

# Cumulative effects of nitrogen additions on litter decomposition in three tropical forests in southern China

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**Abstract** A field-scale experiment with nitrogen (N) addition treatments was performed in three forest types – a pine (*Pinus massoniana* Lamb.) forest, a pine-broadleaf mixed forest (mixed) and a mature monsoon evergreen broadleaf forest (mature) – in tropical China. Two kinds of leaf litter, *Schima superba* Chardn. & Champ. and *Castanopsis chinensis* Hance, were studied using the litterbag technique after more than 2 years of continuous N additions. The objective of this study was to understand the cumulative effect of N addition on litter decomposition in the tropical forests and to determine if the initial effects of N addition changes over time. Results indicated that leaf litter decomposition was significantly faster in the mature forest than in

the mixed or pine forests. The mean fraction of mass remaining after 12-months of decomposition was: mature (0.22) < mixed (0.50) < pine (0.51) for the two litters. Nitrogen addition significantly depressed litter decomposition in the pine forest and the mature forest, but had no significant effect in the mixed forest. These results suggest that N deposition has significant cumulative effect on litter decomposition.

**Keywords** N deposition · Litter decomposition · Cumulative effect · Tropical forest

## Introduction

Nitrogen (N) additions have been shown to affect a range of temperate forest ecosystem properties and processes, such as net primary production and nutrient cycling. Atmospheric deposition of N remains elevated in industrialized regions of the world and are accelerating in many developing regions (Matson et al. 2002; Galloway et al. 2004), increasing concerns about the ecological effects of elevated N deposition on terrestrial ecosystems (Tietema et al. 1998; Galloway et al. 2004). The rate and process of litter decomposition greatly influences the formation of soils and humus and the availability of N and other nutrients to plants and microorganisms in forest ecosystems (Vestgard 2001; Osono and Takeda 2004). Moreover, the amount of carbon (C) returned to the atmosphere as CO<sub>2</sub> from decomposing litter is

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an important component of the global carbon budget (Aerts and De Caluwe 1997). Understanding the relationship between N deposition and litter decomposition is a key to predicting future impacts on N cycling in forest ecosystems. Most studies of the consequences of enhanced N deposition have been performed in temperate forest ecosystem in which biological processes are often limited by N supply. Little information is available about the responses in subtropical and tropical forests to atmospheric N deposition (Matson et al. 2002; Galloway et al. 2004), especially in the forests of China (Mo et al. 2006), which has the third highest N deposition following America and Europe (Holland et al. 1999). Increasing amounts of atmospheric N deposition in China has raised concerns about ecological effects on the functioning of forest ecosystems in the region (Mo et al. 2006, 2007), with litter decomposition a critical part.

Despite widespread acceptance of nutrient availability as a controller of decomposition (Swift et al. 1979), the nature of the nutrients' regulation of decomposition is far from clear (Hobbie and Vitousek 2000), and the research results are inconsistent. A stimulating effect of added N has been reported in some studies (Downs et al. 1996), while other studies have shown either no (Prescott 1995; Vitousek 1998) or a depressing (Prescott 1995; Magill and Aber 1998; Micks et al. 2004) effect on the decay rate. The main reason for the positive effect of N deposition on litter decomposition lies that N stimulated microbial activity (Fog 1998; Neff et al. 2002); and the prevailing hypothesis for the mechanism behind the variable response of decomposition to N addition tied to the interactions of N with the enzymes that catalyze degradation of cellulose and lignin, and hence to the composition of the litter (Carreiro et al. 2000).

Ecosystems have shown different responses to N additions at different N-accumulation stages (Emmett et al. 1998). For example, initially N deposition in the temperate region increased plant growth but chronic high N deposition lead to plant mortality and forest decline (Aber et al. 1998). Litter decomposition may also respond differently as N accumulates in the forest ecosystem. As the long-term effects of elevated atmospheric N inputs on litter decomposition will be a critical factor in forest ecosystem response to N deposition, it is necessary to investigate how the litter decomposition process responds to chronic N addi-

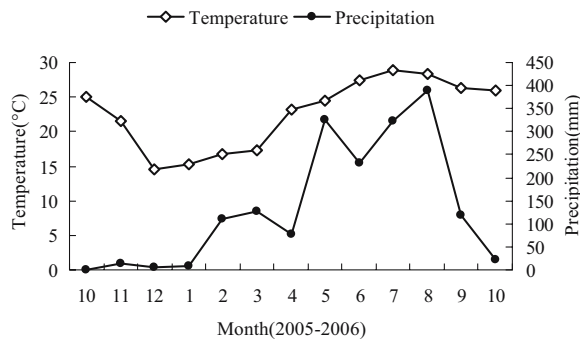
tions. We have found studies with the same litter decomposing at stands with different historic gradient of N deposition during the same time period (Kuperman 1999) or degradation of successive organic strata (litter, fragmented litter and humus) at the same site during the same time period (Berg et al. 1998), but so far to our knowledge we have not found any litter decomposition research with the same litter decomposed at the same site but during different time period after N additions.

A previous litter decomposition experiment carried out in tropical China (Mo et al. 2006) showed that after the first 18 months of N addition, litter decomposition rate increased slightly with increasing N treatment levels in a pine forest and a mixed forest; in a mature forest, some significant rate-suppressing effects were found. Here, we report the results of a litter decomposition study in the same forests, which started after 26 months of N addition. The objective of this study was to understand the cumulative effect of N addition on litter decomposition in the tropical forests and to determine if the initial effects of N addition changes over time.

## Materials and methods

### Site description

This study was conducted in three forest types, a mature monsoon evergreen broadleaf forest (mature), a pine-broadleaf mixed forest and a pine forest in the Dinghushan Biosphere Reserve (DHSBR). The reserve lies in the middle part of Guangdong Province, South China (23°19'N, 112°19'E) and occupies an area of approximately 1,200 ha. The reserve has a monsoon climate and is located in a subtropical moist forest life zone (but still located in the tropical belt; Holdridge 1967). The mean annual rainfall of 1,927 mm has a distinct seasonal pattern, with 75% of it falling from March to August and only 6% from December to February (Huang and Fan 1982). Annual average relative humidity is 80%. Mean annual temperature is 21.0°C, with an average temperature of the coldest (January) and hottest (July) month of 12.6 and 28.0°C, respectively. During the study period, precipitation and temperature largely followed this long-term seasonal pattern (Fig. 1). Nitrogen deposition was 35.6 kg N ha<sup>-1</sup> year<sup>-1</sup> in precipitation



**Fig. 1** Monthly precipitation and temperature of DHSBR during the study period from October 2005 to October 2006. Data are from Dinghushan Forest Research Station, the Chinese Academy of Science

and 39.2 kg N ha<sup>-1</sup> year<sup>-1</sup> in throughfall (below-canopy) in 1990 (Huang et al. 1994). Nitrogen deposition in precipitation was measured to be 34 and 32 kg N ha<sup>-1</sup> year<sup>-1</sup>, respectively in 2004 and 2005, and the NH<sub>4</sub><sup>+</sup>-N/NO<sub>3</sub><sup>-</sup>N ratio was roughly 1.0:1.0.

The mature forest, at about 200–350 m above sea level (meters a.s.l.), occupied 20% of the reserve, the mixed forest, at about 200 m a.s.l, occupied 50%, and the pine forest, 50–200 m a.s.l, occupied about 20% (Mo et al. 2006). The soils in the three study sites are lateritic red earth formed from sandstone with variable soil depths. Soil is deeper than 60 cm in the mature forest, from 30 to 60 cm in the mixed forest, and less than 30 cm in the pine forest (Brown et al. 1995; Mo et al. 2003). The major species in the mature forest were *Castanopsis chinensis* Hance, *Schima superba* Chardn. & Champ., *Cryptocarya chinensis* (Hance) Hemsl., *Cryptocarya concinna* Hance, *Machilus chinensis* (Champ. Ex Benth.) Hemsl., *Syzygium rehderianum* Merr. & Perry in the tree layer and *Calamus rhabdycladus* Burret, *Ardisia quinquegona* Bl. and *Hemigramma decurrens* (Hook.) Copel. in the understory layers (Mo et al. 2006). The major species in the mixed forest were

*Pinus massoniana* Lamb, *S. superba*, *C. chinensis*, *Craibiodendron kwangtungense* S. Y. Hu, *Lindera metcalfiana* Allen, *Cryptocarya concinna* in the tree layer (Mo et al. 2006). The pine forest was dominated by *P. massoniana* (Mo et al. 2006).

The monsoon evergreen broadleaf (mature) forest has been protected by monks in the temples from human impacts for more than 400 years (Mo et al. 2003, 2006). The pine-broadleaf mixed forest originated from a planted pine forest that was naturally invaded and colonized by broadleaf species and is a transitional forest from pine to monsoon evergreen broadleaf forest. The pine forest was planted in about 1930. It has been under constant human pressures most of the time since it was planted (generally the harvesting of understory and litter; Mo et al. 2003). The original site of both pine and mixed forests was badly eroded and degraded (Brown et al. 1995; Mo et al. 2003). Thus, these forests vary both in level of human impacts as well as stages of succession, site conditions, and species assemblages (Mo et al. 2003, 2006). The mean annual litter production was 8.3, 8.5 and 3.3 Mg ha<sup>-1</sup> year<sup>-1</sup>, respectively in the mature, mixed and pine forests (Zhang et al. 2000). Standing floor litter was 8.9, 20.0 and 23.7 Mg ha<sup>-1</sup>, respectively in the mature, mixed and pine forests (Fang et al. 2006). During the study period, the annual mean soil moisture (5 cm below surface) was measured to be 16.1±1.0 cm<sup>3</sup> H<sub>2</sub>O cm<sup>-3</sup> soil, 15.4±1.3 cm<sup>3</sup> H<sub>2</sub>O cm<sup>-3</sup> soil and 23.2±0.8 cm<sup>3</sup> H<sub>2</sub>O cm<sup>-3</sup> soil in the pine, mixed and mature forest, respectively (Mo et al. 2007). The mature forest had higher total C, total N and available P, but lower soil pH, C/N ratio and soil bulk density than pine and mixed forests (Table 1, from Mo et al. 2006).

#### Experimental design and treatments

A block experiment with thirty plots of dimensions 20 by 10 m surrounded by a 10 m-wide buffer strip was

**Table 1** Mineral soil properties (0–10 cm depth) in the control plots of pine (*Pinus massoniana* Lamb.) forest, pine-broadleaf mixed forest and mature monsoon evergreen broadleaf forest in tropical China

Forest	PH (H <sub>2</sub> O)	Total C (mg g <sup>-1</sup> )	Total N (mg g <sup>-1</sup> )	C/N	Available P (mg kg <sup>-1</sup> )	Soil bulk density (g cm <sup>-3</sup> )
Pine	3.93(0.08)	22.7(3.1)	1.3(0.1)	17.01(1.35)	3.59(0.28)	1.16(0.05)
Mixed	3.91(0.03)	17.3(1.2)	1.2(0.1)	14.39(1.03)	4.21(0.30)	1.22(0.01)
Mature	3.76(0.01)	32.1(2.7)	2.5(0.2)	12.84(2.27)	4.96(0.16)	0.98(0.06)

Data are cited from Mo et al. (2006). Values are mean, SE in parentheses; n=3 for all samples; measured in July 2004.

established in the three different forests in the reserve; 12 plots in mature, 9 in mixed and 9 in pine forest. Four N addition treatments (three replicate plots per treatment) were established in the mature forest, control (without N added), low-N ( $5 \text{ g N m}^{-2}$  per year), medium-N ( $10 \text{ g N m}^{-2}$  per year) and high-N ( $15 \text{ g N m}^{-2}$  per year), but only three treatments were established in mixed and pine forests (control, low-N and medium-N). All plots and treatments were laid out randomly. A  $\text{NH}_4\text{NO}_3$  solution was sprayed monthly by hand onto the forest floor (including the litter bags) of these plots as 12 equal applications over the whole year beginning in July 2003. Fertilizer was weighed, mixed with 20 l of water, and applied to the plots using a backpack sprayer below the canopy. Two passes were made across each plot to ensure an even distribution. The Control plots received 20 l water without N addition (Mo et al. 2006).

#### Litter collection

*S. superba* and *C. chinensis* are the main tree species and contribute most of the leaf litter in the three forest ecosystems (Mo et al. 2006). Leaf litter was collected using litter traps and nylon mesh placed on the forest floor under the trees in the study sites during May and June 2005, the season of peak litterfall (Zhang et al. 2000; Mo et al. 2006). All the litters were air-dried to a constant weight (Mo et al. 2006).

#### Litter decomposition and sampling

Each species of leaves was mixed to obtain a uniform mixture before filling the mesh bags. Litter bags were made of  $25 \times 25$  cm polyvinyl with  $0.5 \times 0.5$  mm openings in the bottom and  $2 \times 2$  mm in the top. Six sub-samples (about 12.00 g per sub-sample) from each kind of litter were analyzed for initial N and P concentrations. Bags were filled with 12.00 g air-dried mass. Only one litter type was put in each bag. We strung together (using nylon chord) litter bags to be harvested at a particular date. Each string contained two litter bags. On 18 October 2005, these litter bags were evenly distributed among each plot of the three forests.

Litter bags were randomly retrieved from each string at 3-month intervals. Two litter bags of each species (a total of 120 bags on each collection) were collected from each plot (12 plots for mature forest, 9

plots for pine forest and 9 plots for mixed forest) at each sample time, yielding a sample size of six for each species in each treatment. After collecting, we removed litter from litterbags and gently cleaned it of roots, soil and other extraneous materials (Mo et al. 2006). Leaf residues were oven dried at  $45^\circ\text{C}$  for 48 h and weighed (Mo et al. 2006). Initial N concentration of litter for decomposition was determined with semimicro-Kjeldahl digestion (Bremner and Mulvaney 1982) followed by detection of ammonium with a Wescan ammonia analyzed colorimetrically (Anderson and Ingram 1989).

#### Soil sampling and processing

Soil samples were collected at the end of March and of June 2006, for determining soil microbial biomass carbon (C). Litter layers were removed before soil sampling. Two composite samples of eight cores to 10 cm depth were collected using a standard soil probe (2.5 cm inside diameter) from each plot, yielding a sampling size of six cores for each treatment. The composite samples were gently mixed and stored at  $4^\circ\text{C}$  for further processing. Soil samples were passed through a 2 mm mesh sieve after removing large roots, wood and litter. Microbial biomass C was based on the difference between dissolved organic C extracted with 0.5 M  $\text{K}_2\text{SO}_4$  for 1 h on a shaker from chloroform-fumigated and unfumigated soil samples using a *Kc* factor of 0.33 (Jenkinson 1987; Vance et al. 1987).

Subsamples of litter and soil materials were dried to  $105^\circ\text{C}$ , and all results are reported on  $105^\circ\text{C}$  basis.

#### Statistics

Decomposition was determined by the fraction of the original mass remaining at different times. One-way analysis of variance (ANOVA) with LSD was used to test the difference in decomposition rate among forests and N treatments for each sampling date (Prescott et al. 1992; Mo et al. 2006). For litter decomposition, with samples collected and analyzed continuously throughout the study period, a repeated measure ANOVA with Tukey's HSD test was also performed to examine the difference in decomposition rate (values of  $X/X_0$ ) among N treatment effects, forest types and litter types (Mo et al. 2006). One-way ANOVA with LSD was also used to test the difference

in microbial biomass carbon among N treatments for each sampling date. All analyses were conducted using SPSS 10.0 for windows. Statistical significant differences were set with  $P$  values  $<0.05$  unless otherwise stated.

## Results

### Initial litter quality

Initial chemical composition was different between these two litter types. *S. superba* leaf litter had higher total N concentration and *C. chinensis* leaf litter had higher total P concentration (Table 2).

### Patterns of leaf litter decomposition in control plots

Patterns of leaf litter decomposition in control plots reflect the natural process without N additions. Decomposition rates varied depending on forest, species and the length of exposure of plant litter on the forest floor (Fig. 2). Significant differences between the mixed/pine and the mature forests were found at the sampling date after nine and twelve months for *S. superba* leaf litter, significant difference between pine and mature forests were existed at every sampling date for *C. chinensis* leaf litter ( $P<0.05$ ). When averaged over the entire study period, the mass remaining in the mature forest was significantly lower than means of the mixed and pine forests ( $P<0.05$ ), the latter two not different from each other.

After 12-months of decomposition, the mean fraction of mass remaining of the two litters was 0.22, 0.50 and 0.51, respectively in the mature, mixed and pine forests, and the difference between the mature and pine or mixed forests was significantly different ( $P<0.001$ ; Table 3). The mean fraction of mass

remaining of *C. chinensis* leaf litter was significantly lower than *S. superba* in the mature forest ( $P<0.05$ ), but no such significant difference between these two species in the pine or mixed forests (Table 3).

### Responses of litter decomposition to simulated N deposition

Litters in different forests responded differently to N additions. In the pine forest, decomposition rate decreased with increasing N treatment levels. The mean mass remaining was medium-N (0.55) $>$ low-N (0.53) $>$ control (0.51), and the difference between control and medium-N plots was significant different ( $P<0.05$ , Table 3). Decomposition of *S. superba* litter was significantly slower in the medium-N plots than in the control plots ( $P<0.05$ , Table 3). There was no statistically significant difference among treatments in *C. chinensis* litter although the mass remaining showed: control $<$ low $<$ medium (Table 3). The effect of N addition on litter decomposition rate varied depending on the length of their exposure. For *S. superba* litter, there was a significant difference in decomposition rate between control and medium plots at the time after 6 and 12 months ( $P<0.05$ , Fig. 2). To *C. chinensis* litter, a significant difference in decomposition rate between control and medium plots existed at the time after 6 months ( $P<0.01$ , Fig. 2). No such significant difference was found among treatments in the remaining sampling dates (Fig. 2).

In the mixed forest, the mean mass remaining showed: low-N (0.50) $<$ medium-N (0.52) $<$ low-N (0.54) (Table 3). Repeated ANOVA showed that there was no significant difference to any of the litter types among treatments over the entire study period due to the variation in decomposition rates between sampling dates and between treatments. One way ANOVA showed that there was no significant overall N effect on decomposition rates except for *S. superba* litter, which decomposed significantly faster in the medium-N treated plots than in the control plots (Fig. 2) at the sampling date after 3 months.

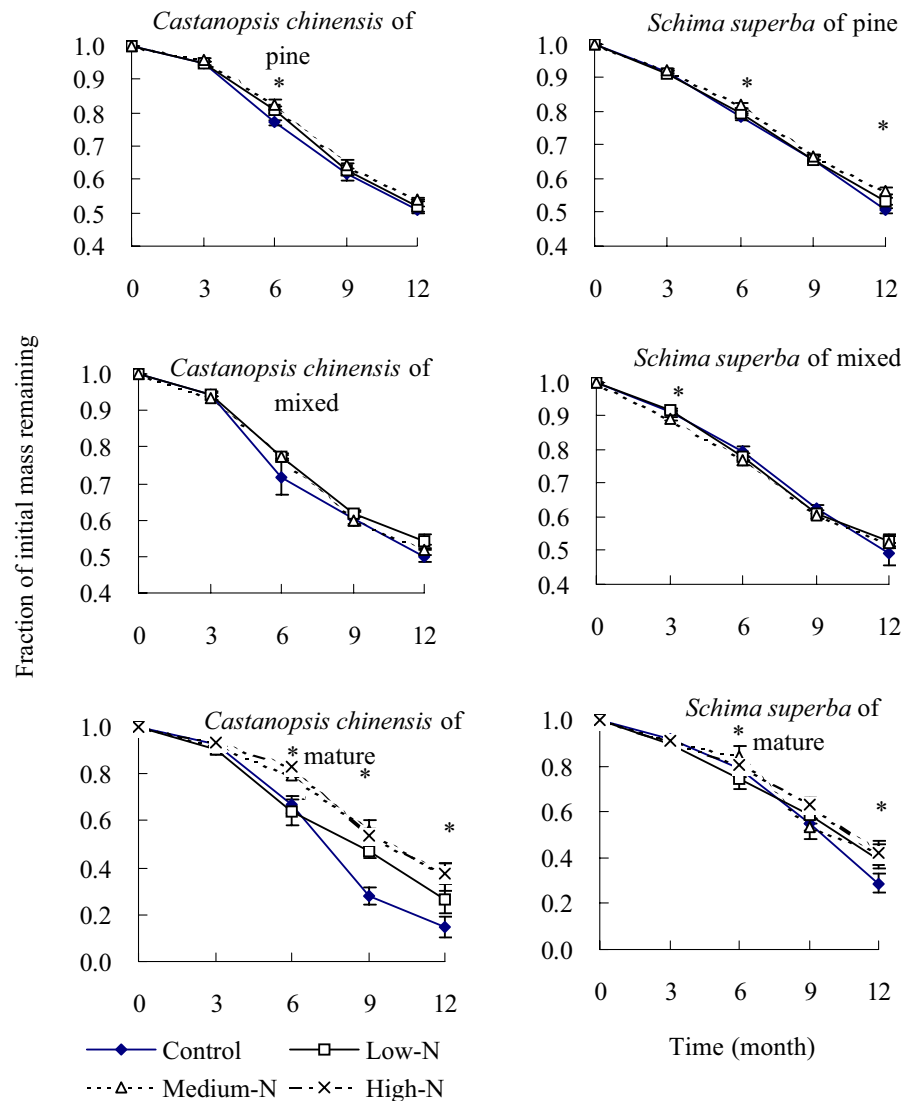
In the mature forest, decomposition of both kinds of litter decreased with increasing N treatment levels (Table 3). Decomposition of litter was significantly slower in the medium/high-N plots than in the control plots for both species ( $P<0.05$ , Table 3). The effect of N addition on litter decomposition varied depending on the length of their exposure. To *S. superba*, there was a

**Table 2** Initial chemical characteristics of leaf litter

Litter types	Total C (mg g <sup>-1</sup> )	Total N (mg g <sup>-1</sup> )	Total P (mg g <sup>-1</sup> )	C/N	C/P
<i>Schima superba</i>	445(8)	14.8(0.3)	0.56(0.01)	30.2 (0.9)	802 (20)
<i>Castanopsis chinensis</i>	445(8)	13.8(0.2)	0.70(0.01)	32.1 (0.2)	633 (6)

Data are means (SE);  $n=6$ .

**Fig. 2** Mass loss of decomposing leaf litter of two dominant tree species in various N treatments in a pine, a mixed and a mature forest. Asterisk indicates significant different at least between two treatments at  $P < 0.05$



significant difference in decomposition rate between low-N and medium-N plots at the time after six months; at the time after 12 months there were significant differences between control and medium-N and between control and high-N (Fig. 2). To *C. chinensis* litter, the depressing effect of N on litter decomposition was more pronounced and there were significant differences among N treatment levels at the sampling date after six, nine and twelve months ( $P < 0.01$ , Fig. 2).

#### Microbial biomass carbon

Soil microbial biomass C increased along the gradient of forest succession (Fig. 3). Soil microbial biomass C among the N treatments was not significantly differ-

ent in the pine and mixed forests. On the other hand, in the mature forest it was significantly lower in high-N plots than in the other ones ( $P < 0.05$ , Fig. 3).

## Discussion

### Decay pattern

Within the overall weight-loss pattern, the mass loss at the beginning was relatively slow, with less than 10% of the initial weight for both the two litter species in the first 3 months. The decay pattern was different from that of a previous leaf litter decomposition experiment, in which most of the leaf litters lost

**Table 3** The mass remaining after 12 months of leaf litters decomposition

Forest type	Species/litter type	Mass remaining (after 12 months)			
		Control	Low-N	Medium-N	High-N
Pine	<i>Schima superba</i>	0.51(0.02)a	0.53(0.03)ab	0.56(0.03)b	
	<i>Castanopsis chinensis</i>	0.51(0.02)a	0.52(0.05)a	0.54(0.02)b	
	Mean	0.51(0.02)a	0.53(0.04)ab	0.55(0.03)b	
Mixed	<i>Schima superba</i>	0.49(0.08)a	0.53(0.04)a	0.52(0.05)a	
	<i>Castanopsis chinensis</i>	0.50(0.04)a	0.54(0.05)a	0.52(0.04)a	
	Mean	0.50(0.06)a	0.54(0.05)a	0.52(0.04)a	
Mature	<i>Schima superba</i>	0.29(0.10)a	0.39(0.10)ab	0.43(0.10)b	0.42(0.09)b
	<i>Castanopsis chinensis</i>	0.15(0.09)a	0.27(0.10)ab	0.36(0.11)b	0.38(0.09)b
	Mean	0.22(0.10)a	0.33(0.10)ab	0.40(0.11)b	0.40(0.09)b

Data are means (SE);  $n=6$ ; Different letters denote significant difference ( $P<0.05$ ) between treatments.

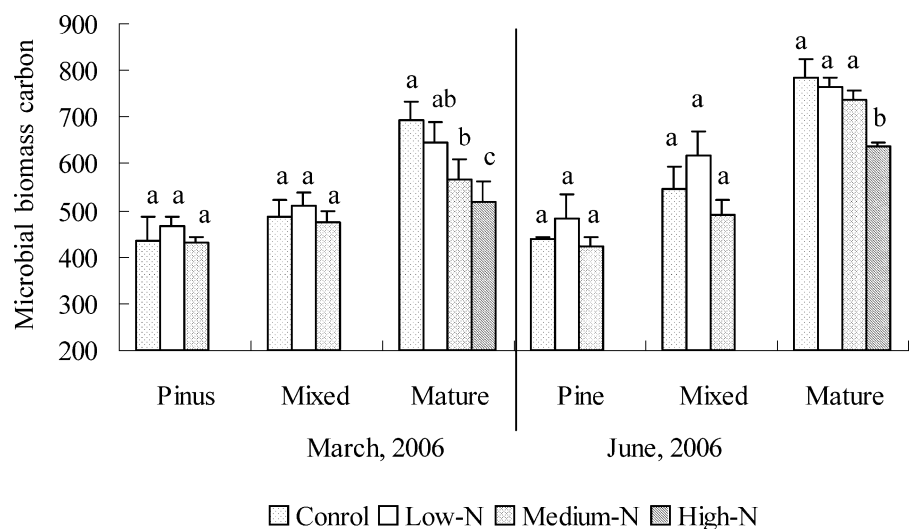
more than 20% and some even lost more than 40% of their initial mass (Mo et al. 2006). The precipitation in DHSBR was uneven and the rain season was also the warmer season (Fig. 1). The litter was placed out in October, the beginning of the dry and cool season, so the low initial decay rate was probably due to low moisture, low temperature and reduced microbial activity.

#### Mass loss: exogenous N additions

Fog (1998) concluded that N additions generally have either no effect or a negative one on litter decomposition in the long term. Our results are consistent with this conclusion: N additions had a negative effect on litter decomposition in pine and mature forest, but no effect in mixed forest.

At the beginning of this experiment, N addition increased litter decomposition rate in the pine forest (Mo et al. 2006), the decline in decomposition after more than 2 years of N addition would be due to the accumulative effect of N additions. The original site of pine forest was badly eroded and degraded (Mo et al. 2006). For example, the organic matter in the upper 20 cm soil was  $27.3 \text{ g kg}^{-1}$  in the pine forest, significantly lower than  $53.5 \text{ g kg}^{-1}$  in the mature forest (Xu et al. 2006), and so may have been N-limited. It has been suggested that chronic N additions to N-limited forests soil will initially stimulate soil microbial activity, but over time would result in a carbon-limited state after microbial demand for N was satisfied (Aber et al. 1989). An earlier experiment in the same plots showed that there were significant

**Fig. 3** Soil microbial biomass C in N-treated and control plots in a pine, a mixed and a mature forest. Soil samples (0–10 cm) for soil microbial biomass C were collected in March and June 2006. Different letters denote significant difference ( $P<0.05$ ) between treatments



differences in abundance and diversity of soil fauna between the mature and pine forests at the start of the N treatment and N additions significantly increased these indices in the pine forest; these differences disappeared after three months of low-N treatment (Xu et al. 2006).

Nitrogen addition may also have created a nutrient imbalance in the pine forest. A balance between concentrations of N and P is important because decomposition requires balanced proportions of nutrient necessary for microbial growth (Berg and Matzner 1997; Kuperman 1999). An experiment in the same area showed that N additions decreased the number of fungi (amount of mycelium) and eventually decreased the amount of bacteria (Xue et al. 2006). An experiment conducted in the same experiment plots in pine forest during the same time period showed that annual mean soil respiration rate was 5% lower in low-N plots ( $63 \pm 3 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ) and 8% lower in medium-N plots ( $61 \pm 3 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ) than in the control plots ( $66 \pm 4 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ; Mo et al. 2007), indicating that microbial activity may decrease as chronic N being accumulated in the forest. The third reason is that demand for N by decomposers may be low relative to supply, even in low-N site, because of the poor carbon quality of the litter produced at these sites (Adersson et al. 2004). Furthermore, fertilization with N may directly suppress the degradation of lignin, offsetting any stimulatory effect of N on decay of more labile carbon fractions (Fog 1998). Several studies in temperate system have demonstrated such inhibition of lignin degradation by N in situ (Berg 1986; Conn and Day 1996).

As to the mixed forest, the previous experiment showed that N addition initially accelerated litter decomposition (Mo et al. 2006), but there was no clear tendency for N addition to accelerate or reduce litter decomposition rate in this experiment. We surmise that after two years of N addition, the depressing effect of N on microorganism activity was relieved. Microbial biomass C was also not significantly different among the N treatments (Fig. 3) in the mixed forest, indicating that this forest is not yet N saturated.

In the mature forest, N additions depressed litter decomposition further than that in the previous experiment (Mo et al. 2006). Nitrogen addition did not reduce decomposition rate of *C. chinensis* leaf litter in the previous experiments but significantly

reduced in this experiment. The results for the mature forest and the difference between the two experiments may be explained by the followings. First, the mature forest is likely to be N saturated before N addition with the soil extractable inorganic N in the upper 10 cm mineral soils being  $14.9 \text{ mg kg}^{-1}$  compared with  $5.9 \text{ mg kg}^{-1}$  in pine forest and  $6.4 \text{ mg kg}^{-1}$  in mixed forest (Mo et al. 2006), so the earlier litter decomposition experiment showed no positive effect or some negative effect (Mo et al. 2006). Continuous N additions lowered the soil pH value from 3.76 in control plots to 3.70 in the high-N plots (data not published), and depressed the activity of microorganism, thus further depressing litter decomposition. Microbial biomass C decreased along the increasing N additions in the mature forest, and high-N had a significantly lower microbial biomass C than that in the control plots (Fig. 3). The adverse effect in litter decomposition may be also due to adverse effects of N addition treatment on soil and litter fauna. After N additions, the greatest decrease in abundance and diversity of soil fauna occurred in the mature forests (Xu et al. 2006). During the period of October 2005 to September 2006, annual mean soil respiration rate was 2% lower in the low-N plots ( $69 \pm 3 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ), 11% lower in the medium-N plots ( $63 \pm 3 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ) and 18% lower in the high-N plots ( $58 \pm 2 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ) than in the control plots ( $70 \pm 3 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$ ; data not published), also indicating that microbial activity was decreased as N accumulate in the forest.

Our results was supported by some study results showed that chronic N additions generally have either no effect or negative effect on litter decomposition in the long term (Fog 1998; Prescott 1995). The reasons might be that enhanced atmospheric N addition aggravates C-limitation for microbial degradation (Berg et al. 1998), depressed microbial activity (Fog 1998), depressed soil fauna activity (Xu et al. 2006), and more rapid formation of recalcitrant material (Ågren et al. 2001).

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## References

- Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in northern forest ecosystem: hypothesis and implications. *Bioscience* 39:378–386
- Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea K, McNulty S, Currie W, Rustad L, Fernandez I (1998) Nitrogen saturation in temperate forest ecosystems. *Bioscience* 48:921–934
- Adersson M, Kjoller A, Struwe S (2004) Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. *Soil Biol Biochem* 36:1527–1537
- Aerts R, De Caluwe H (1997) Nutritional and plant-mediated controls on leaf litter decomposition of *Carex* species. *Ecology* 78:244–260
- Ågren GI, Bosatta E, Magill AH (2001) Combining theory and experiment to understand effects of inorganic N on litter decomposition. *Oecologia* 128:94–98
- Anderson JM, Ingram JSI (1989) Tropical soil biology and fertility, A handbook of methods. CBA international, Wallingford, Oxford, England
- Berg B (1986) Nutrient release from litter and humus in coniferous forest soils, a mini review. *Scand J For Res* 1: 359–370
- Berg B, Matzner E (1997) Effect of nitrogen deposition on decomposition of plant litter and soil organic matter in forest ecosystems. *Environ Rev* 5:1–25
- Berg MP, Kniese JP, Zoomer R, Verhoef HA (1998) Long-term decomposition of successive organic strata in an N saturated Scots pine forest soil. *For Ecol Manag* 107: 159–172
- Bremner JM, Mulvaney CS (1982) Nitrogen total. In: Page AL, Miller RH, Keeney DR (eds) *Methods of soil analysis, Part 2: Chemical and microbial properties*, Agronomy monograph 9. Agronomy Society of America, Madison, Wisconsin
- Brown S, Lenart MT, Mo JM, Kong GH (1995) Structure and organic matter dynamics of a human-impacted pine forest in a MAB reserve of subtropical China. *Biotropica* 27: 276–289
- Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81:2359–2365
- Conn CE, Day FP (1996) Response of root and cotton strip decay to nitrogen amendment along a barrier island dune chronosequences in Hawaii. *Can J Bot* 74:176–284
- Downs MR, Nadelhoffer KJ, Melillo JM, Aber JD (1996) Immobilization of a N-labeled nitrate addition by decomposing forest litter. *Oecologia* 105:141–150
- Emmett BA, Boxman D, Bredemeier M, Gundersen P, Kjonaas OJ, Moldan F, Schleiippi P, Tietema A, Wright RF (1998) Predicting the effects of atmospheric nitrogen deposition in conifer stands, evidence from the NITREX ecosystem-scale experiments. *Ecosystems* 1:352–360
- Fang YT, Zhu WX, Mo JM, Zhou GY, Gundersen P (2006) Dynamics of soil inorganic nitrogen and their responses to nitrogen additions in three subtropical forests, South China. *J Environ Sci (China)* 18:752–759
- Fog K (1998) The effect of added nitrogen on the rate of decomposition of organic matter. *Biol Rev* 63:433–462
- Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vorosmarty CJ (2004) N cycles, past, present, and future. *Biogeochemistry* 70:153–226
- Hobbie SE, Vitousek PM (2000) Nutrient limitation of decomposition in Hawaiian forests. *Ecology* 81:1867–1877
- Holdridge LR (1967) Life zone ecology. Tropical Science Center, San Jose, Costa Rica
- Holland EA, Dentener FJ, Braswell BH, Sulzman JM (1999) Contemporary and pre-industrial global reactive nitrogen budgets. *Biochemistry* 46:7–43
- Huang ZF, Fan ZG (1982) The climate of Dinghushan. *Tropical and Subtropical Forest Ecosystem* 1:11–23 (in Chinese with English abstract)
- Huang ZL, Ding MM, Zhang ZP, Yi WM (1994) The hydrological processes and nitrogen dynamics in a monsoon evergreen broad-leaved forest of Dinghushan. *Acta Phytocologia Sinica* 18:194–199. (in Chinese with English abstract)
- Jenkinson DS (1987) Determination of microbial biomass carbon and N in soil. In: Wilson JR (ed) *Advances in N cycling in agricultural ecosystem*. CBAT National, London, pp 368–386
- Kuperman RG (1999) Litter decomposition and nutrient dynamics in oak–hickory forests along a historic gradient of nitrogen and sulfur deposition. *Soil Biol Biochem* 31:237–244
- Magill AH, Aber JD (1998) Long-term effects of experimental nitrogen additions on foliar litter decay and humus formation in forest ecosystems. *Plant Soil* 203:301–311
- Matson PA, Lohse KA, Hall SJ (2002) The globalization of nitrogen deposition, consequences for terrestrial ecosystems. *Ambio* 31:113–119
- Micks P, Down MR, Magill AH, Nadelhoffer KJ, Aber JD (2004) Decomposition litter as a sink for 15N-riched additions to an oak forest and a red pine plantation. *For Ecol Manag* 196:71–87
- Mo JM, Brown S, Peng SL, Kong GH (2003) Nitrogen availability in disturbed, rehabilitated and mature forests of tropical China. *For Ecol Manag* 175:573–583
- Mo JM, Brown S, Xue JH, Fang YT, Li ZA (2006) Response of litter decomposition to simulated nitrogen deposition in disturbed, rehabilitated and mature forests in subtropical China. *Plant Soil* 285:135–151
- Mo JM, Zhang W, Zhu WX, Fang YT, Li DJ, Zhao P (2007) Response of soil respiration to simulated N deposition in a disturbed and a rehabilitated tropical forest in southern China. *Plant Soil* 296(1–2):125–135
- Neff JC, Townsend AR, Gleixner G, Lehman SJ, Turnbull J, Bowman WD (2002) Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature* 419: 915–917
- Osono T, Takeda H (2004) Accumulation and release of nitrogen and phosphorus in relation to lignin decomposition in leaf litter of 14 tree species. *Ecol Res* 19:593–602
- Prescott CE (1995) Dose nitrogen availability controls rates of litter decomposition in forest? *Plant Soil* 168–169:83–88
- Prescott CE, Corbin JP, Parkinson D (1992) Immobilization and availability of N and P in the forest floors of fertilized Rocky Mountain coniferous forests. *Plant Soil* 143:1–10

- Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. University of California Press, Berkeley
- Tietema A, Boxman AW, Bredemeier M, Emmett BA, Moldan F, Gundersen P, Schleppi P, Wright RF (1998) Nitrogen saturation experiments (NITREX) in coniferous forest ecosystems in Europe, a summary of results. *Environ Pollut* 102:433–437
- Vance ED, Brookes SA, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* 19:703–707
- Vestgarden LS (2001) Carbon and N turnover in the early stage of Scots pine (*Pinus sylvestris* L.) needle litter decomposition: effects of internal and external nitrogen. *Soil Biol Biochem* 33:465–474
- Vitousek PM (1998) Foliar and litter nutrients, nutrient resorption, and decomposition in Hawaiian *Metrosideros polymorpha*. *Ecosystems* 1:401–407
- Xu GL, Mo JM, Zhou GY, Fu SL (2006) Preliminary response of soil fauna to simulated N deposition in three typical subtropical forests. *Pedosphere* 16:596–601
- Xue JH, Mo JM, Li J (2006) The short-term response of soil microorganism number to simulated N deposition. *Guangxi Botany* 27(2):174–179 (in Chinese with English abstract)
- Zhang DQ, Ye WH, Yu QF, Kong GH, Zhang YC (2000) The litter-fall of representative forests of successional series in Dinghushan. *Acta Ecologica Sinica* 20:938–944 (in Chinese with English abstract)