

# Litter decomposition in a subtropical plantation in Qianyanzhou, China

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**Abstract** A long-term (20 months) bulk litter decomposition experiment was conducted in a subtropical plantation in southern China in order to test the hypothesis that stable isotope discrimination occurs during litter decomposition and that litter decomposition increases concentrations of nutrients and organic matter in soil. This was achieved by a litter bag technique. Carbon (C), nitrogen (N) and phosphorus (P) concentrations in the remaining litter as well as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  during the experimental period were measured. Meanwhile, organic C, alkali-soluble N and available P concentrations were determined in the soils beneath litter bags and in the soils at the control plots. The dry mass remaining (as % of the initial mass) during litter decomposition exponentially declined ( $y = 0.9362 e^{-0.0365x}$ ,  $R^2 = 0.93$ ,  $P < 0.0001$ ), but total C in the remaining litter did not decrease significantly with decomposition process during a 20-month period. By comparison, total N in the remaining litter significantly increased from  $5.8 \pm$

$1.7 \text{ g kg}^{-1}$  dw litter in the first month to  $10.1 \pm 1.4 \text{ g kg}^{-1}$  dw litter in the 20th month. During the decomposition,  $\delta^{13}\text{C}$  values of the remaining litter showed an insignificant enrichment, while  $\delta^{15}\text{N}$  signatures exhibited a different pattern. It significantly depleted  $^{15}\text{N}$  ( $y = -0.66x + 0.82$ ,  $R^2 = 0.57$ ,  $P < 0.0001$ ) during the initial 7 months while showing  $^{15}\text{N}$  enrichments in the remaining 13 months ( $y = 0.10x - 4.23$ ,  $R^2 = 0.32$ ,  $P < 0.0001$ ). Statistically, litter decomposition has little impact on concentrations of soil organic C and alkali-soluble N and available P in the top soil. This indicates that nutrient return to the topsoil through litter decomposition is limited and that C cycling decoupled from N cycling during decomposition in this subtropical plantation in southern China.

**Keywords** Litter decomposition · Subtropical plantation ·  $\delta^{13}\text{C}$  ·  $\delta^{15}\text{N}$

## Introduction

Litter decomposition plays an important role in nutrient cycles and controls primary production in forest ecosystems (Clark et al. 1998; Onyekwelu et al. 2006; Pandey et al. 2007), because it is a key ecological process responsible for the transformation of organic matter and energy from the vegetation to the soil in a variety of ecosystems. Among these ecosystems, tropical and subtropical forests are important vegetation types on the Earth, therefore a large number of studies have recently been conducted in these forests in order to understand C and N cycling through investigations of litter decomposition (Wen et al. 1989; Wu et al. 1990; Wu 1991; Zhao et al. 1991; Didham 1998; Gurvich et al. 2003; Cleveland et al.

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2006; Liao et al. 2006; Zou et al. 2006; Li et al. 2007; Kaspari et al. 2008).

Recently, discrimination of C and N isotopes during litter decomposition has been employed as a strong tool for a better understanding of C flows and nutrient availability in terrestrial ecosystems (Högberg et al. 1995; Bernoux et al. 1998; Giocchini et al. 2006). It has been shown that some litter types exhibited  $^{13}\text{C}$  depletion (Benner et al. 1987; Asada et al. 2005; Osono et al. 2008), whereas others demonstrated  $^{13}\text{C}$  enrichment (Wedin et al. 1995; Osono et al. 2006, 2008) or no significant variations (Mellilo et al. 1989; Schweizer et al. 1999; Connin et al. 2001). Numerous studies have suggested that  $^{15}\text{N}$  enrichment occurred in the remaining litter with decomposition time (Nadelhoffer and Fry 1988; Asada et al. 2005). However, a non-linear pattern was observed in a red pine plantation during a 77-month period (Mellilo et al. 1989) while significant  $^{15}\text{N}$  depletion was found during 18 months of decomposition of belowground parts in salt-marsh sediments (Benner et al. 1991). Over a period of 6 years, there was no clear pattern for  $^{15}\text{N}$  dynamics during litter decomposition (Connin et al. 2001). The discrepancy in discrimination of C and N isotopes during litter decomposition is ascribed to the difference in the relative contribution of different mechanisms such as preferential use of light isotopes by decomposers, selective retention of recalcitrant components, and incorporation of exogenous substrate (Nadelhoffer and Fry 1988; Ågren et al. 1996). Microbial-mediated fractionations or preferential retention of  $^{15}\text{N}$ -enriched recalcitrant compounds was used to explain  $^{15}\text{N}$  enrichments during litter decomposition, while selective preservation of recalcitrant components accounted for  $^{13}\text{C}$  patterns during decomposition (Connin et al. 2001; Osono et al. 2008). This means that discrimination of C and N isotopes during litter decomposition may be dependent on ecosystem types.

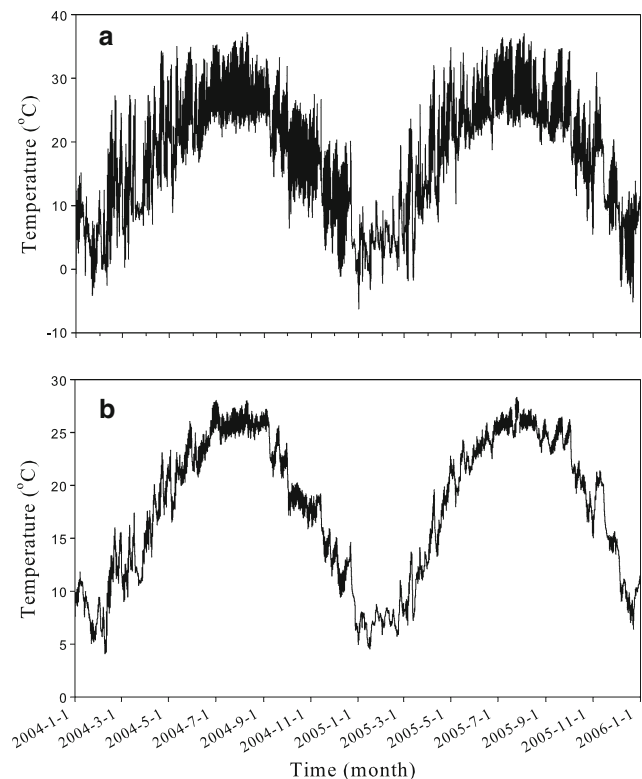
A large-scale campaign of afforestation was launched in the 1970s in the tropical and subtropical forests in southern China, and a larger area of plantations was established during the 1980s (Li and Li 1996). Previous studies in tropical and subtropical forests in southern China showed that broad-leaved trees are better than coniferous trees in improving soil physical and chemical characteristics (Wu et al. 1990). Among different litter components, foliar litter decomposed faster than twig (Xue and Kuang 1990). Reciprocal experiments showed that increasing temperature significantly enhanced foliar litter decomposition in tropical and subtropical forests (Liu et al. 2005). Xu and Hirata (2005) suggested that P is an important factor controlling litter decomposition in a subtropical forest, while Wang et al. (2009) reported that the mass loss of leaf litter is positively correlated with initial N concentration. However, little is known about C and N dynamics in the decomposition of bulk litter in subtropical forests in southern China.

A 20-month bulk litter decomposition experiment was conducted in a subtropical plantation to test two hypotheses: first, that discrimination of C and N isotopes occurs during litter decomposition if microbial-mediated fractionations play a more important role in litter decomposition (Nadelhoffer and Fry 1988); and, second, that litter decomposition increases concentrations of nutrients (e.g., alkali-soluble N and available P) and soil organic C through leaching because litter decomposition is a key ecological process returning nutrients bound in organic material to mineral form in soils (Swift et al. 1979).

## Materials and methods

### Site description

This study was conducted in a subtropical plantation at Qianyanzhou station of the Chinese Academy of Sciences (26°44'N, 115°04'E, 100 m a.s.l.), where it is characterized by a typical subtropical monsoon climate. Annual temperature averaged 17.9°C from 1985 to 2005 while annual mean precipitation was 1,494 mm during this period. During the experimental period, air temperature at 1.6 m and soil temperature at 5 cm depth are presented in Fig. 1. Volumetric soil water content at 5 cm depth was



**Fig. 1** Air temperature at 1.6 m above the ground (a) and soil temperature at 5 cm depth (b) during the incubation period

measured by a TDR probe (model CS615-L; Campbell Scientific), which is shown in Fig. 2 together with precipitation. Soil is classified as Haplic Acrisol (WRB 1998) and its basic soil properties are presented in Table 1. The studied plantation was established around 1985, and is dominated by *Pinus massoniana* Lamb, *Pinus elliottii* Engelm, and *Cunninghamia lanceolata* Hook (Yang 1998; Hu et al. 2005).

#### Litter collection and decomposition

Freshly fallen litter including twigs and leaves was collected using litter traps (1 m × 1 m). Collected litterfall was taken to the laboratory and classified as twigs and leaves. Afterwards, they were dried in an oven at 80°C and then were cut into 2-cm segments. Litter decomposition was studied using a litter bag technique described by Falconer et al. (1933). Based on the ratio of twig to leaf in the bulk litterfall, 130 g of dried litterfall, including 10 g of

twigs and 120 g of leaves, was placed into nylon litterbags (bag and mesh size 35 × 35 cm and 1 mm, respectively). Litter bags were sealed and tied together with a nylon rope.

At the end of December 2003, five strings of bags (36 bags in each) were placed on the soil surface of five separate plots in a subtropical plantation, with a distance about 25 m between them. Each nylon rope was fixed to a tree nearby. Close to these plots, an additional three plots were selected and regarded as the controls. One litter bag was randomly collected from each plot every month after they were buried. Collected litter samples were taken to the laboratory and dried at 80°C in an oven over 48 h. They were weighed and ground into fine powder for analyzing total C, N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

In order to assess the effects of the placement of litterbags on soil nutrients, the soils beneath litter bags (referred as the soils with litter) and the soils in the control plots (referred as the soils without litter) was collected up 0–5 cm when litter bags were harvested. Soil samples were air-dried and ground into fine powder for measurements of organic C, alkali-soluble N and available P contents.

#### Analysis

Total C, N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the remaining litter was measured by continuous flow gas isotope ratio mass spectrometry (IRMS). IRMS is consisted of an elemental analyzer (EA 1110; CE Instruments, Milan, Italy) interfaced via a ConFlo II device (Finnigan MAT, Bremen, Germany) to the gas isotope ratio mass spectrometry (Finnigan MAT253).

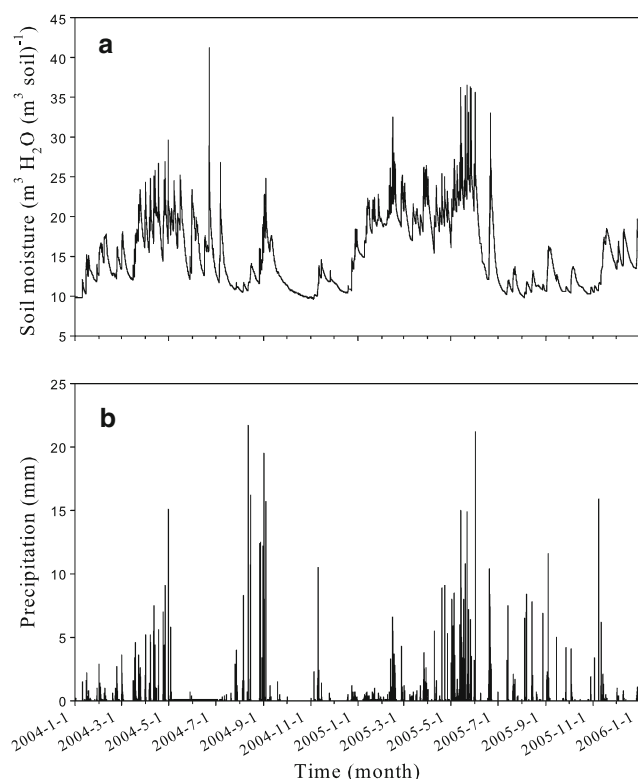
Stable isotope abundances were reported as

$$\delta^{13}\text{C} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000\text{‰} \text{ or}$$

$$\delta^{15}\text{N} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000\text{‰}$$

where  $R$  is the ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  of either the sample or reference standard. Urea was used as an internal reference standard ( $\delta^{13}\text{C} = -43.14\text{‰}$ ,  $\delta^{15}\text{N} = -1.42\text{‰}$ ). The standard deviation of repeated measurements of laboratory standards is  $\pm 0.15\text{‰}$  for  $\delta^{15}\text{N}$  and  $\pm 0.20\text{‰}$  for  $\delta^{13}\text{C}$ .

Soil organic C was measured by the dichromate digestion method (Kalembasa and Jenkinson 1973). Alkali-soluble N was determined by alkali-hydrolyzed reduction



**Fig. 2** Soil moisture at 5 cm depth (a) and precipitation (b) during the incubation period

**Table 1** Basic soil properties (means  $\pm$ 1SE)

Soil depth (cm)	pH	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Available P (mg kg <sup>-1</sup> )
0–5	4.49 $\pm$ 0.02	31.8 $\pm$ 0.9	1.1 $\pm$ 0.0	25.4 $\pm$ 1.1
5–10	4.56 $\pm$ 0.02	16.6 $\pm$ 0.6	0.7 $\pm$ 0.0	13.1 $\pm$ 0.8

diffusing method. In brief, alkali (NaOH) and ferrous sulfate were added into soil, followed by liberation and diffusion of ammonia into acid in Conway dishes for the measurement by nesslerization. Available P was analyzed by colorimetry in NaHCO<sub>3</sub> extracts (Lu 1999). Total N in soils was converted to ammonia by Kjeldahl digestion and was then measured through steam distillation followed by titration with a sulfuric acid standard solution against Methyl Red–Bromocresol Green mixed indicator (Lu 1999). Soil pH values were determined using a glass electrode using a 1:2 soil-to-water ratio.

Calculation and statistics

The decay constant (*k*) was calculated as a negative exponential decay model:  $y = \alpha e^{-kt}$ , where *y* is the amount remaining (as % of the initial dry weight) at time *t*, *t* is decomposition time (month),  $\alpha$  is a modified coefficient and *k* is the decay constant (Olson 1963). Based on our data during a 20-month period, the decay model was estimated to be  $y = 0.9362 e^{-0.0365x}$  (Fig. 3a).

As a result, the time to decompose 50% (*t*<sub>0.5</sub>) and 95% (*t*<sub>0.95</sub>) of the initial dry mass was calculated by the following equation:

$$t_{0.5} = -\ln(0.5/0.9362)/0.0365;$$

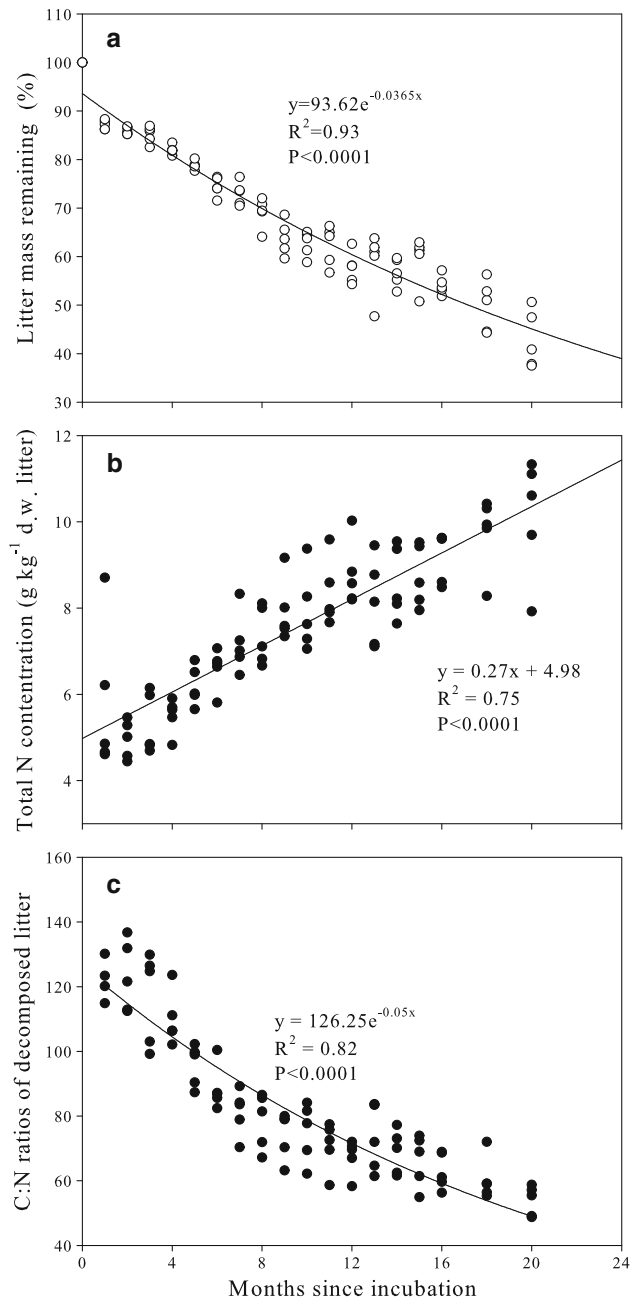
$$t_{0.95} = -\ln(0.05/0.9362)/0.0365$$

Hereafter, the values given are means ± standard errors. Regressions were calculated between incubation time and total C, total N and C:N ratios as well as stable isotope abundance in the remaining litter using a Sigmaplot 9.0 software package.

Results

Dry mass, total C and N concentrations of the remaining litter

The dry mass remaining (as % of the initial mass) during litter decomposition exponentially declined ( $y = 0.9362 e^{-0.0365x}$ ,  $R^2 = 0.93$ ,  $P < 0.0001$ ). It decreased from 87% in the first month to  $43 \pm 5.9\%$  in the 20th month (Fig. 3a). Total C of the remaining litter did not demonstrate significant changes with decomposition time during the 20-month period, whereas total N concentrations of the remaining litter significantly increased with decomposition time. Total N increased from  $5.8 \pm 1.7 \text{ g kg}^{-1} \text{ dw litter}$  in the first month to  $10.1 \pm 1.4 \text{ g kg}^{-1} \text{ dw litter}$  in the 20th month ( $y = 0.27x + 4.98$ ,  $R^2 = 0.75$ ,  $P < 0.0001$ ; Fig. 3b). During litter decomposition, C:N ratios of the remaining litter declined steeply from  $117.5 \pm 6.4$  in the first month to  $48.9 \pm 4.7$  in the 20th month

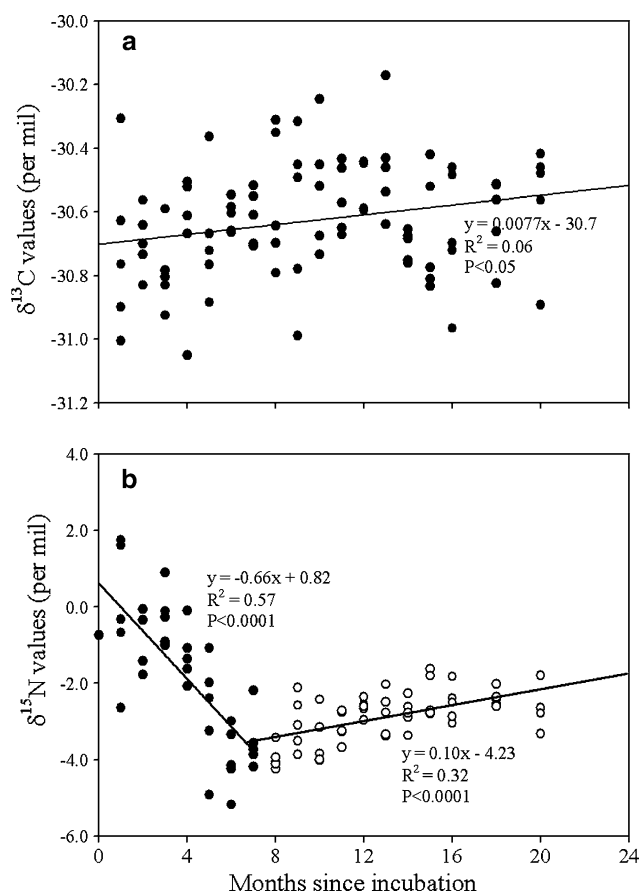


**Fig. 3** Changes in the remaining dry mass as % of the initial mass (a), total N concentrations (b) and C:N ratios (c) of the remaining litter during the incubation period of 20 months

( $y = 126.25 e^{-0.05x}$ ,  $R^2 = 0.82$ ,  $P < 0.0001$ ; Fig. 3c). The time to decompose a half (*t*<sub>0.5</sub>) and 95% (*t*<sub>0.95</sub>) of the initial litter mass was estimated to be about 17 and 80 months, respectively.

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of the remaining litter

$\delta^{13}\text{C}$  values of the remaining litter showed a slight enrichment during decomposition (Fig. 4a). The lowest

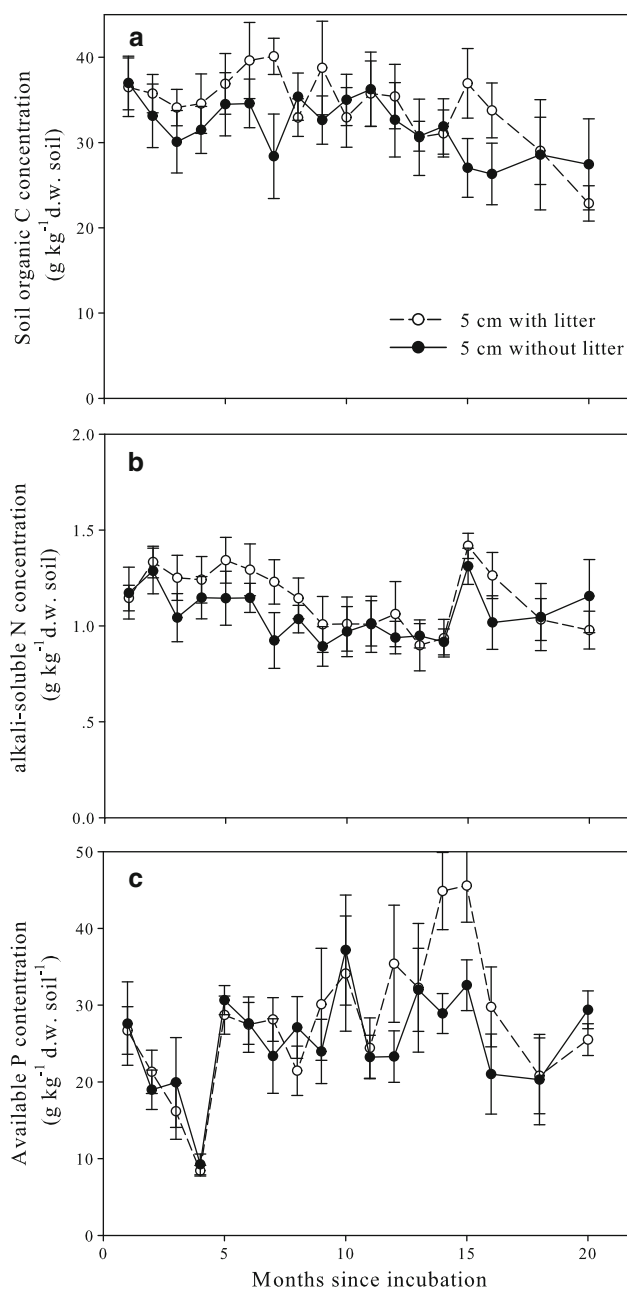


**Fig. 4** Changes in  $\delta^{13}\text{C}$  values (a) and  $\delta^{15}\text{N}$  values (b) of the remaining litter during a 20-month period. Based on an inflexion at the 7th month, the line in b was divided into parts at the 8th month for a better understanding of N cycling during decomposition

$\delta^{13}\text{C}$  value of the remaining litter was  $-31.06\text{‰}$  while the highest was  $-30.19\text{‰}$ . By comparison,  $\delta^{15}\text{N}$  signatures of the remaining litter showed a different pattern during litter decomposition. During the initial 7 months, the remaining litter significantly depleted  $^{15}\text{N}$  ( $y = -0.66x + 0.82$ ,  $R^2 = 0.57$ ,  $P < 0.0001$ ; Fig. 4b), while it greatly enriched in  $^{15}\text{N}$  starting from the eighth month ( $y = 0.10x - 4.23$ ,  $R^2 = 0.32$ ,  $P < 0.0001$ ; Fig. 4b).

#### Effects of litter decomposition on soil organic C, alkali-soluble N and available P concentrations

Although there was no significance in organic C concentrations in the soils between with and without litter, the soils with litter showed higher organic C concentrations within the initial 8 months (Fig. 5a). During the 15th and 16th months, organic C concentrations in the soils with litter were significantly higher than in the soils without litter (Fig. 5a). During the initial 10 months except the first



**Fig. 5** Organic C concentration (a), alkali-soluble N concentration (b), and available P concentrations (c) in the top 5 cm of soil beneath litter bags and at the control plots. Values and error bars are the mean and 1SE, respectively, of five replicates

month, alkali-soluble N concentrations in the soils with litter were higher than in the soils without litter, though only the significant difference was shown in the 15th and 16th months (Fig. 5b). During the initial 5 months, available P concentrations were similar in the soils between with and without litter (Fig. 5c). Available P concentrations in the soils with litter were significantly higher than in the soils without litter in the 12th, 14th and 15th months (Fig. 5c).

## Discussion

Decomposition of bulk litter in a subtropical plantation was investigated using a litter bag technique. Our estimates of  $t_{0.5}$  and  $t_{0.95}$  are within the range estimated by Li et al. (2007) at the same site for four different forest types, who reported that  $t_{0.5}$  ranged from 11.4 to 26.3 months while  $t_{0.95}$  ranged from 53.9 to 114.9 months. Our estimates are also comparable with those ( $t_{0.5} = 19.5$  and  $t_{0.95} = 84.5$  months) observed for coarse wood in tropical forests in Xishuangbanna in southern China (Zheng et al. 2006). By comparison, litter decomposed faster in a subtropical evergreen broad-leaved forest in Japan, where  $t_{0.95}$  of the four tree species varied from 32 to 54 months (Alhamd et al. 2004). On 1-year scale, 42% of the litter dry mass was lost in this study (Fig. 3a). In an evergreen broad-leaved forest, 28% of the dry litter mass of bulk litter was lost (Zhao et al. 1991), while in a subtropical forest about 60% of the dry mass of a foliar mixture of 10 plant species was lost (Liu et al. 2004). The difference in decomposition rates among these forests indicates that litter decomposition is strongly dependent on ecosystem types.

The decay constant ( $k$ ) was estimated to be about 0.0365 on an experimental period in this study (Fig. 3a). This number was 0.0395 in the first year, lower than in the second year (0.0459). These values were similar to those estimated by Li et al. (2007) at the same study site. In this study, soil temperature and soil moisture demonstrated a clear seasonal change, but only a small difference (0.0064) was shown between 2 years. Besides, our estimates were comparable with those observed in tundra, but far lower than in tropical forests (Zhang et al. 2008). This indicates that litter quality could play more important role in litter decomposition in subtropical forests.

A growing body of studies showed that organic C concentrations of the remaining litter decrease with decomposition time (e.g., Swift et al. 1979; Li et al. 2007, 2008). However, organic C concentrations in the remaining litter did not change significantly in this study, indicating C loss in a similar proportion during litter decomposition within a 20-month period. Another possible explanation is that a part of exogenous organic matter could be incorporated into the remaining litter and supplement lost organic C. The dynamics of N concentrations in the remaining litter observed in our study are consistent with previous studies in that N concentrations increased and C:N ratios decreased with decomposition time (Connin et al. 2001). This implies that C and N cycles in litter decomposition are uncoupled in this subtropical plantation.

It has been suggested that discrimination against  $^{13}\text{C}$  or  $^{15}\text{N}$  by microbial-mediated processes is an important mechanism responsible for isotope enrichment in the remaining litter (Blair et al. 1985; Nadelhoffer and Fry

1988; Ågren et al. 1996). Numerous studies provided evidence that some litter types exhibited  $^{13}\text{C}$  enrichment (Wedin et al. 1995; Osono et al. 2006, 2008). However, other studies showed  $^{13}\text{C}$  depletion (Benner et al. 1987; Asada et al. 2005; Osono et al. 2008) or no significant  $^{13}\text{C}$  variations (Mellilo et al. 1989; Schweizer et al. 1999; Connin et al. 2001) during decomposition. Additional mechanisms were put forward to explain observed  $^{13}\text{C}$  patterns; e.g., Connin et al. (2001) ascribed no discernable  $^{13}\text{C}$  discrimination in the remaining litter to selective preservation from decomposition. In this study,  $^{13}\text{C}$  in the remaining litter showed a slight enrichment with litter decay (Fig. 4a;  $R^2 = 0.06$ ), increasing 0.4‰ relative to the initial litter  $\delta^{13}\text{C}$  values. This could be caused by the movement of dissolved organic compounds into the surrounding soils (Nadelhoffer and Fry 1988), because of higher organic C concentrations in the soils with litter relative to the soils without litter in the early stages of decomposition (Fig. 5a). Besides, it has been shown that litters with high quality can result in a greater increase in the  $\delta^{13}\text{C}$  signature of the remaining residues than litters with low quality (Schweizer et al. 1999). Therefore, insignificant  $\delta^{13}\text{C}$  enrichments in the remaining litter in this study also indicate low quality of bulk litterfall in this site.

Observed  $^{15}\text{N}$  patterns with decomposition time in this study were inconsistent and rare in subtropical forests. Studies have suggested that  $^{15}\text{N}$  enrichment occurs during decomposition of litter or soil organic matter (Nadelhoffer and Fry 1988; Connin et al. 2001; Asada et al. 2005). Mellilo et al. (1989) showed a non-linear pattern in a 77-month period, with  $^{15}\text{N}$  depletion during the initial 22 months and slight  $^{15}\text{N}$  enrichment during the remainder of 55 months. Benner et al. (1991) reported that significant  $^{15}\text{N}$  depletion occurred during a period of 18 months. Connin et al. (2001) did not show a clear pattern for  $^{15}\text{N}$  dynamics during litter decomposition over 6 years. However, in this study, a non-linear pattern was found during a 20-month period, with  $^{15}\text{N}$  depletion during the initial 7 months, and  $^{15}\text{N}$  enrichment from the 8th to the 20th months during litter decomposition. One possible explanation for  $^{15}\text{N}$  depletion in the early stage is that soil microbes need additional N to attack litter with high C:N ratios (Hodge et al. 2000). Incorporation of exogenous N via soil microbes maybe lead to  $^{15}\text{N}$  depletion in the remaining litter (Nadelhoffer and Fry 1988). In the subsequent stage, microbial discrimination could play a more important role and thus lead to  $^{15}\text{N}$  enrichments in the remaining litter. In addition, Benner et al. (1991) reported that there was a significant correlation between  $^{15}\text{N}$  and N content in the remaining litter during an 18-month period in a wetland ecosystem, although our study did not show a clear correlation during a period of 20 months. Connin

et al. (2001) also did not find a tight correlation over 6 years in an arid ecosystem. This indicates N dynamics are very complicated and different functional microbial groups may be involved in N transformations which result in different  $^{15}\text{N}$  signatures during litter decomposition, but this needs a future investigation.

Although litterfall has been suggested to be important process responsible for incorporation of organic matter into soils, litter decomposition had little impact, statistically, on soil nutrients and soil organic matter in this study (Fig. 5). Two possible explanations are put forward. Firstly, in this study, we did not remove litterfall over the control plots. Litterfall on the soil surface could release some nutrients and organic compounds to soils, which would lead to insignificant differences between the soils with and without litter. Secondly, there was a high precipitation at the studied site (Fig. 2b). More nutrients or dissolved organic matter may be leached into the layer beneath the topsoil by rainfall because it has been shown that litter decomposition in tropical forests may be dominated by leaching (Cleveland et al. 2006). Considering that the plots placed with litter bags were also covered by litter, we suggest that the second explanation is more reasonable.

In summary, decomposition of bulk litter in our observed subtropical plantation is comparable with other studies in similar plantations in southern China, and nutrient return to the topsoil through litter decomposition is limited. Observed patterns of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the remaining litter with time provide a clear implication that C cycling decoupled from N cycling during decomposition and that different mechanisms were responsible for N transformation at different stages of litter decomposition in our observed subtropical plantation.

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