Changes of tannin and nutrients during decomposition of branchlets of *Casuarina equisetifolia* plantation in subtropical coastal areas of China

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ABSTRACT

A litterbag experiment was conducted to investigate the changes of tannins and nutrients in branchlets at different decomposition stages of *Casuarina equisetifolia* in southern subtropical coastal zone, China, using the colorimetric assays. The time required for the loss of half of the initial dry weight (t_{50}) was 9.13 months. Total phenolics (TP), extractable condensed tannins (ECT), protein-bound condensed tannins (PBCT), total condensed tannins (TCT) and protein precipitation capacity (PPC) of branchlets litter decreased rapidly, while fibre-bound condensed tannins (FBCT) increased during decomposition. Nitrogen and phosphorus concentration of the branchlet litter both increased gradually during decay. Negative correlations between TP and nitrogen or phosphorus, as well as TCT and nitrogen or phosphorus were found. These chemical changes enhanced the current knowledge on the potential ecological role of nutrient transformation in tannins in *C. equisetifolia* plantations.

Keywords: total phenolics; condensed tannin; protein precipitation capacity; nitrogen; phosphorus; litter decomposition

Casuarinas are commonly used in agroforestry plantations for soil stabilization, reclamation and coastal protection. At present, casuarina plantations cover about 300 000 hectares in the coastline of southern China (Zhong et al. 2005). They act as pioneer trees for degraded sites and for soil improvement. There is no other species that can replace them at the foreshores. Low soil fertility will slow growth. However, *Casuarina equisetifolia* is characterized by high primary productivity in coastal sandy soils. High tannin production may be an important strategy for *C. equisetifolia* in

coastal environments (i.e. arid, nutrient limitation) (Zhang et al. 2008, 2009).

Phenolic compounds, including tannins, are a significant component of plant secondary metabolites (Míka et al. 2005, Lin et al. 2006, Lachman et al. 2011). Because high tannin concentrations in plants are often associated with infertile site conditions (Northup et al. 1995), it has been suggested that there is an evolutionary advantage to higher tannin production. Tannins may provide a nutrient conservation mechanism by reducing decomposition rates of litter and decreasing N leaching potential (Lin et al. 2010).

Previous studies suggested that litter material with high tannin concentration was commonly associated with decreased decomposition rates (Gallardo and Merino 1992, Kraus et al. 2003, Semwal et al. 2003). High levels of CTs in *Kandelia obovata* leaf litter might retard the decomposition process and lack of CTs in *Avicennia marina* leaves was responsible for the fast rate of decay (Zhou et al. 2010, 2012). However, tannin content was unrelated or positively correlated with decay rate in other studies (Meentemeyer 1978, Gallardo and Merino 1993).

Despite the growing knowledge of the physiological basis and ecological consequences of leaf phenolics in plant tissues, there is scant information on detailed changes of leaf phenolics and nutrient concentrations during leaf decomposition (Schofield et al. 1998, Hernes et al. 2001, Lin et al. 2006). This variability may determine important aspects of nutrient cycling in terrestrial ecosystems (Northup et al. 1995, Lin et al. 2007, Zhou et al. 2012). The aim of this study was to examine changes in tannins and nutrients in branchlets at different decomposition stages of *C. equisetifolia* in southern subtropical coastal zone, Fujian, China.

MATERIAL AND METHODS

Site description. The experiments were carried out in the Chihu Forestry Center of Huian County (23°45'N, 118°55'E), Fujian province, China. The climate of the region belongs to southern subtropical maritime monsoon climate, with annual temperature ranging from 2.2°C to 37.0°C. Mean annual precipitation and evaporation are 1029 mm and 2000 mm, respectively. The soils are coastal sandy, barren, pH 5.0. In the study site, the *C. equisetifolia* plantations were artificial, pure forests which were planted in 1978.

Litterbag decomposition experiment. For the decomposition studies, the gray colored branchlets were randomly hand-picked from the trees. All samples were taken to the laboratory after sampling and cleaned with distilled water. The branchlets were air-dried for 12 h so that no surface water remained and then 20 g were placed into nylon litter bags (25 × 25 cm) with a mesh size of 1 × 1 mm. The experiment was started on December 22, 2010, and three litterbags were sampled randomly every month. These samples of branchlet litter were returned to the laboratory where they were gently washed with distilled water in a sieve to remove soil. The washed branchlets litter was immediately freeze-dried for 48 h and the final dry mass was recorded, and then ground to pass through the 40-mesh sieve. The samples were stored at −20°C prior to the chemical analyses.

Determinations of tannin concentrations. All chemicals used for analyses of tannin concentrations were analytical reagent purity grade (Sinopharm, Shanghai, China). An additional standard, here denoted purified tannin, was extracted from *C. equisetifolia* branchlets and purified on Sephadex LH-20 (Amersham, USA) according to the procedure previously described by Hagerman (2002). The condensed tannins standard was freeze-dried and stored at −20°C until required.

Procedures described by Lin et al. (2006) were used to determine total phenolics (TP), extractable condensed tannins (ECT), protein bound condensed tannins (PBCT), and fibre bound condensed tannins (FBCT) and protein precipitation capacity (PPC) in the branchlet litter. TP concentrations were measured by the Foline Ciocalteu method (Zhou et al. 2010), ECT, PBCT and FBCT were assayed by the butanol-HCl method (Terrill et al. 1992), using purified tannins from *C. equisetifolia* branchlets as the standard. Total condensed tannin (TCT) content was calculated by adding the respective quantities of ECT, PBCT and FBCT (Terrill et al. 1992). A radial diffusion assay was used to determine the protein precipitation capacity (PPC) (Hagerman 1987).

Determinations of carbon, nitrogen and phosphorus concentrations. Total C and N content of litter were determined on ground subsamples by the Elementar CHNS analyzer model Vario EL III (Vario EL, Elementar Analyser systeme GmbH, Hanau, Germany). Plant samples were digested with sulfuric acid and hydrogen peroxide, then P content was determined by the ascorbic acid-

antimony reducing phosphate colorimetric method (Nanjing Institute of Soil Science 1978).

Statistical analyses. Mean and standard deviation values of triplicate samples were calculated. A one-way analysis of variance (ANOVA) was performed with the decomposition stage as the treatment factor. The Student-Newman-Keuls multiple comparison method was used to test significant differences among the decomposition stages. All analyses were performed by the SPSS 20.0 for Windows.

RESULTS AND DISCUSSION

Mass loss during branchlet litter decomposition. The decay pattern showed rapid early losses in mass followed by a slower decrease for the remaining period of the experiment (Figure 1). There were about 41.53% mass losses during the first 6-month decomposition. The initial rapid decrease of litter mass may be related to leaching of soluble organic materials (Van der Valk and Attiwill 1984) and inorganic compounds (Steinke et al. 1983). But the decomposition rate became slower after 6 months, which reflected the loss of more resistant materials. In this study, t_{50} (half-life) of *Casuarina equisetifolia* branchlet litter was 9.13 months, which was longer than other results (Van der Valk and Attiwill 1984, Zhou et al. 2010, 2012). However, these studies were carried out under the different field conditions.

Changes in tannin concentrations and PPC during branchlet litter decomposition. TP and ECT decreased rapidly in the first 5 months of decomposition and remained the same thereafter (Figures 2a–b). PBCT increased with branchlet decay firstly, but decreased after 2 months of decomposition (Figure 2c). FBCT did not show any significant change in the first 4 months (around 13 mg/g) but increased rapidly over 6 months and then increased gradually at later stages (Figure 2d). At the different stages of decomposition, the level of TCT decreased gradually (Figure 2e), this was similar with ECT. The dynamics of PPC was similar with TP and ECT (Figure 2f).

During decomposition, a steady decrease of TP, ECT, PBCT and TCT was observed, while FBCT increased across the decay. On the one hand, the rapid loss of TP, ECT and TCT in the first 5 months might be due to leaching, because leaching is an important pathway for loss of phenolics from the

leaves (Hernes et al. 2001). On the other hand, degradation or oxidation might also play an important role. However, the increase of FBCT may be suggested an alternate fate for CTs, which could be polymerisation and/or the formation of stable complexes with plant cell wall polysaccharides (Haase and Wantzen 2008).

Some previous studies showed that litter material high in tannin content was commonly associated with reduced decomposition rates (Gallardo and Merino 1992, Kraus et al. 2003, Semwal et al. 2003). However, Gallardo and Merino (1993) suggested that tannin content was unrelated or was positively correlated with rate of decay. In the present study, tannin content decreased significantly in its branchlet litter.

Changes in nutrient concentrations during branchlet litter decomposition. The concentration of nitrogen and phosphorus in branchlet litter decreased after one month for decomposition, then increased gradually and remained the same towards the end of the experiment (Figures 3a,b). N and P concentrations in litter correlated positively during decay ($r = 0.836$; $P < 0.001$; $n = 39$).

On the one hand, the increase in nitrogen concentrations in leaf litter may be due to greater loss of carbon and mass than nitrogen, and on the other hand, the immobilization of external nitrogen, such as microbial biomass (He et al. 1988, Wang et al. 2012) and dissolved organic nitrogen (Maie et al. 2008), and internal nitrogen, such as leaf litter protein and amino acids (Gallardo and Merino 1992). We observed a negative correlation between TP and nitrogen contents at various stages of decay (Table 1), which is indicative of humification

$[Mass]_t = 19.8459 + 82.535e^{-0.1103t}$
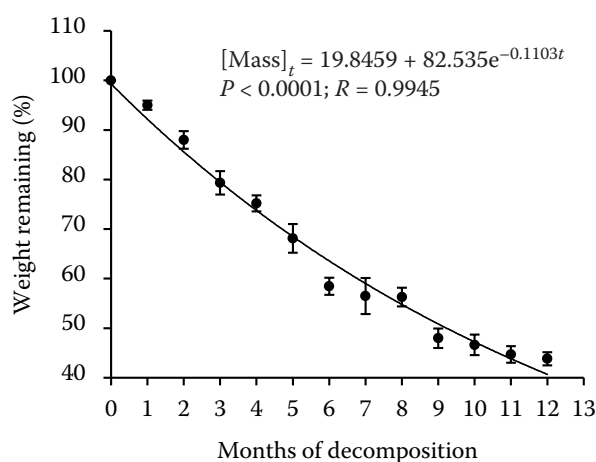$P < 0.0001; R = 0.9945$

Figure 1. Mass remaining of *Casuarina equisetifolia* branchlets after various decomposition times, as % of initial dry weight, in litter bags experiment
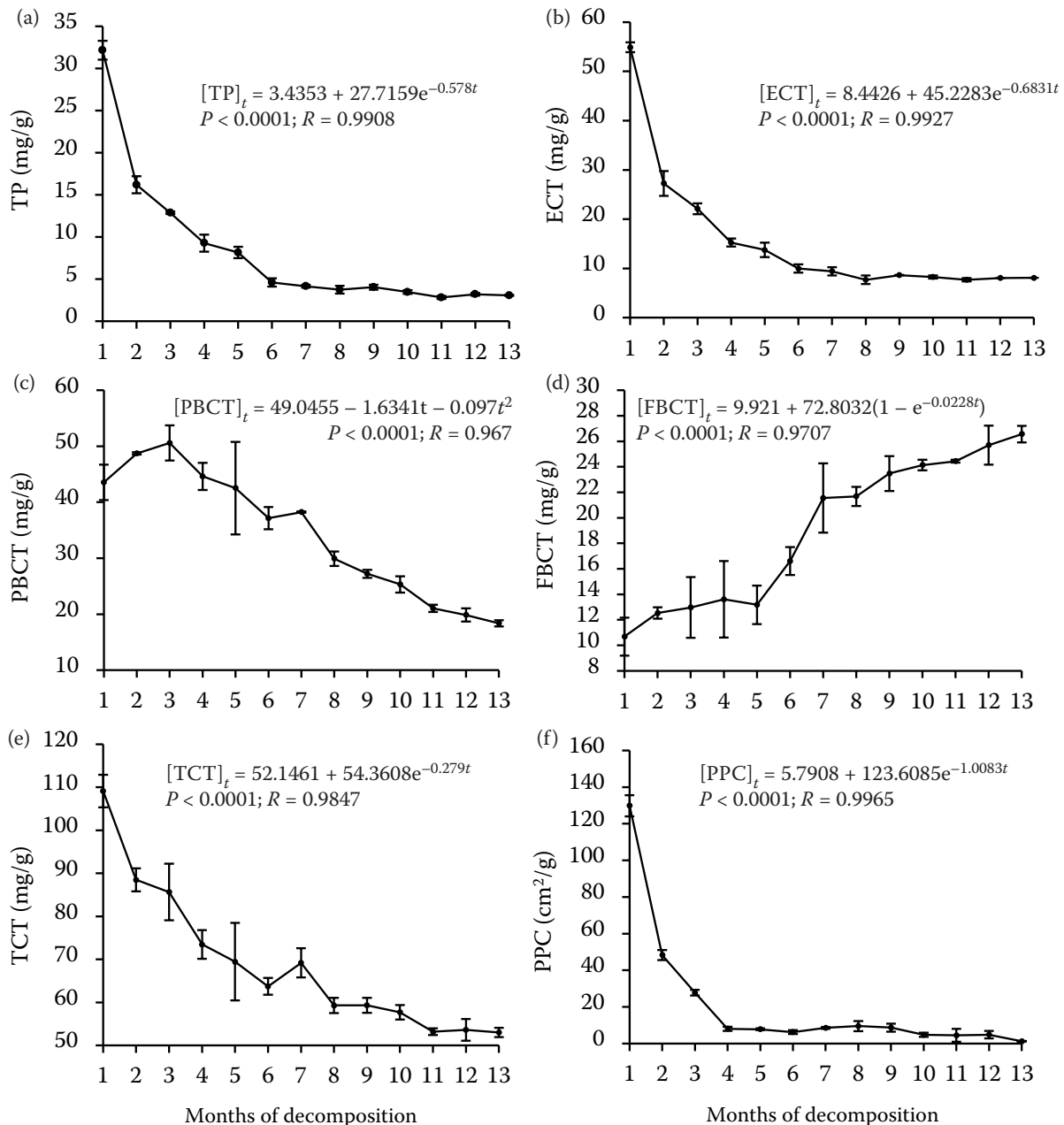
Figure 2. Changes in the total phenolics (TP); extractable condensed tannins (ECT); protein-bound condensed tannins (PBCT); fibre-bound condensed tannins (FBCT), and total condensed tannins (TCT) contents and protein precipitation capacity (PPC) in the decomposition of branchlets litter of *Casuarina equisetifolia*

reactions. Our results are consistent with that of Hernes et al. (2001). However, significantly positive correlations were found between TP or TCT concentrations and PPC. Therefore, the binding of tannins and nitrogenous substance may be an important mechanism of nitrogen accumulation in leaf litter (Adamczyk et al. 2011).

Additionally, nitrogen levels might also play a role in controlling the decomposition of leaf litter (Schimel and Hättenschwiler 2007, Abramovich

et al. 2010). Fenchel et al. (1998) suggested that high initial nitrogen concentrations would result in high microbial assimilation efficiencies and an increase in mineralization, but it would lead to a decrease in immobilization, leading to a fast decay rate.

Fewer studies examined dynamics of phosphorus concentration across decomposition of litter compared with nitrogen. The phosphorus concentration declined in *Bruguiera gymnorrhiza* leaves

(a)

$$[N]_t = 10.9089 + 21.2793(1 - e^{-0.0602t})$$
$$P < 0.0001; R = 0.9773$$

(b)

$$[P]_t = 0.192 + 0.1949(1 - e^{-0.1516t})$$
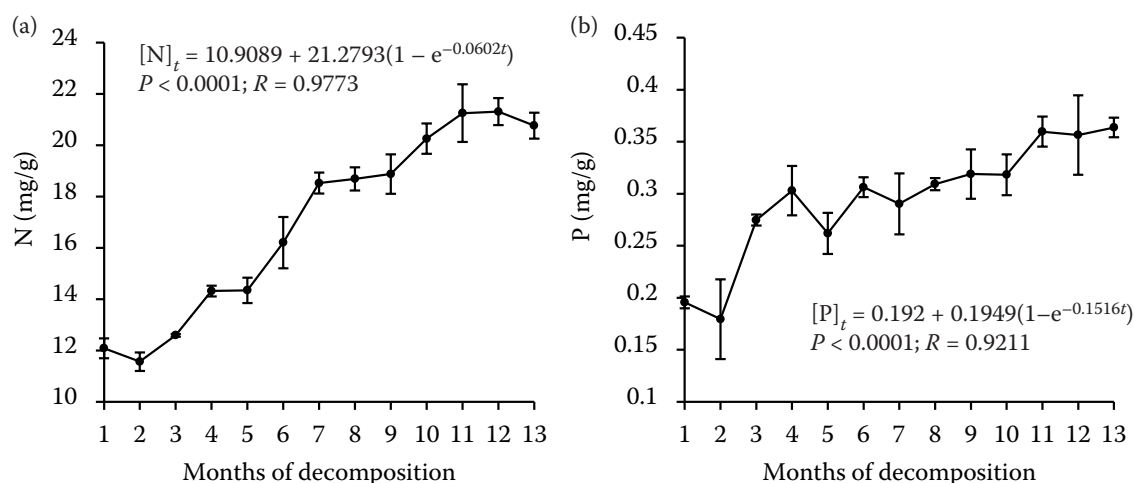$$P < 0.0001; R = 0.9211$$

Figure 3. Changes in the N and P concentrations in the decomposition of branchlets litter of *Casuarina equisetifolia*

during the first 4–6 weeks of decomposition, but either remained the same or gradually increased thereafter, for about 10–24 weeks, suggesting phosphorus immobilization (Steinke et al. 1983). The phosphorus concentration increased gradually and remained the same during decomposition in the current study. Significant correlations were also found between TP or TCT and phosphorus concentration.

In conclusion, *C. casuarina* branchlet litter showed higher initial level of nitrogen and lower tannins than the reported values of other studies, while its decay rate was lower. Different environments might be responsible for these differences. The lower rates of mass loss of *C. casuarina* branchlet litter can be attributed to lower activity of microbial decomposer communities in drier sandy soil. In addition, different tannin structures of varied species might also lead to different decay rate. The slow decomposition rate of *C. equiseti-*

folia branchlet litter prevents nutrient loss from infertile sandy soils. This reveals the nutrient conservation strategy for *C. equisetifolia* to survive in coastal sandy soils with low nutrient and moisture availability.

Table 1. Correlative coefficient of selected branchlet litter data

Variable	*r*	*n*	*P*
TP-N	−0.770	39	< 0.001
TP-P	−0.797	39	< 0.001
TCT-N	−0.861	39	< 0.001
TCT-P	−0.832	39	< 0.001
TP-PPC	0.965	39	< 0.001
TCT-PPC	0.869	39	< 0.001

TP – total phenolics; TCT – total condensed tannins; PPC – protein precipitation capacity

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