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LITTERFALL RESPONSE TO NITROGEN AND PHOSPHORUS FERTILIZATION IN SUBTROPICAL EVERGREEN BROAD-LEAVED FOREST OF 80-YEAR-OLD

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Abstract

Production and nitrogen: phosphorus (N : P) ratios in litterfall organs were studied following N and P additions in two blocks of subtropical evergreen broad-leaved forest of 80-year-old. Three plots (30 × 40 m) at each forest block were studied as not fertilized (CK), fertilized with N and N + P for 5 consecutive years. For litterfall production, leaves and reproductive organs was better than branches to predict total litterfall biomass in linear relationships. Litterfall biomass were not significantly different at each organ ($p > 0.05$). For N : P ratios, each organ was significantly different in treatments ($p < 0.001$). N fertilization significantly increased N : P ratios in all organs, and blocks only influenced reproductive organs. Moreover, N : P ratios of leaves and reproductive organs responded more strongly to soil nutrient availability than did N : P ratios of branches, and the response patterns between are similar at 0 - 10 cm and 10 - 20 cm soil depth. This result could predict litterfall production and soil nutrient in subtropical evergreen broad-leaved forest using litterfall biomass and N : P ratios of leaves and reproductive organs.

Introduction

Litterfall production and nutrients are correlated to forest growth and nutrient cycles in forest soil and serve as indicator of forest condition (Stewart *et al.* 1990, Tutua *et al.* 2008). The relationship also reflects the interaction between biological heredity of trees and physicochemical properties of soil. Early studies in this respect focused mainly on stand types, seasonal variations of litterfall decomposition and nutrient retention and recession (Eklabya and Ambasht 1987, Davis 1991, Mohan and Deepu 1992). As fossil fuel combustion elevates CO_2 and atmospheric N deposition and intensive agriculture inputs nitrogen (N) oxides, litterfall production and nutrient recycling in the forest ecosystems have been studied extensively (Hartemink 2005, Wang *et al*. 2014). N and P input could produce variation in different organ types and species of litterfall (Uselman *et al.* 2012) and increase organic carbon mineralization and microbial community. Variation in the availability of one nutrient could alter plant nutrient ratios relative to the other (Güsewell 2004, Yuan and Chen 2015, Shaver and Melillo 1984).

On the basis of environmental and artificial factors affected on N and P addition in forest ecosystem, the forest ecologists have extended the variation between production and N : P ratios at different stands years of litterfall. Maggs (1985) gave earlier discussion on litterfall and retranslocation of nutrients in burned *Pinus elliottii* plantation. Lopez-Zamora *et al.* (2001) examined the fertilization effects on N and P concentrations of needles litter in 13-year-old slash pine. Campo *et al.* (2007) studied litter N and P dynamics in 10- and 60-year-old tropical dry forests. Santiago *et al.* (2012) compared N and P concentrations in five species of 200-year-old tropical forest. The N : P ratios could increase in difference organ types, such as either an increase in N availability or a decrease in P availability (Güsewell 2004, Townsend *et al.* 2007). N : P ratios of foliage is used to indicate relative nutrient availability to plants and soils (Olsen and Bell 1990, McGroddy *et al.* 2004).

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Studies mentioned above potentially contributed to the biomass variation in growth organs of litterfall. For example, the variation of stem, leaf and branch was apparent in N and P addition. In addition, the N : P ratios of growth organs could be a response indicator to soil nutrient. Besides, stem, root and older leaf N : P ratios are more responsive indicators of soil nutrient availability than new foliage (Schreeg *et al.* 2014). However, it is rarely involved the biomass of reproductive organs to fertilization, and the N : P ratios of litterfall response to nutrient of different soil depth. Therefore, it was necessary to obtain the variation of vegetative and reproductive organs biomass with fertilization and N : P ratios of organs could indicate the N : P ratios of soil at different depth.

Seasonal dynamics of litterfall biomass under specific conditions is preferred. However, it is known that these conditions may vary depending on the species and growth phase. It is also preferred, which N : P ratios of growth organs is a better indicator response to soil nutrient. In this study, the variability of biomass and N:P ratios of vegetative and reproductive litter in a 80-year-old evergreen broad-leaved forest in the northern subtropics has been studied. The relationship between total litterfall and organs biomass and the N : P ratios of each organ in relation to N : P ratios of soil at different depth has also been investigated.

Materials and Methods

The study was conducted in mature subtropical forest at National Zhawan Forestry Farm (29°35′N, 117°12′E) in southern Anhui Province, China. In the area, mean annual precipitation is 1700 mm and mean annual temperature is 17ºC. Complete block design was set on mid-slope and top-slope because dominant tree species parallel the gradient. The dominant tree species of the two blocks were *Castanopsis eyrei*, *C. eyrei* and *Cyclobalanopsis glauca,* respectively. Both the selected blocks of the forests were 80 years old (Table 1). Within each blocks, N addition, N and phosphorus (N + P) addition and control (CK) treatments were designed. Replication was not set in each treatments because stand ages parallel the gradient. The six experimental plots were all set as 30 × 40 m and the minimum distance between them was 500 m.

The fertilization experiment, along with responses of litter productivity to fertilizer additions, was started in 2010. The factorial experiment of N and P was applied to 30 × 40 m plots at rates of 100 and 50 kg P/ha/y, respectively. N was added as NH_4NO_3 and P as triple superphosphate ($Ca(H_2PO_2)_2$), with fertilizer added by hand during growth season (in middle May, July and September each year). No fertilizer was added in CK plots.

After one year of fertilization, litterfall was measured using 12 litter traps placed regularly in each plot. These traps had a circular opening of 1.2 m in diam. and was positioned 1.2 m above the soil surface. The litterfall in the traps was collected at two monthly intervals from September 2011 to July, 2014. Collected litterfalls were separated as leaves, branches (d 3 cm), reproductive organs and then weighted. The sub-samples were placed in labeled and sealed bags for laboratory analysis. All subsamples were oven dried to a constant weight at 60°C to determine the percentage of moisture and to calculate the litterfall biomass of individual organs. Meanwhile, soil samples were also taken at 0 - 10 and 10 - 20 cm from each plot, and air-dried, sieved, and stored in labeled and sealed bags. N and P concentrations were analyzed for all components of litterfall and soil. The concentration of total N was determined by dry combustion with EA-3000 CN Analyser (Vector, Italy). The total P was determined by HNO_3-$HCLO_4$ digestion and detection by induced coupled plasma spectrometer (ICPS-2000, Shimadzu Co., Japan).

Three analyses were performed in the study: (1) Seasonal dynamics of total litterfall biomass, which was done to investigate its various pattern and relationships between total and individual organ biomass and finally to see which organ is better predictor of total litterfall biomass. (2) ANOVA was used to test the variance of biomass and N : P ratios for each organs. Differences in variance between the blocks and treatments were evaluated using a two-way factorial ANOVA. Three F tests, which compared two groups (blocks and treatments), were used to examine the variance in different organ litterfall. (3) Linear mixed models, to evaluate the N : P ratios of each organ to soil at 0 - 10 and 10 - 20 cm depth. Log likelihood ratio tests were used to select among nested models. Fixed effects included in different years (from 2011 to 2014). Random effects included blocks and treatments. An initial model included all fixed effects. Blocks and treatments were added to this initial model using log likelihood ratio tests (Zuur *et al.* 2009). All analysis was conducted in R sofeware (Team 2012).

Table 1. Structure characteristics of the experimental forest.

Blocks (dominant tree)	Treatments	Age (year)	DBH (cm)	Height (m)	Density ($n \cdot hm^{-2}$)
	CK	80	17.8	17.6	1267
Castanopsis eyrei	N	80	17.5	16.5	1308
	N+P	80	18.0	17.2	1334
Castanopsis eyrei + *Cyclobalanopsis glauca*	CK	80	15.0	14.1	1311
	N	80	15.3	14.6	1284
	N+P	80	15.5	15.3	1295

Results and Discussion

The seasonal dynamics of total litterfall biomass showed similar pattern in the three treatments (Fig. 1). The annual variation between the highest and lowest peak occurred in November and March, of which the peaks were caused by annual growth of forest stand. Leaf fall determined the pattern of total litterfall contributed 58.3, 61.9 and 61.5% of CK, N and N + P treatments, respectively. The seasonal dynamics of litterfall production did not change with N and P additions.

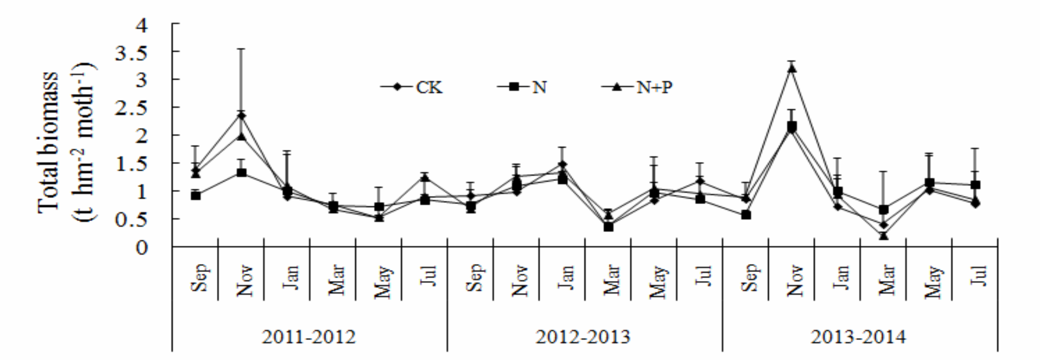

Fig. 1. Seasonal dynamics of litterfall biomass indifferent treatment.

In linear relationships, leaves, branches and reproductive organs were used to fit total litterfall biomass (Fig. 2). P values of leaves and reproductive organs were < 0.001, whereas the P value of branches was 0.331 ($p > 0.05$). R^2 of reproductive organs and leaves were 0.542 and 0.376, respectively. From P values, both leaves and reproductive organs were better indicator than branches in predicting total litterfall biomass in linear relationships. Thus, leaves and reproductive organs were more responsive to predict total litterfall biomass than branches.

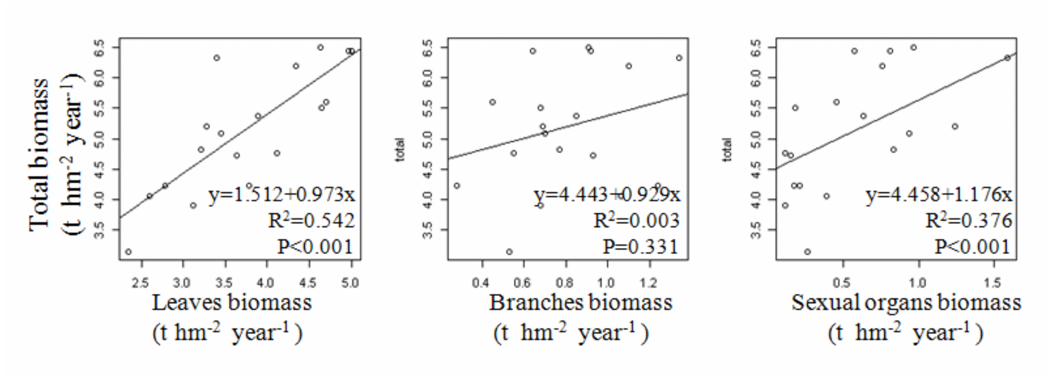

Fig. 2. Linear relationships between tissue and total litterfall biomass.

For each organ, differences of litterfall biomass were not significant ($p > 0.05$), but differences of N : P ratios were extremely significant between treatments ($p < 0.001$, Table 2). The average biomasses were 3.772, 0.796 and 0.615 t hm^{-2} $year^{-1}$ for leaves, branches and reproductive organs, respectively. The average N : P ratios of leaves and branches were 23.18 and 37.88 in N addition treatment. But the average values were 15.92, 20.50 and 17.708, 17.41 in CK and N + P addition treatments, respectively.

For reproductive organs, the differences of N : P ratios were significant both in blocks and treatments ($p < 0.001$). The average N : P ratios of reproductive organs were 20.52, 31.04 and 20.18 in the treatments of CK, N addition, N + P addition, respectively. However, the average values were 32.57 and 15.26 in difference blocks. Thus, N : P ratios were significantly increased with N addition in all organs, and blocks only influenced reproductive organs.

In linear mixed models, the N : P ratio of each organ was better responses than soil's N : P ratios in 0 - 10 and 10 - 20 cm depth. Log likelihood ratios were used to compare the variance in fixed and random effects. For fixed effects of different years, variances were not significant among organ types ($p > 0.05$, Table 3) at two soil depth. The random effect of block was highly significant ($p < 0.001$) in each organ at 0 - 10 cm and in leaves and branch at 10 - 20 cm, which was only significant ($p < 0.01$) in reproductive organs at 10 - 20 cm. The random effect of treatment was significant in leaves and reproductive organs ($p < 0.01$). Thus, the N : P ratios of organ responded to soil were not different at different depth, and N : P ratios of leaves and reproductive organs was more responsive indicators of soil nutrient than branches.

Litterfall is usually shown strong seasonal pattern which is driven by climatic conditions (McGrath *et al.* 2000). The highest peak and lowest peak in present study occurred consistently in November and March, particularly in the 2011 and 2013 year. The climatic temperature and precipitation were mainly the factors to affect the peak patterns (Gerdol *et al.* 2011). From November to March, temperature and precipitation were lowest and as a result the peak occurred during this period. The peak was not evident in 2012, because in this year the temperature and precipitation were higher than the other years. The seasonality of litter biomass in this study did not agree with the results reported in other subtropical evergreen broad-leaved forests (Xu *et al.* 2004, Xu 2013). These reports were located in Japan, and litterfall production was mainly driven by typhoon. But the location of the present study has rarely been effected by typhoon.

The litterfall contains leaves, branches and reproductive organs. Biomass of each organ did not vary among the blocks and treatments. It indicated that stand types (dominant tree) and fertilization had no effect on litterfall biomass in subtropical evergreen broad-leaved forest. The litter biomass in the present study was 6.32 t/hm^2/year raging from 5.76 to 6.55 t/hm^2/year in average. It is slightly lower than in other subtropical forests (Xu *et al.* 2004, Tutua *et al.* 2008, Xu 2013). Most of litterfall was contributed by leaves, which was amount to 60.7% in total biomass. However, needle litterfall is an available variable to predict litterfall and above-ground biomass in pine stand (Huebschmann *et al.* 1999, Starr *et al.* 2005, Gonçalves Jr *et al.* 2014). Results from the present research indicate both leaves and reproductive organs are variables to predict litterfall production in linear relationships.

Table 2. Variance of biomass and N : P ratios among blocks and treatments.

Tissue types	Factors	Biomass			N : P ratios		
		Df	F value	Pr (>F)	DF	F value	Pr (>F)
Leaves	Blocks	1	0.383	0.683	1	0.268	0.606
	Treatments	2	0.09	0.765	2	94.508	<0.001
	Blocks : Treatments	5	0.357	0.701	5	0.009	0.991
Branches	Blocks	1	0.439	0.646	1	0.203	0.653
	Treatments	2	0.017	0.897	2	94.508	<0.001
	Blocks : Treatments	5	1.701	0.188	5	0.339	0.713
Reproductive organs	Blocks	1	0.814	0.446	1	489.68	<0.001
	Treatments	2	1.176	0.281	2	94.508	<0.001
	Blocks : Treatments	5	0.198	0.82	5	19.11	<0.001

Table 3. Log likelihood ratios test significant fixed and random effects in linear mixed-model.

Soil depth	Tissue type	Fixed effects	Random effects	
			Treatments	Blocks
0-10 cm	Leaves	1.851	15.861**	121.851***
	Branches	1.043	1.521	293.929***
	Reproductive organs	0.962	10.237**	30.655***
10-20 cm	Leaves	1.826	16.343**	113.585***
	Branches	0.982	1.122	121.851***
	Reproductive organs	0.654	8.0314**	8.865**

*p < 0.05,**p < 0.01, ***p < 0.001.

Although the litterfall production was not different between the blocks and treatments, differences of N : P ratios were significant in treatments. Present results showed that litterfall N : P are higher in N treatment than in CK and N + P treatment, and litterfall N : P in CK is similar to that in N + P treatment. Because N increases are higher than P increases with N fertilization, resulting in higher N : P and P fertilization can induce a shift in N : P (Yuan and Chen 2015). Global changes have drastically affected the biogeochemical cycles of nutrient elements of earth's ecosystems (Gruber and Galloway 2008). Globally, the input of N in the fields occurs from intensive agricultrure and fossil fuel combustion but P input occurs only from fertilization of crop lands (Peñuelas *et al.* 2012, Peñuelas *et al.* 2013). N : P ratios of litterfall has a increasing trend in subtropical evergreen broad-leaved forest.

Contrasting variation of N : P ratios in growth organs, differences of N : P ratios of reproductive organs are significant in various blocks. The nutrient concentrations of litterfall organs vary among sampling stands of subtropical evergreen broad-leaved forest (Xu 2013). The sampling stands are mainly different in the dominant tree, and our two sampling blocks were *Castanopsis eyrei* and *Cyclobalanopsis glauca*. In addition, interaction of sampling stands and fertilization also effects the N : P ratio of reproductive organs. The effects of N : P supplied on reproductive organs correlate closely with species, suggesting tha it is not only because of fertilization (Güsewell 2004). The reproductive organs of litterfall is mixed flowers and fruits in our studies, so the variation of N : P ratios is not clearly between flower and fruit.

In the present investigation, N : P ratios of litterfall are used to infer N : P ratios of soil at 0 - 10 and 10 - 20 cm depth. There are similar response patterns at different soil depth. N : P ratios of plant organs inferring soil nutrient is availability and popularity method in forest studies (Koerselman and Meuleman 1996, Santiago *et al.* 2012, Turner *et al.* 2013). As the search for the most appropriate plant organ for linking N : P ratios to soil nutrient availability progresses (Sayer *et al.* 2012), stem, root, and older leaf N : P ratios are more responsive indicators of soil nutrient availability than new foliage in five species tree (Schreeg *et al.* 2014). Our results show that N : P ratios of leaves and reproductive organs responded more strongly to soil nutrient availability than did N : P ratios of branches, and response patterns are similar at different soil depth.

It is concluded that N : P ratios of litterfall are more susceptible to fertilization than litterfall production. Moreover, biomass and N : P ratios of both leaves and reproductive organs reflect better on litterfall production and soil nutrient availability than does the N : P ratio of branches. It is clear that the N : P ratios of response patterns between litterfall organs and soil are similar at 0 - 10 and 10 - 20 cm soil depth.

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