Nutrient Resorption and Phenolics Concentration Associated with Leaf Senescence of the Subtropical Mangrove *Aegiceras corniculatum*: Implications for Nutrient Conservation

Hui Chen [1,2,†], **Benbo Xu** [1,†], **Shudong Wei** [1,2,*], **Lihua Zhang** [3,4,*], **Haichao Zhou** [2] **and Yiming Lin** [2]

1   College of Life Science, Yangtze University, Jingzhou 434025, China; chenhui_17@126.com (H.C.);
    benboxu@yangtzeu.edu.cn (B.X.)
2   Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University,
    Xiamen 361005, China; seapass2004@163.com (H.Z.); linym@xmu.edu.cn (Y.L.)
3   Key Laboratory of Coastal Environmental Processes and Ecological Remediation,
    Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS), Yantai 264003, China
4   Shandong Provincial Key Laboratory of Coastal Zone Environmental Processes, YICCAS,
    Yantai 264003, China
*   Correspondence: weishudong2005@126.com (S.W.); lhzhang@yic.ac.cn (L.Z.); Tel.: +86-716-806-6858 (S.W.)
†   These authors contributed equally to this work.

Abstract: *Aegiceras corniculatum* (L.) Blanco, a mangrove shrub species in the Myrsine family, often grows at the seaward edge of the mangrove zone in China. In the present study, seasonal dynamics of nutrient resorption and phenolics concentration associated with leaf senescence of *A. corniculatum* were investigated in order to evaluate its possible nutrient conservation strategies in the subtropical Zhangjiang river estuary. It was found that the nitrogen (N) and phosphorus (P) concentrations in mature leaves showed similar seasonal changes with the highest concentrations in winter and the lowest in summer, and were significantly higher than those in senescent leaves. The N:P ratios of mature leaves through the year were found to be less than 14, indicating that the *A. corniculatum* forest was N-limited. The nitrogen resorption efficiency (NRE) was higher than phosphorus resorption efficiency (PRE), and N resorption was complete. In addition, *A. corniculatum* leaves contained high total phenolics (TPs) and total condensed tannin (TCT) levels (both above 20%). TPs concentrations in mature and senescent leaves were all inversely related to their N or P concentrations. TPs:N and TCT:N ratios in senescent leaves were significantly higher than those in mature leaves. The obtained results suggested that high NRE during leaf senescence and high TPs:N and TCT:N ratios in senescent leaves might be important nutrient conservation strategies for the mangrove shrub *A. corniculatum* forest growing in N-limited conditions.

Keywords: *Aegiceras corniculatum*; nutrient resorption; phenolics; leaf senescence

1. Introduction

Mangrove species that flourish in low-nutrient environments have very efficient mechanisms for retaining and recycling nutrients [1]. *Aegiceras corniculatum* (L.) Blanco is a cryptoviviparous mangrove species, often grows at the seaward edge of the mangrove zone in China. This species seems most characteristic of the seaward mangal fringe and occurs typically as an isolated low shrub [2]. Compared with other mangrove species, *A. corniculatum* shrubs have high annual litter fall production in subtropical China [3]. The resulting litter from the leaf fall must be decomposed and the nutrients contained in that litter must be remineralized to become available again for plant uptake. However,

most of this leaf fall is washed away by tides [4]. Such a high leaf fall rate will inevitably bring about a great loss of nutrients. Therefore, mangrove soils are generally low in nutrient concentrations [5], which can lead to the slow growth of mangrove species. However, mangroves are characterized by their high primary productivity [6]. To sustain the high levels of productivity, a substantial amount of nutrients is required. How mangrove plants, including *A. corniculatum*, cope with these conditions has provoked little attention [7].

Many previous studies showed that the concentrations of nutrients in the mangrove leaves varied with season and species [8,9]. In particular, seasonal changes in foliar nutrients occur in response to resorption before senescence [6]. Nutrient transfer from senescing leaves is the ecophysiological process by which plants withdraw nutrients from these leaves, making those nutrients available for later investment in new structures [10]. According to the reports by Lin et al. [8], Wei et al. [9], and Lin and Sternberg [11], the process of retranslocation is closely associated with leaf senescence and conservation of nutrients, and thus it is considered to be an important mechanism enabling plants to maintain growth in nutrient-poor sites. Nutrients may be used more efficiently at nutrient-poor sites, and this efficient nutrient use could reduce plant dependence on soil nutrient supply [12]. Feller [13], Reef et al. [14], and McKee et al. [15] suggested that nitrogen (N) and phosphorus (P) availability are important factors responsible for mangrove growth. Plant N:P ratio is a useful variable for consideration in ecological research because it reflects the gradual and dynamic nature of nutrient limitation rather than the fixed characteristics, such as N-limited versus P-limited [16].

Phenolic compounds, including tannins, are a significant component of plant secondary metabolites. In mangrove species, tannins are an abundant component, constituting as high as 20% dry weight [17]. Due to tannins being complex and energetically costly molecules to synthesize, their widespread occurrence and abundance indicate that tannins play an important role in plant function and evolution [18]. High tannin concentrations in plants are often associated with infertile site conditions. It has been suggested that there is an evolutionary advantage to higher tannin production. By reducing decomposition rates and decreasing N leaching potential, tannins may provide a nutrient conservation mechanism. In comparison to foliar nutrients, the detailed changes of leaf tannins between seasons and during leaf senescence of the mangrove species has been less intensively studied [8,9,19,20]. According to the reports by Serrano [21] and Northup et al. [22], this variability may determine not only the susceptibility of plants to herbivore attack, but also important aspects of nutrient cycling in terrestrial and aquatic ecosystems.

Therefore, nutrient resorption and high tannin production may be important for *A. corniculatum* to conserve nutrients in coastal environments. The objective of the present study was to evaluate possible nutrient conservation strategies of the mangrove shrub species *A. corniculatum* under low nutrient conditions. The questions asked here were regarding whether (1) leaf N and P concentrations follow a similar seasonal pattern, and decrease during leaf senescence; (2) a strong nutrient limitation will show higher resorption of the limiting nutrients; and (3) the production of phenolics will increase during leaf senescence under nutrient limited conditions.

2. Materials and Methods

2.1. Study Area

The experiments were carried out in Zhangjiangkou National Mangrove Nature Reserve (23°55′ N, 117°24′ E), which is located in the gulf of Gulei, Yunxiao, Fujian Province, in southern China. This nature reserve occupies 2358 hectares, and consists mostly of mangroves and salt marshes along the coastline of the Zhangjiang estuary. This nature reserve is the northernmost national nature mangrove in China, and it was included into the Ramsar List in 2008. The climate of the region is characteristic of a southern subtropical maritime monsoon climate. Based on Yunxiao Meteorological Administration, during 2001–2008 the mean air temperature was 21.15 ± 0.68 °C, 28.13 ± 0.28 °C, 24.07 ± 0.54 °C, and 15.34 ± 0.60 °C for spring, summer, autumn, and winter, respectively. The mean

annual precipitation and evaporation were 187.21 mm and 1718.4 mm, respectively. The soil is clay, and the concentration of organic matter is 39.60 ± 1.90 mg/g. N and P concentrations in the soil are 2.20 ± 0.10 mg/g and 0.40 ± 0.00 mg/g, respectively [23]. The tide is irregular semidiurnal, the soil can be flooded for 1–2 h per day during spring tides, but the soil can be exposed for 3–7 days during the neap tide. The salinity of the soil is above 10‰, and the salinity of tidal water salinity ranges between 1‰ and 22‰ [24]. In the study site, *A. corniculatum* was the dominant mangrove species with a few *Kandelia candel*. The mean height of *A. corniculatum* plants was 2.8 m and the canopy density was 0.90.

2.2. Sample Collection

Leaf samples of *A. corniculatum* were collected from Zhangjiangkou National Mangrove Nature Reserve, Yunxiao, Fujian Province, China in 15 July 2009 (summer), 13 October 2009 (autumn), 15 December 2009 (winter), and 17 March 2010 (spring). Thirty trees with similar height and growth conditions were selected and labeled. The thirty trees were divided into five groups (six trees in one group) as five replications. The developmental stages of leaves were demarcated into two stages, green mature leaf and yellow senescent leaf. The green mature leaf was the third pair of developmentally matured leaves and did not show any sign of senescence. The yellow senescent leaf was collected by gently tapping the petiole. Only those senescent leaves that could be detached by this method were collected. Leaves damaged by insects and disease or mechanical factors were avoided. All samples were taken to the laboratory immediately after sampling, cleaned with distilled water, and then freeze dried using a desktop freeze-dryer at $-56\ ^\circ$C for 72 h. The freeze-dried leaves were ground finely and stored at $-20\ ^\circ$C prior to analysis.

2.3. Measurement of N and P Concentrations

The freeze-dried leaf samples were digested with sulfuric acid and hydrogen peroxide. The N concentration was determined based on the micro-Kjeldahl method described by Yoshida et al. [25], and the P concentration was evaluated according to the ascorbic acid-antimony reducing phosphate colorimetric method [26].

2.4. Determination of Total Phenolics (TPs), Extractable Condensed Tannins (ECT), Protein-Bound Condensed Tannins (PBCT), and Fiber-Bound Condensed Tannins (FBCT)

Established procedures demonstrated by Lin et al. [20] were used. TPs were measured with the Prussian blue method [27], and ECT, PBCT, and FBCT were determined by the butanol-HCl method [28] using purified tannins from *A. corniculatum* leaves as the standard. The concentration of total condensed tannins (TCT) was calculated by adding the respective quantities of ECT, PBCT, and FBCT [28].

2.5. Calculations

Resorption efficiency (RE) is the percentage of N or P recovered from the senescing leaves [29,30]. RE was calculated using the following equation: RE (%) = $(A_1 - A_2)/A_1 \times 100\%$. Where A_1 is N or P concentration in mature leaves, and A_2 is N or P concentration in senescent leaves.

Nutrient resorption proficiency referred to the minimum level to which a plant can reduce an element in senescing leaves. N and P resorption proficiency values were determined as absolute nutrient concentrations in senescent leaves [30]. Lower final nutrient concentrations correspond to higher proficiencies, which are usually expressed as percentages. This index's proxy seems to be more closely correlated than RE to the nutrient status of a population [31].

2.6. Statistical Analysis

All measurements were replicated five times. A one-way analysis of variance (ANOVA) was performed with season as the treatment factor. The Student-Newman-Keuls multiple comparison

method was used to test significant differences between any two seasons. All analyses were performed by SPSS13.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Seasonal Changes of N and P Concentrations, N:P Ratios, and Nutrient Resorption in Mature and Senescent Leaves of A. corniculatum

The N and P concentrations in mature leaves showed similar changes, with the highest concentrations in winter (17.94 ± 0.48 mg/g and 1.56 ± 0.05 mg/g) and the lowest in summer (9.27 ± 0.54 mg/g and 0.85 ± 0.04 mg/g), respectively. The N concentrations in senescent leaves ranged from 3.83 ± 0.15 mg/g to 4.77 ± 0.10 mg/g, with the lowest in spring and no significant differences among other three seasons. The P concentrations in senescent leaves were higher in summer (0.60 ± 0.04 mg/g) and autumn (0.59 ± 0.05 mg/g) than in winter (0.52 ± 0.03 mg/g) and spring (0.49 ± 0.03 mg/g) (Figure 1). Statistical results showed that the concentrations of N and P in mature leaves were significantly higher than those in senescent leaves in all seasons.
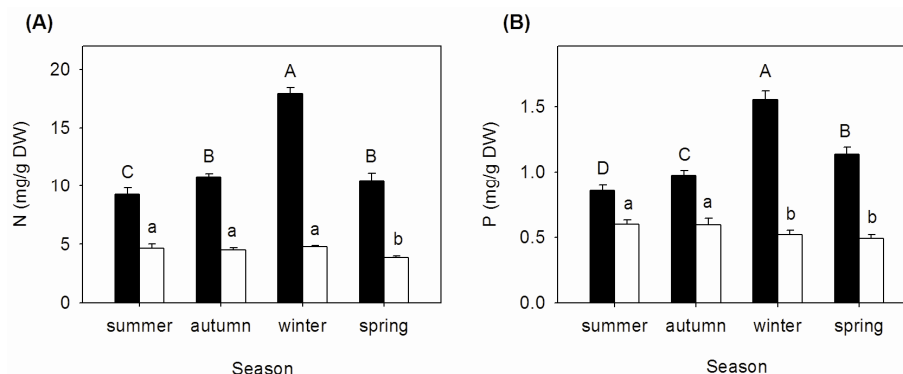
Figure 1. Seasonal changes in the (**A**) nitrogen (N) and (**B**) phosphorus (P) concentrations in leaves of *A. corniculatum*. Symbols are black bars for mature leaves and white bars for senescent leaves. Different capital letters indicate significant differences among seasons for N and P concentrations in mature leaves at $p < 0.05$. Different lowercase letters indicate significant differences among seasons for N and P concentrations in senescent leaves at $p < 0.05$.

The N:P ratios in mature leaves ranged from 9.23 ± 0.92 to 11.55 ± 0.55 with the lowest in spring. The nitrogen resorption efficiency (NRE) was significantly higher than phosphorus resorption efficiency (PRE) during leaf senescence in every season. The NRE and PRE followed the identical pattern, reaching the highest in winter (73.39% ± 1.06% and 66.51% ± 2.37%) and the lowest in summer (49.87% ± 4.39% and 30.01% ± 4.43%), respectively (Table 1).

The N concentrations in senescent leaves were all below 0.48%, and the P concentrations in senescent leaves were all above 0.05%, respectively (Table 1).

Table 1. Seasonal changes in the nitrogen and phosphorus ratios (N:P), nitrogen resorption efficiency (NRE), phosphorus resorption efficiency (PRE), nitrogen resorption proficiency (NRP), and phosphorus resorption proficiency (PRP) of *A. corniculatum* leaves.

Season	$N_m{:}P_m$	NRE (%)	PRE (%)	NRP (%)	PRP (%)
Summer	10.90 ± 1.13 a	49.87 ± 4.39 d	30.01 ± 4.43 d	0.46 ± 0.04 a	0.06 ± 0.00 a
Autumn	11.09 ± 0.41 a	58.18 ± 2.33 c	38.84 ± 5.94 c	0.45 ± 0.02 a	0.06 ± 0.00 a
Winter	11.55 ± 0.55 a	73.39 ± 1.06 a	66.51 ± 2.37 a	0.48 ± 0.01 a	0.05 ± 0.00 b
Spring	9.23 ± 0.92 b	63.36 ± 2.03 b	57.08 ± 3.48 b	0.38 ± 0.02 b	0.05 ± 0.00 b

N_m, P_m correspond to the N and P concentrations in mature leaves, respectively; Different letters in the same column indicate significant differences among seasons at $p < 0.05$.

There were significantly positive correlations between N and P concentrations in mature and senescent leaves. N concentrations in mature leaves and P concentrations in mature and senescent leaves were correlated with NRE and PRE, respectively. Meanwhile, the correlation between NRE and PRE was also highly significant (Table 2).

Table 2. Correlations between nitrogen (N) and phosphorus (P) concentrations, N and nitrogen resorption efficiency (NRE), P and phosphorus resorption efficiency (PRE), and NRE and PRE of *A. corniculatum* leaves.

Correlation	*F*	*R*	*p*
N_m-P_m	108.070	0.926	**<0.001**
N_s-P_s	5.765	0.493	**0.027**
N_m-NRE	55.731	0.869	**<0.001**
N_s-NRE	0.117	−0.080	0.737
P_m-PRE	91.214	0.914	**<0.001**
P_s-PRE	32.805	−0.804	**<0.001**
NRE-PRE	82.361	0.906	**<0.001**

N_m, P_m correspond to the N and P concentrations in mature leaves, respectively; N_s, P_s correspond to the N and P concentrations in senescent leaves, respectively. *p* values less than 0.05 are marked as bold numbers.

3.2. Seasonal Changes of Tannins Concentrations in Mature and Senescent Leaves of A. corniculatum

TPs concentrations in senescent leaves were higher than those in mature leaves. TPs concentrations reached the lowest in winter (210.19 ± 5.67 mg/g) for mature leaves. However, TPs concentrations in senescent leaves were the highest in spring (318.72 ± 12.66 mg/g) (Figure 2). TPs concentrations in mature and senescent leaves were all inversely correlated to N or P concentrations. A significant negative correlation between TPs concentrations in senescent leaves and N:P ratios in mature leaves was also observed (Table 3).
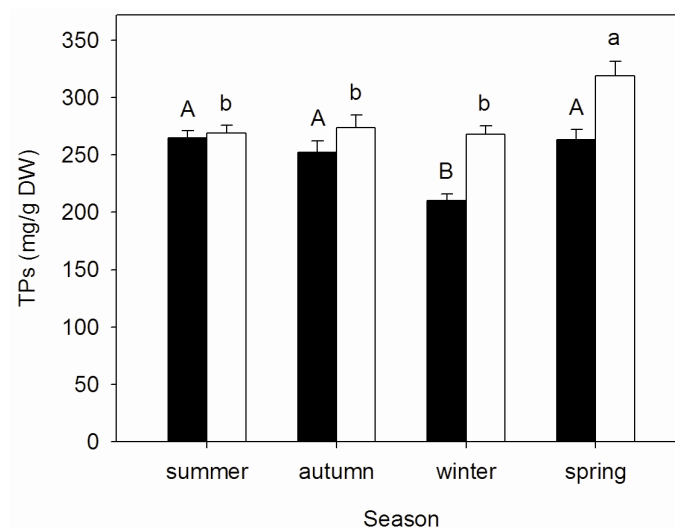
Figure 2. Seasonal changes in the total phenolics (TPs) concentrations in leaves of *A. corniculatum*. Symbols are black bars for mature leaves and white bars for senescent leaves. Different capital letters indicate significant differences among seasons for TPs concentrations in mature leaves at $p < 0.05$. Different lowercase letters indicate significant differences among seasons for TPs concentrations in senescent leaves at $p < 0.05$.

Table 3. Correlations between total phenolics (TPs) and nutrient concentrations (N and P), and N:P ratios of *A. corniculatum* leaves.

Correlation	*F*	*R*	*p*
TPs_m-N_m	122.338	−0.934	**<0.001**
TPs_s-N_s	34.399	−0.810	**<0.001**
TPs_m-P_m	56.098	−0.870	**<0.001**
TPs_s-P_s	11.631	−0.627	**0.003**
TPs_m-N_m:P_m	3.940	−0.424	0.063
TPs_s-N_m:P_m	12.143	−0.635	**0.003**

N_m, P_m correspond to the N and P concentrations in mature leaves, respectively; N_s, P_s correspond to the N and P concentrations in senescent leaves, respectively; TPs_m, TPs_s correspond to the TPs concentrations in mature and senescent leaves, respectively. *p* values less than 0.05 are marked as bold numbers.

ECT concentrations in mature leaves ranged from 190.77 ± 17.21 mg/g to 230.18 ± 33.10 mg/g, and remained relatively stable between the seasons. ECT concentrations in senescent leaves were significantly higher than those in mature leaves in winter and spring (Figure 3A).

PBCT in mature and senescent leaves were both higher in summer and winter than those in autumn and spring (Figure 3B). However, FBCT concentrations did not significantly change during leaf senescence, except in spring, and FBCT in mature leaves was the highest (1.56 ± 0.17 mg/g) in autumn (Figure 3C).

TCT concentrations had a similar changing trend to that of ECT concentrations during leaf senescence through the seasons (Figure 3D).
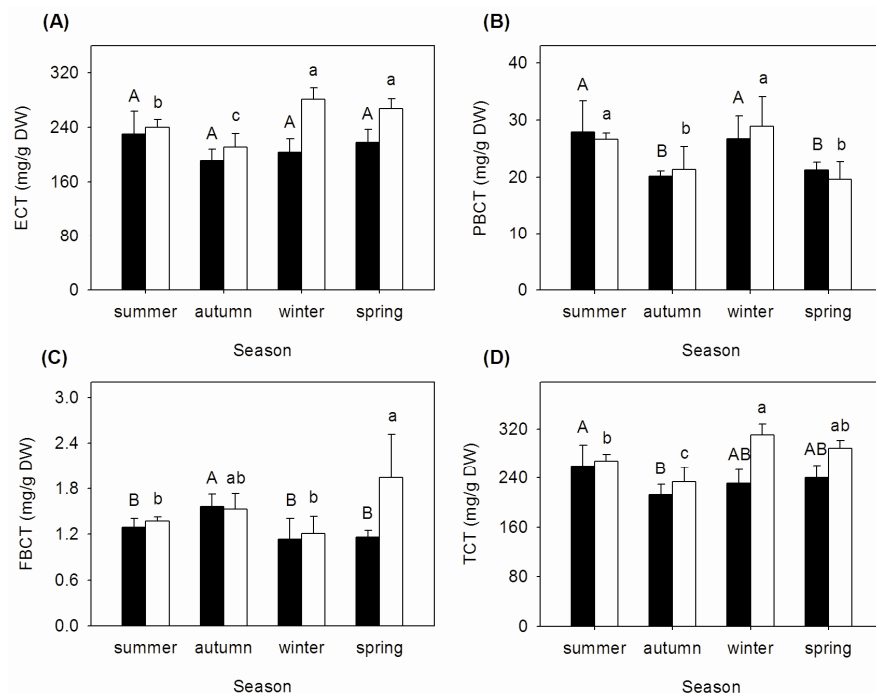

Figure 3. Seasonal changes in the concentrations of (**A**) extractable condensed tannins (ECT); (**B**) protein-bound condensed tannins (PBCT); (**C**) fiber-bound condensed tannins (FBCT); and (**D**) total condensed tannins (TCT) in leaves of *A. corniculatum*. Symbols are black bars for mature leaves and white bars for senescent leaves. Different capital letters indicate significant differences among seasons for ECT, PBCT, FBCT, and TCT concentrations in mature leaves at *p* < 0.05. Different lowercase letters indicate significant differences among seasons for ECT, PBCT, FBCT and TCT concentrations in senescent leaves at *p* < 0.05.

3.3. Seasonal Changes of TPs:N and TCT:N Ratios in Mature and Senescent Leaves of A. corniculatum

Seasonal changes of TPs:N and TCT:N ratios during leaf senescence are shown in Figure 4. TPs:N and TCT:N ratios in senescent leaves were significantly higher than those in mature leaves for all seasons. TPs:N and TCT:N ratios in mature leaves reached the highest in summer and the lowest in winter, while in senescent leaves they were higher in spring than in the other three seasons.
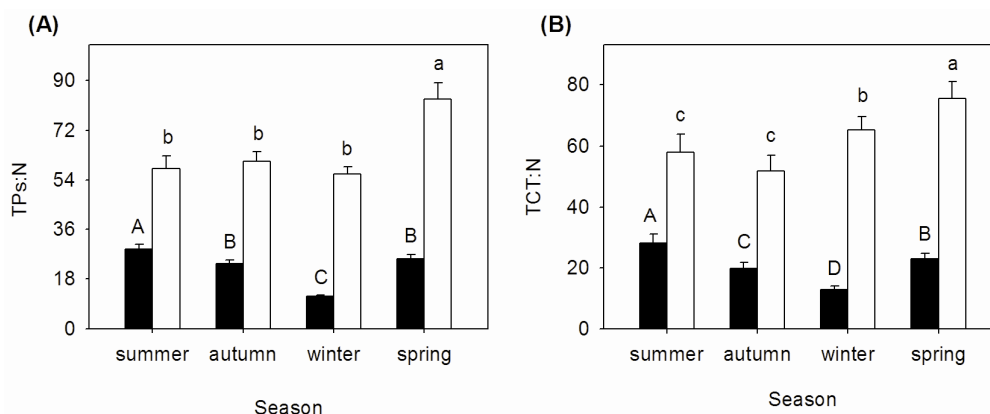
Figure 4. Seasonal changes of (**A**) total phenolics and nitrogen ratios (TPs:N) and (**B**) total condensed tannins and N ratios (TCT:N) in leaves of *A. corniculatum*. Symbols are black bars for mature leaves and white bars for senescent leaves. Different capital letters indicate significant differences among seasons for TPs:N and TCT:N ratios in mature leaves at *p* < 0.05. Different lowercase letters indicate significant differences among seasons for TPs:N and TCT:N ratios in senescent leaves at *p* < 0.05.

4. Discussion

N and P are the most common nutrients limiting plant growth [32]. The N and P concentrations in mature leaves of *A. corniculatum* followed a similar seasonal pattern, with the highest concentrations in winter and the lowest in summer (Figure 1). This was in agreement with the observations made in a study by Wang et al. [6], which showed higher N and P concentrations in cold seasons and lower concentrations in warm seasons for mature leaves. Similarly, Aerts et al. [33] also suggested that summer warming reduced N concentrations of mature and senescent leaves in *Rubus*. First, a portion of N and P were allocated to other plant parts (e.g., roots and fruits). Second, N and P concentrations were diluted by leaf mass accumulation during summer when *A. corniculatum* grew rapidly.

Nutrient resorption during leaf senescence serves to reduce the plant's dependence on current nutrient supply, and has been recognized as one of the most important strategies used by plants to conserve nutrients [8,9,34,35]. It has been estimated that approximately 50% of the nutrients (N and P) are resorbed during leaf senescence [29,36]. In the present study, the averaged NRE and PRE values were 61.20% ± 9.10% and 48.11% ± 15.31%, respectively (Table 1). These high N and P resorption values indicated that internal cycling of N and P can supply a significant fraction of the required nutrients for *A. corniculatum* growth. In addition, both NRE and PRE values were the highest in winter and the lowest in summer. This may indicate that *A. corniculatum* mainly resorbed N and P from senescent leaves in winter, but absorbed N and P from the soil in summer to adapt to low nutrient environments.

The N:P ratios of mature leaves are in response to natural variations in N and P supply, reflecting variation in N and P concentrations or both together [16]. A review of 40 fertilization studies revealed that an N:P ratio > 16 indicates P limitation on a community level, while an N:P ratio < 14 is indicative of N limitation [37]. As critical N:P ratios are successfully used to establish the nature of nutrient limitation in aquatic and agricultural ecosystems, the general concept may be applicable to a much wider range of ecosystems. N:P ratios in mature leaves of *A. corniculatum* in the Zhangjiang river estuary ranged from 9.23 to 11.55, and the lowest N:P ratio occurred in spring (Table 1). These results, both less than 14,

suggested the presence of strong community level N limitation in this forest. Accordingly, N-limited *A. corniculatum* forest had significantly higher NRE than PRE. This result supported the hypothesis that a strong nutrient limitation will show higher resorption of the limiting nutrient.

Killingbeck [30] modified the concepts of nutrient resorption efficiency and proposed the use of "resorption proficiency", which is the level to which nutrient concentrations are reduced in senescent leaves. One of the important features of knowing the levels to which N and P can be reduced in senescent leaves is that these values offer an objective gauge by which resorption can be measured [30]. The potential or ultimate resorption proficiency, the lowest levels to which a nutrient is reduced in senescent leaves, is a reflection of biochemical limits to resorption and, unlike efficiency, is not subject to the temporal variations in nutrient concentrations present in green leaves and the timing of sampling [30,38]. For these reasons, resorption proficiency has been argued to be the more definitive measure of resorption success than efficiency [39]. By analyzing N and P levels in the senescent leaves of 89 species of deciduous and evergreen woody perennials, Killingbeck [30] proposed that in evergreen species, nutrient concentrations reduced below 0.7% N and 0.04% P or above 1.0% N and 0.05% P in senescent leaves are believed to represent complete or incomplete N and P resorption, respectively. In the present study, N concentrations in senescent leaves ranging from 0.38% to 0.48% were indicative of complete resorption of N; P concentrations in senescent leaves were all above 0.05%, reflecting that P resorption was incomplete (Table 1). Alongi et al. [5] argued that mangrove soils are generally low in nutrient concentrations, especially N. Our results suggested that there is greater need to resorb N from senescent leaves in N-limited mangrove forests. All the same, NRP and PRP values were lowest in spring when *A. corniculatum* grew rapidly. This is in agreement with the findings by Wei et al. [9], which suggested that lower N and P concentrations of senescent leaves in growing seasons in comparison to other seasons make it difficult to resorb additional nutrients.

N and P concentrations correlated with each other in mature and senescent leaves (Table 2). Chapin and Kedrowski [40] found a direct correlation between proportional nutrient retranslocation from the leaves during senescence and nutrient concentration in tree leaves. The significant positive correlation between N concentrations in mature leaves and NRE was consistent with the findings of Chapin and Kedrowski [40]. The significant negative correlation between P concentrations in senescent leaves and PRE was consistent with results reported in a previous study [8]. NRE had a significant positive correlation with PRE. This correlation suggested that N resorption may be controlled by biochemical processes similar to those that control P resorption, in accordance with the results of studies of wood species in tropical forests [41,42] and wetland graminoids [43]. However, other studies have found no significant correlations between the N and P resorption efficiencies of certain plants [40].

Traditionally, justification for the high metabolic cost associated with the production of tannins is attributed to improved herbivore defense [44]. In mangrove species, tannin is an abundant component as high as 20% dry weight which prevents damage from herbivory [17]. *A. corniculatum* leaves had high total phenolics and total condensed tannin levels (both above 20%), which increased during leaf senescence (Figures 2 and 3). The observed changes were in agreement with the findings reported for another mangrove species, *Rhizophora stylosa* [8]. As polyphenols are water-soluble and susceptible to leaching [45], leaching of polyphenols or tannins from green leaves by sporadic rain might be a cause for the net enrichment in senescent leaves [46]. However, Mafongoya et al. [47] indicated that much of the soluble carbon compounds, including polyphenols, are expected to be translocated from leaves during senescence. Constantinides and Fownes [48] found both an increase and a decrease in concentrations of polyphenols with the development and senescence of leaves, while Kuhajek et al. [49] observed no significant effects of leaf age on both condensed tannins and total phenolics. As for bound condensed tannins, only the FBCT concentrations in spring were significantly higher in senescent leaves than in mature leaves. These results were not exactly the same as the findings reported by Zhang et al. [34], which indicated that PBCT concentrations are significantly higher in senescent leaves than in mature leaves in all seasons.

The TPs concentrations in mature leaves were lowest in winter, and in senescent leaves concentrations were highest in spring (Figure 2). The TPs concentrations in mature and senescent leaves were all inversely related to N or P concentrations (Table 3). It is common to find a negative correlation between N and secondary compound concentrations, such as phenolics and tannins [50]. This pattern lends support to source-sink hypotheses, such as the carbon-nutrient balance (CNB) hypothesis and the growth-differentiation (GDB) hypothesis that predict increased C allocation to secondary C compounds under low nutrient conditions. Under nutrient limited circumstances, the diversion of C to secondary compounds may be essentially cost-free with respect to growth or reproduction [51]. Kandil et al. [52] suggested that the production and accumulation of the polyphenolics is favored in the high light, high temperature, high salinity, and low nutrient environment in which the mangroves occur, particularly when carbon used for growth or reproduction is precluded by lack of nutrient resources.

TPs:N and TCT:N ratios in senescent leaves were significantly higher than those in mature leaves (Figure 4). Condensed tannins are considered to have an important impact for N immobilization in soils, in particular during fall when fresh litter materials are introduced into soils [53]. Through both litter deposition and foliar leaching, soils in tannin rich plant communities receive appreciable tannin inputs. Because high tannin concentrations in plants are often associated with infertile site conditions [22], it has been suggested that there is an evolutionary advantage to higher tannin production. By reducing decomposition rates and decreasing N leaching potential, tannins may provide a nutrient conservation mechanism [8,54]. Therefore, parameters such as TPs:N and TCT:N ratios may be the best predictors of litter quality [55]. In the present study, higher TPs:N and TCT:N ratios in senescent leaves may slow down the rate of litter decomposition, thereby increasing the nutrient use efficiency and may be, in part, an adaptation to nutrient limited conditions.

In conclusion, N:P ratios of mature leaves are a good indicator for nutrient limitation in mangrove ecosystems. The results confirmed that resorption efficiency during leaf senescence depends on the type of nutrient limitation, and NRE was much higher than PRE in N-limited habitat. The resorption proficiency of N and P indicated that N resorption was complete and P resorption was incomplete. *A. corniculatum* leaves contained high total phenolics, extractable condensed tannin, and total condensed tannin levels. TPs:N and TCT:N ratios in senescent leaves were significantly higher than those in mature leaves. Our results indicated that high NRE during leaf senescence and high TPs:N and TCT:N ratios in senescent leaves might be important nutrient conservation strategies for the mangrove shrub *A. corniculatum* forest growing under the N-limited conditions. Furthermore, it has also been previously reported that mangroves are highly plastic in their responses to multiple abiotic and biotic factors such as destructive weather, herbivory, etc. As such, further work is required for understanding a more thorough temporal variation of nutrient resorption and phenolics concentration for *A. corniculatum* in long-term observations.

Author Contributions: Hui Chen, Benbo Xu, Shudong Wei, and Haichao Zhou designed and performed the experiments; Hui Chen, Shudong Wei and Lihua Zhang analyzed the data; Benbo Xu and Yiming Lin contributed reagents/materials/analysis tools; Hui Chen wrote the paper; Shudong Wei and Lihua Zhang revised the paper and modified the language.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Twilley, R.W.; Lugo, A.E.; Patterson-Zucca, C. Litter production and turnover in basin mangrove forests in southwest Florida. *Ecology* **1986**, *67*, 670–683. [CrossRef]

2. Tomlinson, P.B. *The Botany of Mangroves*; Cambridge University Press: New York, NY, USA, 1986.

3. Li, M. Nutrient dynamics of a Futian mangrove forest in Shenzhen, South China. *Estuar. Coast. Shelf. Sci.* **1997**, *45*, 463–472. [CrossRef]

4. Odum, W.E.; Heald, E.J. The detritus-based food web of an estuarine mangrove community. In *Estuarine Research*; Cronin, L.E., Ed.; Academic Press: New York, NY, USA, 1975; pp. 265–286.

5. Alongi, D.M.; Boto, K.G.; Robertson, A.I. Nitrogen and phosphorus cycles. In *Tropical Mangrove Ecosystems*; Robertson, A.I., Alongi, D.M., Eds.; American Geographysical Union: Washington, DC, USA, 1992; pp. 251–292.

6. Wang, W.Q.; Wang, M.; Lin, P. Seasonal changes in element contents in mangrove element retranslocation during leaf senescence. *Plant Soil* **2003**, *252*, 187–193. [CrossRef]

7. Cai, G. Salt content and regulation of mangroves. *Ecol. Sci.* **1990**, *2*, 123–127.

8. Lin, Y.M.; Liu, X.W.; Zhang, H.; Fan, H.Q.; Lin, G.H. Nutrient conservation strategies of a mangrove species *Rhizophora stylosa* under nutrient limitation. *Plant Soil* **2010**, *326*, 469–479. [CrossRef]

9. Wei, S.D.; Liu, X.W.; Zhang, L.H.; Chen, H.; Zhang, H.; Zhou, H.C.; Lin, Y.M. Seasonal changes of nutrient levels and nutrient resorption in *Avicennia marina* leaves in Yingluo Bay, China. *South. For.* **2015**, *77*, 237–242.

10. Millard, P.; Neilsen, G.H. The influence of nitrogen supply on the uptake and remobilization of stored N for the seasonal growth of apple trees. *Ann. Bot.* **1989**, *63*, 301–309.

11. Lin, Y.; Sternberg, L.D.L. Nitrogen and phosphorus dynamics and nutrient resorption of *Rhizophora mangle* leaves in south Florida, USA. *Bull. Mar. Sci.* **2007**, *80*, 159–169.

12. Pugnaire, F.I.; Chapin, F.S. Controls over nutrient resorption from leaves of evergreen Mediterranean species. *Ecology* **1993**, *74*, 124–129. [CrossRef]

13. Feller, I.C. Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora mangle*). *Ecol. Monogr.* **1995**, *65*, 477–505. [CrossRef]

14. Reef, R.; Feller, I.C.; Lovelock, C.E. Nutrition of mangroves. *Tree Physiol.* **2010**, *30*, 1148–1160. [CrossRef] [PubMed]

15. McKee, K.L.; Feller, I.C.; Popp, M.; Wanek, W. Mangrove isotopic (δ^{15}N and δ^{13}C) fractionation across a nitrogen vs. phosphorus limitation gradient. *Ecology* **2002**, *83*, 1065–1075. [CrossRef]

16. Güsewell, S. N:P ratios in terrestrial plants: Variation and functional significance. *New Phytol.* **2004**, *164*, 243–266. [CrossRef]

17. Hernes, P.J.; Benner, R.; Cowie, G.L.; Goni, M.A.; Bergamaschi, B.A.; Hedges, J.I. Tannin diagenesis in mangrove leaves from a tropical estuary: A novel molecular approach. *Geochim. Cosmochim. Acta* **2001**, *65*, 3109–3122. [CrossRef]

18. Zucker, W.V. Tannins: Does structure determine function? An ecological perspective. *Am. Nat.* **1983**, *121*, 335–365. [CrossRef]

19. Lin, Y.M.; Liu, J.W.; Xiang, P.; Lin, P.; Ding, Z.H.; Sternberg, L.D.L. Tannins and nitrogen dynamics in mangrove leaves at different age and decay stages (Jiulong River Estuary, China). *Hydrobiologia* **2007**, *583*, 285–295. [CrossRef]

20. Lin, Y.M.; Liu, J.W.; Xiang, P.; Lin, P.; Ye, G.; Sternberg, L.D.L. Tannin dynamics of propagules and leaves of *Kandelia candel* and *Bruguiera gymnorrhiza* in the Jiulong River Estuary, Fujian, China. *Biogeochemistry* **2006**, *78*, 343–359. [CrossRef]

21. Serrano, L. Leaching from vegetation of soluble polyphenolic compounds, and their abundance in temporary ponds in the Doñana National Park (SW Spain). *Hydrobiologia* **1992**, *229*, 43–50. [CrossRef]

22. Northup, R.R.; Yu, Z.; Dahlgren, R.A.; Vogt, K.A. Polyphenol control of nitrogen release from pine litter. *Nature* **1995**, *377*, 227–229. [CrossRef]

23. Huang, G.M. Growth characteristics of *Spartina alterniflora* and its relative competitive ability with *Kandelia candle* in mangrove areas of the Zhangjiang estuary. Master's Thesis, Xiamen University, Xiamen, China, 2009.

24. Chen, H.; Lu, W.; Yan, G.; Yang, S.; Lin, G. Typhoons exert significant but differential impacts on net ecosystem carbon exchange of subtropical mangrove forests in China. *Biogeosciences* **2014**, *11*, 5323–5333. [CrossRef]

25. Yoshida, S.; Forno, D.A.; Cock, J.H.; Gomez, K.A. *Laboratory Manual for Physiological Studies of Rice*, 2nd ed.; The International Rice Research Institute: Manila, Philippines, 1972; pp. 7–9, 36–38.

26. Nanjing Institute of Soil Science. *Physical and Chemical Analysis of Soils*; Science and Technology Press: Shanghai, China, 1978; pp. 1–320.

27. Graham, H.D. Stabilization of the Prussian blue color in the determination of polyphenols. *J. Agric. Food Chem.* **1992**, *40*, 801–805. [CrossRef]

28. Terrill, T.H.; Rowan, A.M.; Douglas, G.B.; Barry, T.N. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. *J. Sci. Food Agric.* **1992**, *58*, 321–329. [CrossRef]

29. Aerts, R. Nutrient resorption from senescing leaves of perennials: Are there general patterns? *J. Ecol.* **1996**, *84*, 597–608. [CrossRef]

30. Killingbeck, K.T. Nutrients in senesced leaves: Keys to the search for potential resorption and resorption proficiency. *Ecology* **1996**, *77*, 1716–1727. [CrossRef]

31. Rejmánková, E. Nutrient resorption in wetland macrophytes: Comparison across several regions of different nutrient status. *New Phytol.* **2005**, *167*, 471–482. [CrossRef] [PubMed]

32. Lambers, H.; Chapin, F.S.; Pons, T.L. *Plant Physiological Ecology*; Springer: New York, NY, USA, 1998.

33. Aerts, R.J.; Barry, T.N.; McNabb, W.C. Polyphenols and agriculture: Beneficial effects of proanthocyanidins forages. *Agr. Ecosyst. Environ.* **1999**, *75*, 1–12. [CrossRef]

34. Zhang, L.H.; Ye, G.F.; Lin, Y.M.; Zhou, H.C.; Zeng, Q. Seasonal changes in tannin and nitrogen contents of *Casuarina equisetifolia* branchlets. *J. Zhejiang Uni. Sci. B* **2009**, *10*, 103–111. [CrossRef] [PubMed]

35. Ye, G.F.; Zhang, S.J.; Zhang, L.H.; Lin, Y.M.; Wei, S.D.; Liao, M.M.; Lin, G.H. Age-related changes in nutrient resorption patterns and tannin concentration of *Casuarina equisetifolia* plantations. *J. Trop. For. Sci.* **2012**, *24*, 546–556.

36. Aerts, R.; Chapin, F.S. The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. *Adv. Ecol. Res.* **2000**, *30*, 1–67.

37. Koerselman, W.; Meuleman, A.F.M. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. *J. Appl. Ecol.* **1996**, *33*, 1441–1450. [CrossRef]

38. Kobe, R.K.; Lepczyk, C.A.; Iyer, M. Resorption efficiency decreases with increasing green leaf nutrients in a global data set. *Ecology* **2005**, *86*, 2780–2792. [CrossRef]

39. Wright, I.J.; Westoby, M. Nutrient concentration, resorption and lifespan: Leaf traits of Australian sclerophyll species. *Funct. Ecol.* **2003**, *17*, 10–19. [CrossRef]

40. Chapin, F.S.; Kedrowski, R.A. Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees. *Ecology* **1983**, *64*, 376–391. [CrossRef]

41. Lal, C.B.; Annapurna, C.; Raghubanshi, A.S.; Singh, J.S. Effect of leaf habit and soil type on nutrient resorption and conservation in woody species of a dry tropical environment. *Can. J. Bot.* **2001**, *79*, 1066–1075.

42. Cai, Z.; Bongers, F. Contrasting nitrogen and phosphorus resorption efficiencies in trees and lianas from a tropical montane rain forest in Xishuangbanna, south-west China. *J. Trop. Ecol.* **2007**, *23*, 115–118. [CrossRef]

43. Güsewell, S. Nutrient resorption of wetland graminoids is related to the type of nutrient limitation. *Funct. Ecol.* **2005**, *19*, 344–354. [CrossRef]

44. Feeny, P. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* **1970**, *51*, 565–581. [CrossRef]

45. Hättenschwiler, S.; Vitousek, P.M. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol. Evol.* **2000**, *15*, 238–243. [CrossRef]

46. Teklay, T. Seasonal dynamics in the concentrations of macronutrients and organic constituents in green and senesced leaves of three agroforestry species in southern Ethiopia. *Plant Soil* **2004**, *267*, 297–307. [CrossRef]

47. Mafongoya, P.L.; Giller, K.E.; Palm, C.A. Decomposition and nitrogen release patterns of tree prunings and litter. *Agrofor. Syst.* **1998**, *38*, 77–97. [CrossRef]

48. Constantinides, M.; Fownes, J.H. Nitrogen mineralization from leaves and litter of tropical plants: Relationship to nitrogen, lignin and soluble polyphenol concentrations. *Soil Biol. Biochem.* **1994**, *26*, 49–55. [CrossRef]

49. Kuhajek, J.M.; Payton, I.J.; Monks, A. The impact of defoliation on the foliar chemistry of southern rata (*Metrosideros umbellata*). *N. Z. J. Ecol.* **2006**, *30*, 237–249.

50. Mansfield, J.L.; Curtis, P.S.; Zak, D.R.; Pregitzer, K.S. Genotypic variation for condensed tannin production in trembling aspen (*Populus tremuloides*, Salicaceae) under elevated CO_2 and in high-and low-fertility soil. *Am. J. Bot.* **1999**, *86*, 1154–1159. [CrossRef] [PubMed]

51. Bryant, J.P.; Chapin, F.S., III; Klein, D.R. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* **1983**, *40*, 357–368. [CrossRef]

52. Kandil, F.E.; Grace, M.H.; Seigler, D.S.; Cheeseman, J.M. Polyphenolics in *Rhizophora mangle* L. leaves and their changes during leaf development and senescence. *Trees* **2004**, *18*, 518–528. [CrossRef]

53. Maie, N.; Behrens, A.; Knicker, H.; Kogel-Knabner, I. Changes in the structure and protein binding ability of condensed tannins during decomposition of fresh needles and leaves. *Soil Biol. Biochem.* **2003**, *35*, 577–589. [CrossRef]

54. Kraus, T.E.C.; Dahlgren, R.A.; Zasoski, R.J. Tannins in nutrient dynamics of forest ecosystems—A review. *Plant Soil* **2003**, *256*, 41–66. [CrossRef]

55. Kraus, T.E.C.; Zasoski, R.J.; Dahlgren, R.A. Fertility and pH effects on polyphenol and condensed tannin concentrations in foliage and roots. *Plant Soil* **2004**, *262*, 95–109. [CrossRef]