, protect the bamboo against the depredations of disease organisms and herbivores including insects. Long life span and high silica accumulation appear to be the main adaptations of *Ph. pubescens* under nutrient-limited conditions. RE is independent of status of individuals.

Seasonal changes in chlorophyll content of mature and senescent leaves

Seasonal changes in Chl *a*, Chl *b* and total chlorophyll were curvilinear in style (Fig. 2), with Chl *a* making up the major portion of total chlorophyll (50.44–74.28% for mature leaves and 57.44–75.18% for senescent leaves respectively). Chl *a* content of mature leaves in autumn and winter (from September 2002 to February 2003) was higher than in spring and summer ($P < 0.0005$), different from that of senescent leaves.

Chlorophyll content decreased during leaf senescence. Chlorophyll content of mature leaves ranged from 3.05 to 5.19 mg/g, with the average of 4.49 ± 0.79 mg/g; Chlorophyll content of senescent leaves varied from 1.03 to 3.39 mg/g, average 2.20 ± 0.79 mg/g. Chlorophyll content of mature leaves was significantly higher than that of senescent leaves ($P < 0.0001$), corresponding to the fact that mature leaves have higher photosynthesis capacity than senescent leaves. The ratio of Chl *a* to Chl *b* in the mature leaves ranged from 1.015 to 2.886, average 2.076 ± 0.758 , that in the senescent leaves varied from 1.350 to 3.026, with an average of 2.454 ± 0.568 . Mature leaves had a lower Chl *a*/Chl *b* ratio than senescent leaves ($0.01 < P < 0.05$). The higher Chl *a*/Chl *b* ratio in the senescent leaves resulted from the greater decrease percentage (55.80%) of Chl *b* relative to that (44.64%) of Chl *a* during senescence. Scheumann *et al.* [40] observed that the Chl *a*/Chl *b* ratio of barley seedling increased during senescence from 2.9 at day 0 to 5 at day 8, and suggested that either degradation of Chl *b* was faster than that of Chl *a* or Chl *b* was transformed into Chl *a*. Gossauer and Engel [41] proposed that the conversion of Chl *b* to Chl *a* should precede chlorophyll degradation in higher plants. The

physiological significance of the conversion of Chl *b* to Chl *a* is not yet known. Chl *a* is located only in the reaction centres of the photosystems, while Chl *b* is located both in reaction centres and light harvesting complexes. A change in the Chl *a*/Chl *b* ratio reflects an adaptation mechanism to balance the amount of light captured by the leaf and its utilization for photochemical processes. Figure 2

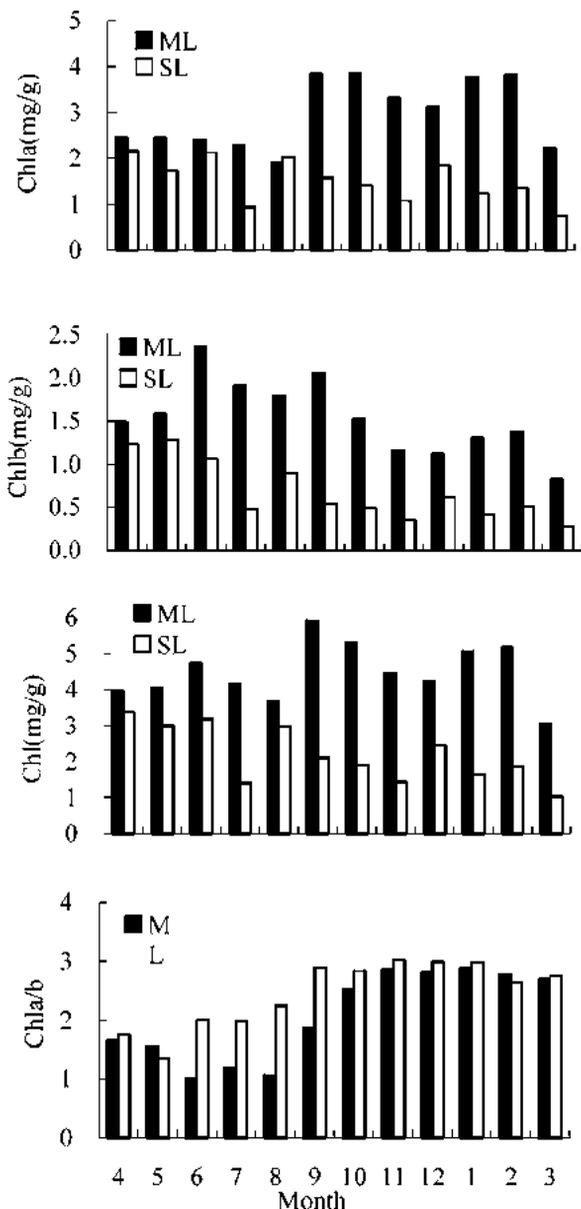


Figure 2. Seasonal changes in photosynthetic pigments of mature leaves (ML) and senescent leaves (SL) of *Phyllostachys pubescens* from April 2002 to March 2003.

shows that Chl *a* increased substantially in winter in mature leaves only, it can be hypothesized that the photosynthetic apparatus of mature leaves adapted to changes in the environment (i.e., temperature) whilst senescent leaves were less sensitive to environmental changes.

Seasonal variations of ash contents in mature and senescent leaves

A notable seasonal variation may be seen in the ash contents of mature and senescent leaves (Fig. 3A). Ash contents of mature and senescent leaves were high in the cold season (in particular in February and March 2003) and low in the warm season,

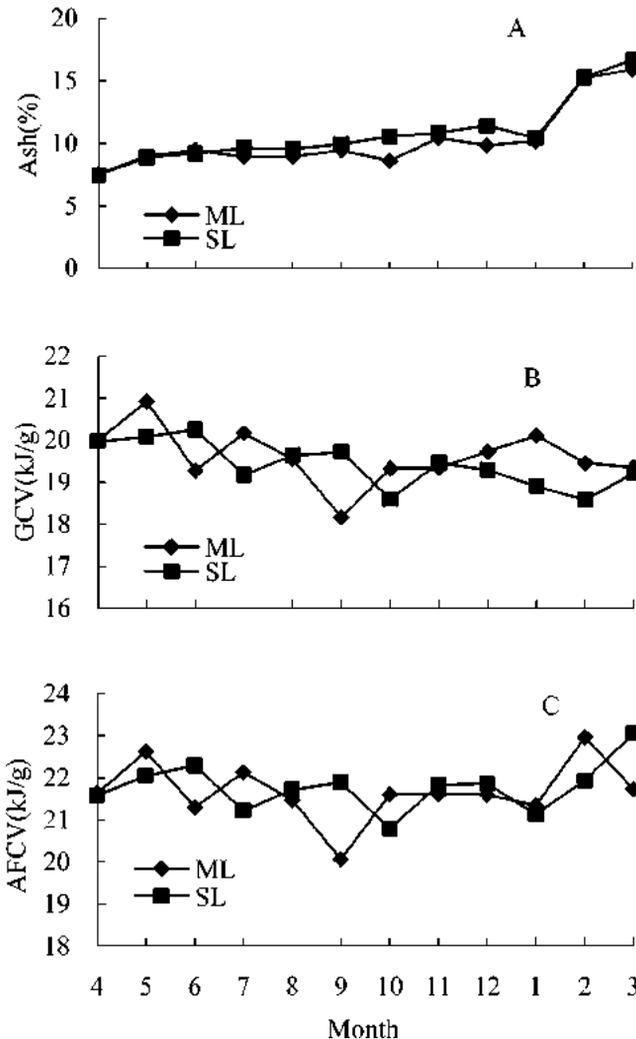


Figure 3. Seasonal changes in ash contents, gross calorific values (GCV) and ash free calorific values (AFCV) of mature leaves (ML) and senescent leaves (SL) of *Phyllostachys pubescens* from April 2002 to March 2003.

which was similar to N concentration. There was a significant linear correlation between ash content and N concentration for mature leaves ($y = 0.6111x + 3.849$, $r = 0.730$, $P < 0.01$), but no significant correlation for senescent leaves ($y = 0.529x + 6.5596$, $r = 0.523$, $P > 0.05$).

Ash contents of mature leaves ranged from 7.56 to 15.91%, with an average of $10.29 \pm 2.59\%$. Those of senescent leaves varied from 7.46 to 16.67%, average $10.82 \pm 2.63\%$. Senescent leaves had higher ash content than mature leaves ($P = 0.0199$).

Obviously, ash content was closely related to element concentrations absorbed by plants. It could reflect the role of plant element enrichment and related with soil condition.

As we know, leaf senescence is not a single process of element retranslocation, but a process accompanied by respiration, hydrolysis of carbohydrate, protein and the translocation of hydrolyzates such as soluble sugar, amino acid out of the senescing leaves [17]. Certain elements such as Ca accumulate in the senescing leaves, too [25, 31]. In the present study, there was an increasing trend in the ash content during leaf senescence, showing that *Ph. pubescens* leaves possibly possessed the mechanism to maintain the nutrition balance.

Li *et al.* [42] observed that the leaf mean ash content of 46 tree-layer species was 6.8% in the tropical mountain rain forest of Jianfengling, Hainan, China. In comparison, *Ph. pubescens* leaves had a relatively high ash content. A pattern can be explained by the presence of high Si concentration in *Ph. pubescens* leaves. Silica accumulation is one of the most prominent characteristics of the Poaceae. Most silica is accumulated in the aerial parts (leaves) as amorphous hydrated silica ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$). Previous studies have reported silica contents of up to 27% in the Poaceae [39, 43] with the highest value (41%) exhibited by a temperate bamboo *Sasa veitchii* [44]. Kaneko observed that *Ph. pubescens* leaves continue to accumulate silica throughout their life after expansion [45]. The long progressive accumulation of silica by *Sasa veitchii* confirmed that leaves can accumulate silica continuously until they are shed [44].

Seasonal changes in gross caloric value and ash free caloric value

Gross caloric values in mature and senescent leaves fluctuated with month from April 2002 to March 2003, with the highest in May and the lowest in September for mature leaves, and the highest in June and the lowest in October for senescent leaves, respectively. The peak of senescent leaves lagged 1 month behind mature leaves (Fig. 3B). Ash free caloric values showed difference, with the highest in February and the lowest in September for mature leaves, and the highest in March and the lowest in October for senescent leaves, respectively (Fig. 3C). The difference in leaf ash content resulted in the discrepancy.

The range of gross caloric values of mature leaves (18.166–20.923 kJ/g, average 19.617 ± 0.666 kJ/g) was similar to that of senescent leaves (18.581–20.249 kJ/g, average 19.407 ± 0.550 kJ/g) ($P > 0.05$), gross caloric value remained almost

constant from mature leaves to senescent leaves. Research on ash free caloric values came to the similar result, which demonstrated that energy was not lost with leaf senescence.

Significant seasonal changes in gross caloric values have been demonstrated for several ecosystem components. Morrison once reported that in many plants percentages of crude protein in the green leaves decreased, those of crude fibre and nitrogen-free extract (mainly carbohydrates) increased, and those of ether extract (partly fat) remained constant through the growing season. Therefore, the gross caloric values of leaves should be higher in spring than in autumn [46]. Many researches showed that seasonal changes in gross caloric values were correlated with phenology of plants and their reaction to environmental factors [47]. Our results support these observations.

Generally supposing, mature leaves might have higher caloric values than senescent leaves due to their more active life, stronger photosynthesis capacity and higher contents of organic matters. The result of this study did not support this hypothesis. We found that gross caloric value and ash free caloric value remained almost constant during leaf senescence ($P > 0.05$), demonstrating that energy was not lost with leaf senescence. Leaf caloric value increment at abscission was once observed by Hughes [24] on seasonal caloric values from deciduous woodland in England. Our results are different but not unique in terms of the trend in caloric value of the leaves.

The mean caloric values for 34 species studied by Bowman and Wilson [48] was 18.84 kJ/g. Interestingly, the energy content of fresh leaves was similar to that of the dead leaves in all species, except for *Callitris intratropica* [49], in which the dead leaves were found to have a substantially lower caloric value than the fresh materials. Total caloric value content reflects both structural and non-structural carbohydrates. The non-structural portion (starches and sugars) would vary seasonally and developmentally in plants, the non-structural component is small relative to the stable structural components (cellulose, hemicelluloses, etc.) and therefore, caloric energy yield is unrelated to plant constituents than can be used by the plant for energy. Thus, caloric value remained relatively stable during senescence.

The differences in caloric values among various components were mainly influenced by the differences in their compositions (i.e., protein, lipid, carbohydrate, etc.), light intensity, length of day, soil types and the ages of plants [24, 50–52].

In this study, gross caloric value was correlated remarkably with ash content for senescent leaves, the regression equation was $y = -0.1227x + 20.735$ ($r = 0.587$, $P < 0.05$); there was no significant correlation between gross caloric value and ash content for mature leaves, the regression equation was $y = -0.0522x + 20.154$ ($r = 0.203$, $P > 0.05$).

Similarly, gross caloric value had a significant correlation with chlorophyll content for senescent leaves, the regression equation was $y = 1.0027x - 17.258$ ($r = 0.701$, $P < 0.05$). There was no significant relationship between gross caloric

value and chlorophyll content for mature leaves ($y = -0.566x + 15.592$, $r = 0.475$, $P > 0.05$).

CONCLUSIONS

There were notable seasonal variations in nutrient concentrations in the mature and senescent leaves. Mature leaves had significantly higher N, P and K concentrations than senescent leaves, while senescent leaves had higher Ca concentration, and Mg concentration changed little during senescence.

Resorption efficiencies on leaf area basis of N, P, and K were positive, and those of Ca and Mg were negative, suggesting that with leaf senescence, N, P and K were translocated out of senescing leaves to other parts of plant, while Ca and Mg accumulated in senescing leaves.

Seasonal changes in Chl *a*, Chl *b*, and total chlorophyll were curvilinear in style, with Chl *a* making up the major portion of total chlorophyll. Chl *a* increased substantially in winter in mature leaves only, reflecting that the photosynthetic apparatus of mature leaves adapted to changes in the environment (i.e., temperature) whilst senescent leaves were less sensitive to environmental changes. Chlorophyll content decreased but the Chl *a*/Chl *b* ratio increased during leaf senescence.

Ph. pubescens leaves had relatively high ash contents, $10.29 \pm 2.59\%$ for mature leaves and $10.82 \pm 2.63\%$ for senescent leaves, respectively. There was an increasing trend ($P = 0.0199$) in the ash content during leaf senescence, showing that *Ph. pubescens* leaves possibly possessed a mechanism to maintain the nutrition balance.

Gross caloric value and ash free caloric value remained almost constant during leaf senescence ($P > 0.05$), demonstrating that energy was not lost with leaf senescence.

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