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Tannin dynamics of propagules and leaves of *Kandelia candel* and *Bruguiera gymnorrhiza* in the Jiulong River Estuary, Fujian, China

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Abstract. Changes in the total phenolics, condensed tannins (CT), protein-precipitable phenolics content and protein precipitation capacity were determined on a series of mangrove leaves from two true viviparous mangrove species (*Kandelia candel* and *Bruguiera gymnorrhiza*) at various stages of development and decomposition in the Jiulong River Estuary, Fujian, China. Similar measurements were also done for the propagules at different developmental stages. The results showed that the total phenolics, extractable condensed tannins, total condensed tannins, protein-precipitable phenolics content and protein precipitation capacity in young leaves were higher than those in mature and senescent leaves. Tannin dynamics during leaf decomposition varied with species, and the rapid loss of phenolics observed during decomposition can be ascribed to leaching and degradation. Protein-bound CT and fibre-bound CT tended to increase with leaf decomposition, with CT binding more strongly to protein than to fibre. Protein-bound CT was higher than fibre-bound CT with the exception of mature leaves. Total phenolics, extractable CT and protein-precipitable phenolics contents in flower tissues were relatively lower than those in hypocotyls at different developmental stages. Protein precipitation capacity fluctuated with the development of propagules. Increases in nitrogen in decaying litter, and declines in contents of total phenolics and total condensed tannins of detritus support the general conclusion that decomposing mangrove detritus can be a more palatable heterotrophic substrate than living leaves.

Introduction

Phenolic compounds, including tannins, are a significant component of plant secondary metabolites. Tannins occur in plant leaves, roots, wood, bark, fruits and buds (Kraus et al. 2003), and are estimated to be the fourth most abundant biochemical substance produced by vascular plant tissue after cellulose, hemicellulose and lignin (Hernes and Hedges 2000). Tannin in vascular plants occurs as two types, the condensed and the hydrolysable (Hernes et al. 2001). The condensed tannins are now commonly referred to as proanthocyanidins or more broadly, as polymeric flavanoids. More complete reviews of the structure

and chemistry of proanthocyanidins (PA) can be found elsewhere (Hemingway and Karchesy 1989; Hemingway and Laks 1992). The reactivity of condensed tannins with proteins and formation of complexes has important nutritional and physiological consequences (Naczk et al. 1996; Hagerman et al. 1998). Proanthocyanidins may form complexes with proteins in the soil and influence N release (Howard and Howard 1993; Hernes et al. 2001; Nagamitsu et al. 2003), form complexes with various enzymes (Chung et al. 1998; Bruyne et al. 1999), lead to antimicrobial and antiviral properties (Scalbert 1992; Chung et al. 1993, 1995a, b; Chang et al. 1994), retard plant germination, growth and yield (Rawat et al. 1998), and interfere with the domestic animal's digestion and/or absorption (Chung et al. 1998).

In mangrove species, tannin is an abundant component as high as 20% dry weight which prevents damage from herbivory (Hernes et al. 2001). In addition to having a biomarker potential, tannin greatly contributes to the properties of bulk organic matter including color, astringency and reactivity. Nitrogen immobilized in leaf materials entering aquatic environments is commonly observed but poorly understood. Although most investigators advocate a microbial source for N fixation, direct evidence for this origin has not been given. Lee et al. (1980) indicated that microbial biomass accounted for only a few percent of N that accumulated in decaying tissues. Hernes et al. (2001) hypothesized that tannin–nitrogen interaction was very important in terms of early tannin diagenesis and nitrogen immobilization. Here we test the above hypothesis by examining the dynamics of tannin and nitrogen in two abundant mangrove species in a South China coastline site: *Kandelia candel* and *Bruguiera gymnorrhiza*.

Mangrove species in Rhizophoraceae such as *K. candel* and *B. gymnorrhiza* are viviparous. Normal sexual reproduction produces embryos that have no dormant period and grow out of seed and fruit coat while still attached to the parent trees (Tomlinson 1986), forming stick- or spindle-like viviparous seedlings (hypocotyls). Mature hypocotyls drop off and float in seawater until they anchor in the sediment and their roots sprout and penetrate into the substrate. It is hypothesized that adaptations of hypocotyls to saline conditions form when they are still attached by their absorption of salt from parent trees (Lin 1999). However, the dynamics of important organic compounds, such as tannin, is not known.

The present investigation aims to measure total phenolics, extractable condensed tannins, protein-bound CT, fibre-bound CT, protein-precipitable phenolics and protein precipitation capacity dynamics during the development of leaves and propagules of *K. candel* and *B. gymnorrhiza* in the Jiulong River Estuary, Fujian, China. In addition, tannin dynamics in relation to nitrogen content at various stages of decomposition of leaves were measured and discussed. These results increase our understanding of tannin–nitrogen dynamics of mangroves in the coastal food chain and in particular on two species of true viviparous mangrove species.

Materials and methods

Materials

All samples of *K. candel* and *B. gymnorrhiza* were collected from a mangrove forest in the Jiulong River Estuary (24°24′ N, 117°55′ E), Fujian, China. The climate of this region is typically a southern subtropical marine climate (Figure 1). *K. candel* and *B. gymnorrhiza* belong to Rhizophoraceae, having large leaves ranging from 6 to 22 cm long. The flowers are solitary and a few cm in length. Both species are viviparous and the elongated propagules for both species can be as long as 23 cm long. *K. candel* trees were artificially planted in 1960s and the community characteristics were described in a previous report (see Lin and Chen 1986). *B. gymnorrhiza* was introduced into this forest from Hainan Island in 1980s. Mean height and base diameter of *B. gymnorrhiza* plants were 2.5 m and 8 cm, respectively. Average salinities of soil and seawater were 13.4‰ and 18.2‰, respectively.

Twenty trees of each species similar in height and living conditions were chosen and two branches of each tree were randomly selected from the upper crown for sampling leaves and propagules (including flower and hypocotyls). The different development stages of hypocotyls were demarcated and shown in Table 1, with the last phase (V) being fully mature hypocotyls.

The development stages of leaves were demarcated into three stages, i.e. young leaf (the first pair of immature leaves), mature leaf (the third pair of developmentally matured leaves) and yellow senescent leaf (turning yellow from senescence). Leaves damaged by insects or mechanical factors were avoided. Leaves showing various degrees of degradation, as evidenced by their colors of yellow, orange, brown, and black, were collected from the water and surficial sediment of the Jiulong River Estuary. The sampling numbers of each component were 50∼100 for each species, all samples were taken to the laboratory immediately after sampling and cleaned with distilled water.

Methods

All chemicals were of Analytical Reagent (AR) purity grade. Tannic acid standard was obtained from Sigma. Sephadex LH-20 was purchased from Amersham. An additional standard denoted here as purified tannin, was extracted from *K. candel* and *B. gymnorrhiza* and purified on Sephadex LH-20 according to the procedure previously described by Hagerman (2002). The CT standard was freeze-dried and stored at −20 °C until required.

Extractable condensed tannins (ECT)

Duplicate 100 mg fresh materials were weighed and ground with 7:3 (V) acetone/water solution at 5 °C, a small aliquot (1) from this crude extract was

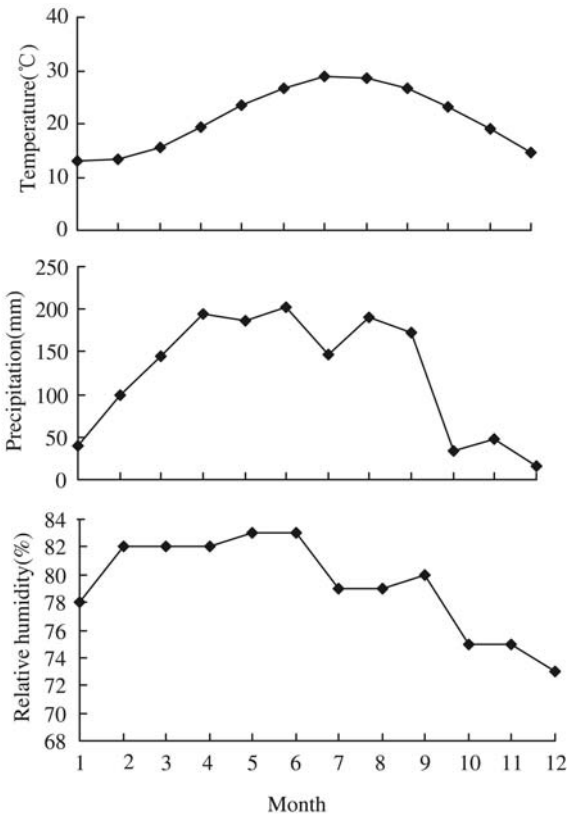

Figure 1. Temperature, precipitation and relative humidity in the Jiulong River Estuary throughout the year (monthly average from 1980 to 1992).

saved and the rest transferred into 5 ml screw-top polyallomer centrifuge tubes and centrifuged (Figure 2). The supernatant was decanted from the pellet and saved, the pellet was reextracted two times with 5 ml 7:3 (V) acetone/water solution. The supernatants were combined to a 50 ml conical flask. After removing a 5 ml of the pooled extracts for total phenolics analysis, the remainder of the extract was adjusted to 0.001 M ascorbic acid to minimize

Table 1. Division of the developmental stages of hypocotyls of *K. candel* and *B. gymnorrhiza* (in cm).

Developmental stage	*K. candel*	*B. gymnorrhiza*
I	0–5	0–4
II	5–10	4–7
III	10–15	7–10
IV	15–20	10–13
V	> 20	> 13

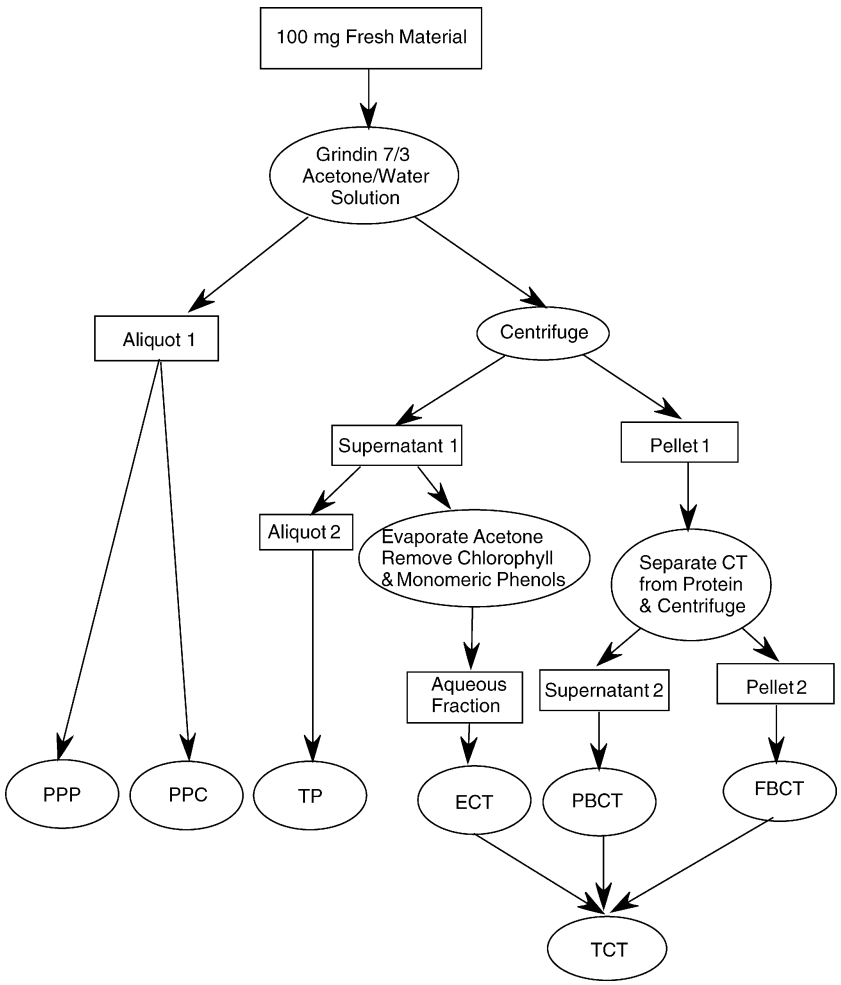

Figure 2. Flow chart for analysis of various forms of tannins in the mangroves.

oxidation (Schofield et al. 1998). The acetone was evaporated with a N_2 stream and the water fraction was purified by liquid/liquid extraction with EtOAc three times in order to remove chlorophyll and monomer phenols. The aqueous fractions were further analyzed by butanol/HCl assay (Terrill et al. 1992).

Protein-bound condensed tannin (PBCT)

To extract protein-bound CT as Terrill et al.(1992) described, 15 ml SDS solution (10 g l^{-1} SDS and 50 g l^{-1} 2-mercaptoethanol in 10 mM Tris/chloride buffer, pH 8.0) was added to pellet 1 from the above centrifugation (Figure 2). The tubes were shaken on a vortex mixer at 100 °C for 45 min., after which

they were cooled to room temperature, centrifuged at 5000 *g* for 15 min., and the supernatant was poured into another 50 ml conical flask. The pellet was reextracted three times and the supernatants combined. Protein-bound condensed tannins were assayed by the butanol/HCl method (Terrill et al. 1992) and purified *K. candel* and *B. gymnorrhiza* leaf tannin as the standard.

Fibre-bound condensed tannin (FBCT)

Fibre-bound CT was determined directly on the residue remaining from the extraction of protein-bound CT (pellet 2). The pellet was washed into a 50 ml glass centrifuge tube with 3.0 ml SDS solution, 21.0 ml butanol/HCl solution was added and maintained at 100 °C for 75 min. Tubes were cooled on ice, centrifuged at 5000 *g* for 15 min., and the absorbance at 550 nm was read on a UV-9200 Spectrophotometer.

Total condensed tannin (TCT)

Total condensed tannin was calculated by adding the respective quantities of extractable CT, protein-bound CT and fibre-bound CT.

Total phenolics (TP)

Total phenolics in the 5 ml aliquot (2) from the supernatant were measured with the Prussian blue method (Graham 1992), using purified tannin and tannic acid as the standards.

Protein-precipitable phenolics (PPP)

One milliliter of crude extract aliquot 1 was added to 3.0 ml of the standard protein solution (1.0 mg BSA ml^{-1}) in a 15 ml glass centrifuge tube for the quantitative determination of protein-precipitable phenolics. The solutions were mixed and allowed to stand at room temperature for about 15 min and then centrifuged for 15 min at 12,000 *g*. The supernatant was discarded, and the surface of the pellet and the walls of the tube were washed with buffer without disturbing the pellet. The precipitate was dissolved in 4 ml of SDS-triethanolamine solution. One milliliter of ferric chloride reagent was added, and the solutions were mixed immediately. Approximately 15–30 min after the addition of the ferric chloride, the absorbance at 510 nm was read on a UV-9200 Spectrophotometer. The average A_{510} of triplicate samples of SDS-triethanolamine solution plus the absorption of the ferric chloride reagent was subtracted from the A_{510} of each sample to correct for background absorbance.

Protein precipitation capacity (PPC)

We used a radial diffusion assay for determining the protein precipitation capacity (PPC) in plant extracts. This assay, developed by Hagerman (1987), measures the protein precipitation capacity of tannins by the formation of insoluble protein-tannin complexes in a protein-containing agar plate. It is most suitable for ecological applications because it is not affected by the solvent (acetone), avoids the problem of varying the tannin-to-protein ratio (Scalbert 1992), requires only small amounts of tannin (0.5 mg) and is relatively simple. Tannin solutions are pipetted into wells punched into the agar plates. The tannins diffuse into the medium and form insoluble complexes with the proteins, forming an opaque ring. The area is known to have an excellent positive correlation with the amount of tannin applied.

N concentration

After digesting the plant materials with sulfuric acid and hydrogen peroxide, the N concentrations were determined with the micro Kjeldahl method (Yoshida et al. 1972)

Statistical analyses

All measurements were replicated three times and analyzed using one-way ANOVA (SPSS11.0 for Windows), with total phenolics, extractable CT, protein-bound CT, fibre-bound CT, protein-precipitable phenolics and protein precipitation capacity.

Results

Comparing results of purified tannin and tannic acid as a standard

There was a significant linear correlation between total phenolics for two different standards, regardless of tissue & species ($y = 2.1537x - 11.685$, $r^2 = 0.9993$, $n = 312$, $p < 0.01$). Leaves and propagules of *B. gymnorrhiza* and *K. candel* showed about twice as much total phenolic content when the purified tannin standard was used rather than the tannic acid standard.

Total phenolic content of different leaf age and decay stages

Total phenolic content in young leaves was higher than those in mature and senescent leaves for *B. gymnorrhiza* (Figure 3). However, in contrast to the

pattern observed in *B. gymnorrhiza*, total phenolic content changes little during different developmental stages of *K. candel* leaves ($p > 0.05$).

At various stages of decomposition, total phenolic content (purified tannin equivalent) ranged from 87.17 ± 16.76 mg g^{-1} in yellow submerged leaves to 90.56 ± 5.36 mg g^{-1} in orange leaves to 52.92 ± 7.30 mg g^{-1} in brown leaves and 13.71 ± 2.49 mg g^{-1} in black leaves of *B. gymnorrhiza*. Total phenolic content remained stable throughout the orange stage (approximately one week in the water), before undergoing noticeable change in the brown and black stages (Figure 3). Total phenolic content decreased from 395.38 ± 44.34 mg g^{-1} to 16.98 ± 6.18 mg g^{-1} during decomposition of *K. candel* leaves. Total phenolic content decreased gradually throughout the orange stage followed by rapid losses in the brown and black stages (Figure 3).

Extractable CT, protein-bound CT, fibre-bound CT and total CT content of different leaf age and decay stages

At different stages of development, extractable CT content of *B. gymnorrhiza* and *K. candel* followed the same order: Young leaves > Mature leaves > Senescent leaves.

At various stages of decomposition, extractable CT content remained stable in yellow submerged leaves and orange leaves for *B. gymnorrhiza*, while increasing in orange leaves for *K. candel*, and followed by an obvious drop in brown and black leaves (Figure 4a).

Protein-bound CT and fibre-bound CT tended to increase with leaf decomposition, with high levels at Stages VI and VII (Figure 4b, c). Total CT had a different trend, the levels of total CT decreased with maturity and senescence. At different stages of decomposition, the level of total CT was the highest in orange leaves (Stage V) and the lowest in black leaves (Stage VII) for both *B. gymnorrhiza* and *K. candel* Figure 4d).

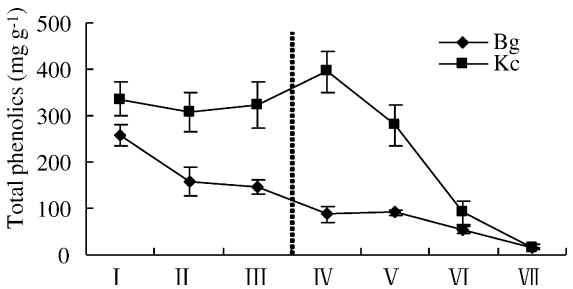

Figure 3. Total phenolic content relative to a purified tannin standard of different leaf age and decay stages of *K. candel* (Kc) and *B. gymnorrhiza* (Bg). I, young leaf; II, mature leaf; III, yellow senescent leaf; IV, yellow submerged leaf; V, orange leaf; VI, brown leaf; VII, black leaf. Stages to the left of the dashed line represent leaves still attached to the plants, whereas those to the right of the line have detached and are decaying.

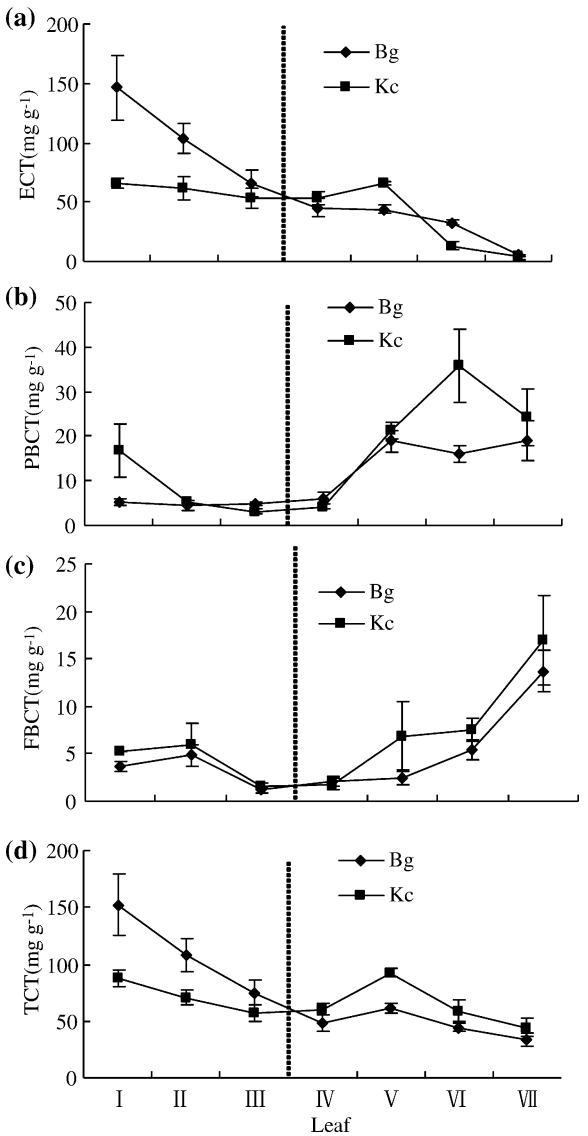

Figure 4. Changes in extractable condensed tannin (ECT), protein-bound CT (PBCT), fibre-bound CT (FBCT) and Total CT (TCT) content of different leaf age and decay stages of *B. gymnorrhiza* (Bg) and *K. candel* (Kc). Leaf stages as described in Figure 3.

Protein-precipitable phenolics and protein precipitation capacity of different leaf age and decay stages

Protein-precipitable phenolics in young leaves were higher than those in mature and senescent leaves for *B. gymnorrhiza* and *K. candel*. At various

stages of decomposition (from Stages IV to VII), protein-precipitable phenolics changed little in yellow submerged leaves and orange leaves, followed by a decline in brown and black leaves of *B. gymnorrhiza*, while the values decreased gradually for *K. candel* (Figure 5a). Protein-precipitable phenolics in *K. candel* leaves were significantly higher than those in *B. gymnorrhiza* leaves ($p < 0.001$).

Protein precipitation capacity in young leaves was 946.73 ± 108.92 cm^2 mg^{-1} for *B. gymnorrhiza* and 655.01 ± 75.79 cm^2 mg^{-1} for *K. candel*, respectively (Figure 5b). While the values in brown or black leaves were low or beyond the limits of detection in this assay.

Total phenolic content of B. gymnorrhiza *and* K. candel *propagules*

Total phenolic content of flower tissue was lower than those of hypocotyls at different developmental stages ($p < 0.0001$) for *B. gymnorrhiza*. While that of flower was also lower than those of hypocotyls at stages I ($p < 0.0001$), II ($p < 0.0001$) and II ($p < 0.05$) for *K. candel*, but close to those of hypocotyls at stages IV ($p = 0.078$) and V ($p = 0.729$). Total phenolic content increased rapidly from flower to hypocotyls at the early development stages, and remained high in *B. gymnorrhiza*, but decreased for *K. candel* (Figure 6).

Total phenolic contents of propagules of *B. gymnorrhiza* at different developmental stages were higher than those of *K. candel* ($p < 0.01$).

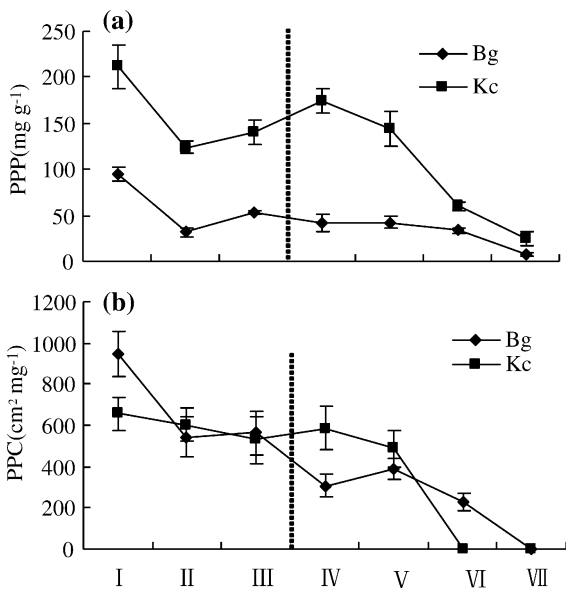

Figure 5. Changes in protein-precipitable phenolics (PPP) content and protein precipitation capacity (PPC) of different leaf age and decay stages of *B. gymnorrhiza* (Bg) and *K. candel* (Kc). Leaf stages as described in Figure 3.

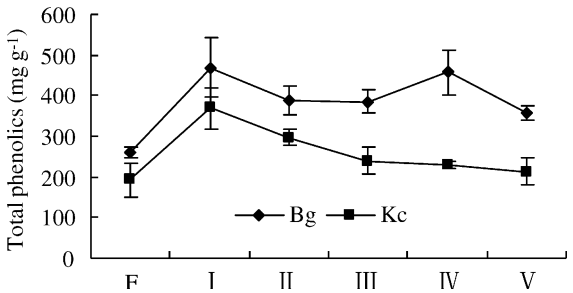

Figure 6. Changes in total phenolic content (purified tannin standard) of propagules of *B. gymnorrhiza* (Bg) and *K. candel* (Kc). F: Flower, Division of the developmental stages of hypocotyls shown in Table 1.

Extractable CT, protein-precipitable phenolics content and protein precipitation capacity of B. gymnorrhiza *and* K. candel *propagules*

Extractable CT and protein-precipitable phenolics content in flower tissue were lower than those in hypocotyls at different developmental stages (both significantly at $p < 0.0001$ levels) for *B. gymnorrhiza*, this result was not in full accordance with the observation of *K. candel*, they changed little ($p > 0.05$) from flower to hypocotyl at Stage I (Figure 7a, b). Protein-precipitable phenolics were highly correlated with extractable condensed tannins ($r = 0.8345$ for *B. gymnorrhiza*, and $r = 0.8463$ for *K. candel*, respectively).

Protein precipitation capacity fluctuated with the development of propagules, and the highest values occurred both in hypocotyls at Stage I (720.37 ± 178.94 cm^2 mg^{-1} for *B. gymnorrhiza* and 713.14 ± 125.59 cm^2 mg^{-1} for *K. candel*, respectively) (Figure 7c). Protein precipitation capacity had not significant relationship with total phenolic content ($p > 0.05$).

Nitrogen concentration of different leaf age and decay stages

N concentration remained almost unchanged ($p > 0.05$) from young leaves to mature leaves, but decreased significantly ($p = 0.0090$ for *K. candel* and $p = 0.0040$ for *B. gymnorrhiza)* during leaf senescence. From yellow senescent leaves to yellow submerged leaves, N concentration decreased 50.30% for *K. candel* leaves and 26.90% for *B. gymnorrhiza* leaves, respectively. During decomposition, however, nitrogen concentration increased (Figure 8).

Discussion

Changes in total phenolic content of different leaf age and decay stages

Total phenolic content showed an immediate and rapid decline between *B. gymnorrhiza* leaves on trees and those in the litter layer, eventually dropping

to low levels (< 2%) in black leaves. Total phenolic content of *K. candel* leaves remained stable during different developmental stages of leaves, and then decreased with decomposition. The results suggest that total phenolics dynamics varies with species. Total phenolic content in young leaves was higher than those in mature and senescent leaves for *B. gymnorrhiza*, the observed changes in total phenolic content associated with different developmental stages are in accordance with the findings reported for different Oak species (Makkar et al. 1988; Rossiter et al. 1988). In contrast to the pattern observed in *B. gymnorrhiza*, total phenolic content changes little during different developmental stages of *K. candel* leaves. The variability of leaf phenolic content during leaf development and senescence may determine not only the susceptibility of plants to herbivore attack, but also important aspects of nutrient cycling in terrestrial and aquatic ecosystems (Northup et al. 1995, 1998; Kandil et al.

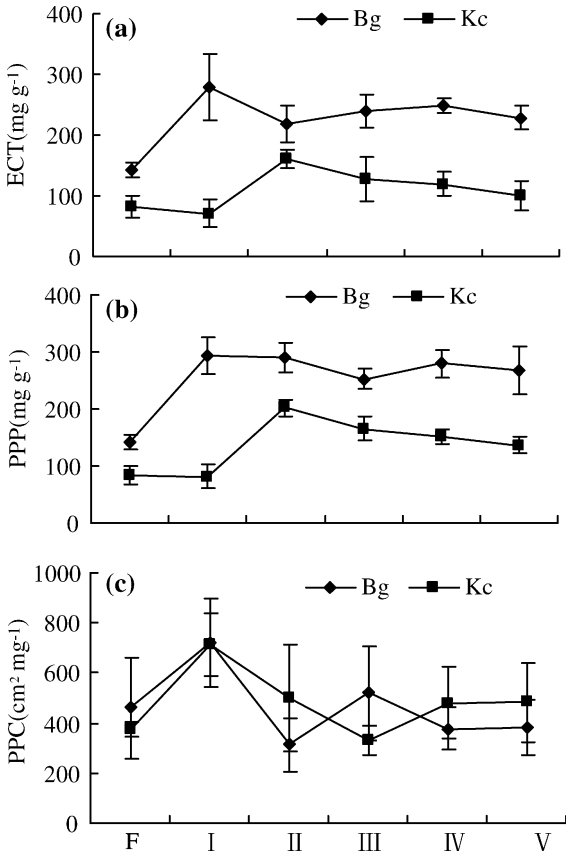

Figure 7. Changes in extractable condensed tannin (ECT), protein-precipitable phenolics (PPP) content and protein precipitation capacity (PPC) of propagules of *B. gymnorrhiza* (Bg) and *K. candel* (Kc). F: Flower, Division of the developmental stages of hypocotyls shown in Table 1.

2004). The rapid loss of phenolics observed during decomposition is probably not only ascribed to leaching. Degradation might also play an important role. In our experiments, mangrove leaf litter, especially *K. candel*, exhibited a high content of total phenolics, but the disappearance of these compounds during decomposition is expected to facilitate faunal consumption, litter decomposition and incorporation into the soil.

The ^{13}C-NMR observations of Hernes et al. (2001) showed constant phenolic concentrations in the leaf samples at about 20% while they were still attached the plant. Total molecular tannin, however, decreased from 7 to $<1\%$ between the orange and brown/black stages of degradation. This trend suggests that tannin is being transformed by condensation and not re-mineralized. Our results support these observations.

Changes in extractable CT, protein-bound CT, fibre-bound CT, total CT content and protein precipitation capacity of different leaf age and decay stages

Maceration of the plants containing condensed tannins (CT) causes CT's to bind to the plant material, with CT binding more strongly to protein than to fibre. There was a higher proportion of protein-bound CT than fibre-bound CT with the exception of mature leaves (Figure 4).

Protein precipitation capacity of young leaves was higher than those of mature and senescent leaves for *B. gymnorrhiza*. The capacity of tannins to bind proteins is related to the molecular size of the tannins (Makkar et al. 1987). The decrease in protein precipitation capacity with maturity and senescence (Figure 5b) and the possible increase in degree of polymerization suggests that, as leaf matures, the tannin active sites specific for the interaction with proteins were becoming fewer by the condensation of the tannin molecules, causing them to become too large to fit the protein orientation for cross-linking.

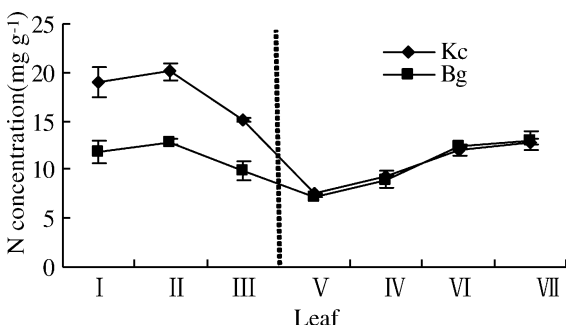

Figure 8. N concentration of different leaf age and decay stages of *K. candel* (Kc) and *B. gymnorrhiza* (Bg). Leaf stages as described in Figure 3.

The results obtained with the radial diffusion assay were highly correlated ($r^2 = 0.7025, n = 84, p < 0.01$) with the results obtained with the precipitation assay for *B. gymnorrhiza* leaves. The radial diffusion assay depended only on the ability of tannin to interact with protein to form a visible precipitate. The protein precipitable phenolics assay depended both on the interaction with protein to form a precipitate and on the reaction with ferric chloride to form a colored complex. Because the two assays were based on different chemistry, it was not surprising that the correlation observed above, although significant, is less than perfect.

BSA was selected as the protein to incorporate in the gel for the radial diffusion assay because it is homogeneous, soluble, and inexpensive. Tannin is a selective protein-precipitating agent, binding tightly to some proteins and more weakly to others (Hagerman and Butler 1981; Hagerman and Klucher 1986). Precipitation assays conducted with inhomogeneous proteins are ambiguous, since the tannins interact selectively with the proteins in the mixture. Both condensed and hydrolysable tannins have moderate affinities for BSA (Hagerman and Butler 1981; Martin and Martin 1983; Hagerman 1987).

Changes in total phenolics, extractable CT, protein-precipitable phenolics content and protein precipitation capacity of B. gymnorrhiza *and* K. candel *propagules*

Total phenolics, extractable CT and protein-precipitable phenolics content of flower tissue was relatively lower than those in hypocotyls at different developmental stages. But the flowers still had high tannin levels (261.77 ± 13.79 mg g^{-1} for *B. gymnorrhiza*, and 191.94 ± 41.00 mg g^{-1} for *K. candel*, respectively). Tannin accumulation occurred with development and maturity of hypocotyls (Figures 6 and 7). The protein precipitation capacity fluctuated with development of propagules. During hypocotyls developments of *K. candel* and *B. gymnorrhiza*, mature hypocotyls (Stage V, namely seedlings) still had the relatively high tannin content.

Nitrogen concentration and potential role of tannin

When leaves of *K. candel* and *B. gymnorrhiza* decompose, an initial phase in which N concentration decrease can be explained by leaching. However, this phase is followed by significant increases in the N concentration of the degrading materials. Although most investigators (e.g., Rice 1982; Melillo et al. 1984; Woitchik et al. 1997) advocate a microbial source for immobilized N, direct evidence for this origin has not been given.

The dilemma of N immobilization can in part be explained by a preservation mechanism. Most investigators suspect that humification reactions are involved due to the fact that humic substances have a high N concentration

relative to vascular plant tissues. The best evidence for this model is the positive correlation between humic substance content and N concentration during immobilization (Rice 1982). The general humification model includes breakdown and oxidation of phenolics and carbohydrates in the plant material with subsequent condensation with nitrogenous materials either endemic to the tissue or of microbial origin such as exoenzymes. Direct evidence for such N immobilization has been limited to a study of *Spartina alterniflora* in which lignin was negatively correlated with N contents (Benner et al. 1991). We observed a negative correlation between total phenolics and N contents for *K. candel* and *B. gymnorrhiza* leaves at various stages of decay, which is indicative of humification reactions (Figure 9). Our results on *K. candel* and *B. gymnorrhiza* leaves at various stages of decay are consistent with that of Benner et al. (1991).

Interest in tannins stems from their ability to bind with protein N during the tanning process. Geochemically, potential N binding and immobilization by tannin is also of great interest. The process of nitrogen immobilization (the incorporation of exogeneous N into organic matters) in sediments and submerged leaves has been poorly understood, and the study of tannin might shed some light on this key process (Hernes et al. 2001).

Increases in nitrogen of decaying litter, and declines in contents of total phenolics and total condensed tannins of detritus occurred in the mangrove swamp studied here. Results from this study support the general suggestion that the decomposing mangrove detritus can be a heterotrophic substrate based on its high nitrogen content and low tannin level compared to the living leaves. The importance of intertidal plants as a main source of matter and energy for the estuarine food web is thus made more significant by the nutritional quality of organic matter provided by mangroves to estuarine consumers.

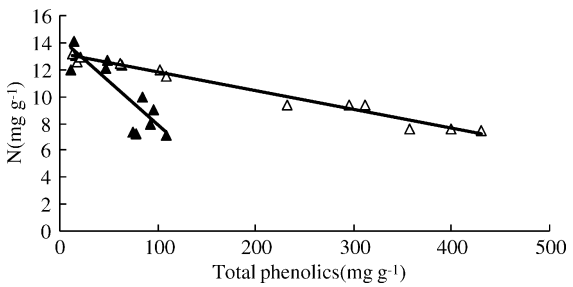

Figure 9. Relationship between tannin (total phenolics) and N contents during leaf decomposition of *B. gymnorrhiza* (Bg) and *K. candel* (Kc). Symbols are: black triangle for Bg leaf; white triangle for Kc leaf. The correlations were both highly significant ($R^2 = 0.7003$, $P < 0.01$ for *B. gymnorrhiza* and $R^2 = 0.9712$, $p < 0.01$ for *K. candel*) with the linear regression equations: $y = -0.0639x + 14.305$ for *B. gymnorrhiza* and $y = -0.0138x + 13.156$ for *K. candel*, respectively.

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