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TITLE:

Flower litters of alpine plants affect soil nitrogen and phosphorus rapidly in the eastern Tibetan Plateau

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Flower Litters of Alpine Plants Rapidly Affect Soil Nitrogen and Phosphorus in the Eastern Tibetan Plateau

Abstract

Litters of reproductive organs have been rarely studied, despite their role in allocating nutrients for offspring reproduction. This study determines the mechanism through which flower litters efficiently increase the available soil nutrient pool. Field experiments were conducted to collect plant litters and calculate biomass production in an alpine meadow of the eastern Tibetan Plateau. Carbon, nitrogen, phosphorus, lignin, cellulose, and their relevant ratios of litters were analyzed to identify their decomposition features. A pot experiment was performed to determine the effects of litter addition on soil nutrition pool by comparison between the treated and control samples. Litter-bag method was used to verify decomposition rates. The flower litters of phanerophyte plants were comparable with non-flower litters. Biomass partitioning of other herbaceous species accounted for 10%–40% of the aboveground biomass. Flower litter possessed significantly higher N and P levels but less C/N, N/P, lignin/N, and lignin and cellulose concentrations than leaf litter. Flower litter fed soil nutrition pool more efficiently because of their faster decomposition rate and higher nutrient contents. Litter-bag experiment confirmed that the flower litters of *Rhododendron przewalskii* and *Meconopsis integrifolia* decomposes approximately three times faster than mixed litters within 50 days. Moreover, the findings of the pot experiment indicated that flower litter addition significantly increased the available nutrient pool. Flower litter influenced nutrition cycling in alpine ecosystems, as evident by its non-ignorable production and significantly faster decomposition. The underlying mechanism can enrich nutrients, which return to the soil, and non-structural carbohydrates, which feed and enhance the transitions of soil microorganisms.

Key words alpine ecosystem, flower litter, chemical property, decomposition rate, nitrogen, phosphorus

The growth and health of plants in their life history have been considerably influenced by variations in the physical, chemical, and biological properties of soil, particularly around the rhizosphere, although soil properties can also be mediated by plants. Plant properties directly affect the productivity and function of an ecosystem (Chapin et al., 1986; Chapin, 2003; Berendse and Aerts, 1987; Grime, 1998). In a natural environment, plants continuously lose N and P in their whole life history and even during litter production and decomposition (Laungani and Knops, 2009; Richardson et al., 2009). N is a major constituent of several important plant substances (Vitousek and Howarth, 1991). Most plants absorb N through soil compounds to support their growth. The plant residue is one principal component of soil organic matter, whose decomposition can supply available N to plants and microorganisms. Similar to nitrogen, P is closely associated with numerous vital plant processes. Nevertheless, in most circumstances, P is limited because of its small concentration in soil; this element is released slowly from insoluble P but is highly demanded by plants and microorganisms (Bieleski, 1973; Richardson et al., 2009). As decomposition is a prolonged process, plants contain concentrated nutrients comparable with soil, which have significant effects on the biogeochemical cycle and feedbacks of plant–soil interaction. However, these nutrients cannot be simply absorbed again to the soil nutrient pool supplied by plants and microorganisms (Bieleski, 1973; Berendse and Aerts, 1987).

In cold life zone ecosystems, plant biomass production is limited by N (Körner, 2003). Litter tends to be recalcitrant in cold environments (Aerts, 1997). In addition, N is a key factor that determines the outcome of interspecific competition in temperate-zone ecosystems (Laungani and Knops, 2009). Several studies reported that litter can mediate the interactions between neighboring plants in infertile communities (Nilsson et al., 1999, Xiong and Nilsson, 1999). In a succulent desert ecosystem in Africa, fertile islands are formed in nutrient enrichment zones beneath shrubs; this formation is attributed to a range of interactions between physical and biotic concentrating mechanisms (Stock et al., 1999). In China, an experiment performed in an alpine meadow ecosystem, the eastern Tibetan Plateau, indicated that soil N

availability and supply rates, as well as microbial biomass, can be enhanced by *Stellera chamaejasme* L., which is an unpalatable poisonous weed that seriously deteriorated the local rangeland (Sun et al., 2009). Another study in the gully region of the Loess Plateau demonstrated that black locust improves most soil properties (Qiu et al., 2010). Plants enhance the microbial immobilization of N when they provide C to soil microorganisms. The nature of litter determines its palatability to soil organisms, thereby influencing their composition and activity levels. Furthermore, a few apparent effects of N may be caused by the low levels of polyphenols, which is associated with high N concentrations in litter (Haynes 1986). The rate of decay and concentrations of nutrients in the litter determine the rate of nutrient release, which creates a positive feedback to site fertility. Hence, the chemical properties of litters from different plant organs and their correlations with decomposition rate must be determined.

Although inflorescences comprise only a small fraction of plant biomass and production in Arctic and alpine vegetation, the inflorescence production can be a significant proportion of the total production of species under certain special circumstances (Martínez–Yrízar et al., 1999, Fabbro and Körner, 2004; Wookey et al., 2009). Reproductive tissues present chemical composition that differs from vegetative parts, resulting in a markedly faster decomposition and nutrient release, with repercussions on nutrient cycling and patchiness (Buxton and Marten, 1989; Lee et al., 2011). High contents of N and P exist in the reproductive organs of plants probably because of their essential roles in plant growth and formation (e.g., high protein content). Alpine ecosystems are thermally restricted and characterized by a low material turnover rate (Körner, 2003). In a high altitude region, plants grow in a harsh habitat that restricted their effective utilization of resources; in this regard, the total available resource is less compared with that of plants in other regions (Fabbro and Körner, 2004; Hautier et al., 2009). In long-term evolution, the allocation of accumulated carbohydrates to reproduction is an adaptation strategy, leading to the partitioning of reproductive organs, that is, the availability and timely mobilization of adequate resources from the vegetative plant body to reproductive structures (Arroyo

et al., 2013). Thus far, probably due to reproductive organs' comparatively minor biomass production and difficult to be collected, studies on their decomposition have been limited particularly compared with those on leaf and other vegetative organs.

A fast decay of N-rich litters suggests that litter decay rates increase with increasing N content. The initial rate of nutrient release is positively correlated with the initial concentrations of N or P (MacLean and Wein, 1978; Aber and Melillo, 1980; Berg and Ekbohm, 1983; Yavitt and Fahey, 1986; Stohlgren, 1988). In agricultural systems, addition of fresh residues can stimulate the decomposition and net release of N from indigenous soil organic matter (Haynes, 1986; Scott et al., 1996). Long-term increases in N availability have also been reported following the additions of C to forests (Groffman, 1999). Recently, a common-garden decomposition experiment in a wide range of subarctic plant types demonstrated that structural and chemical traits are better predictors for several high-turnover organs than structural traits alone (Freschet et al., 2012). Decomposition rate of plant litters slightly differ because of their species-specific traits and various organs, whose chemical qualities vary in a wide range of plant types and environments. Thus, field investigation, pot experiment of litter addition, and litter-bag experiment were conducted in this study to address the following:

1) Should decomposition of flower litter be considered according to inflorescence biomass production, and/or allocation?

2) What are the unique chemical properties of flower litters that influence their faster decomposition rate compared with leaf litters?

3) Is pulsed effect evident on soil available N and P particularly in special temporal period and spatial location as determined through pot experiment?

Materials and Methods

Study area

The field site is located at the foot of Mt. KaKa, which belongs to the middle section of Minshan Mountain, eastern Tibetan Plateau (**Fig. 1**), with a mean annual precipitation of 720 mm. More than 70% of precipitation falls in summer from June to August. Snowfall usually occurs from the end of September to the next early May.

Vegetation presents a typical alpine meadow with numerous and unique alpine plants. Mosses are abundant and cover most of the ground. The moss layer is dominated by *Polytrichum swartzii* and *Trematodon acutus* c. mull. Vascular plants include species mainly belonging to genus *Kobresia* and genus *Carex*. Other common species are *Festuca* spp., *Gentiana* spp., and *Leontopodium* spp.. Plant roots in this ecosystem are generally confined to the surface A-horizon (2–20 cm). A few dwarf shrubs are scattered sporadically in the meadow, e.g., *Rhododendron* and *Salix*. The soil type is dominated by Mat Cry-gelic Cambisols (i.e., silty loam inceptisol, *Chinese Soil Taxonomy Research Group*, 1995).

Plant and soil sampling

During the blooming period from the end of May until mid-June and from the end of July until early August, flower litters of 14 earlier flowering plants species and 15 later flowering plants species were carefully collected in 2012 at two sites, namely, Mt. KAKA (103°42′ E; 32°59′ N, 3500–3900 m a.s.l.) and Bow Ridge Mountain (103°42′ E; 33°1′ N, 3600–3850 m a.s.l.). In the study, 4 litter traps were placed under the crown of each individual shrub, which were processed and modified based on the litterfall monitoring protocol (Muller-Landau and Wright, 2010). The litter trap was composed of 1 cloth bag and 4 support legs. Window screen (with a mesh size of 0.8 mm) was used to seize the cloth bag. Its size was about 50 cm deep and 25 cm length of a side. 4 legs (made by 80 cm PVC pipe) were tied with cloth bag and frame. The frame of opening was made of iron wire with 3 mm diameter. After inserting it into the soil under the shrub's crown, the plant litter was collected twice per week, which was later sorted as flower litter and other types during the blooming period. Due to the small size of herbaceous individuals, flowers were just plucked at the end of flowering phase and their mass ratios to aboveground biomass were calculated. Freshly fallen leaves of different species were collected from the floor of the alpine meadow (i.e. mixed leaf litters, ca. 3950 m a.s.l.). These species were tentatively classified into five groups according to Raunkiaer's life-form system (i.e., chamephyte, geophyte, hemicryptophyte, phanerophyte, and therophyte). These species were divided into earlier flowering species and later flowering species two

groups based on blooming time. According to Raunkiaer's life-form system, earlier flowering species mainly consisted of hemicryptophyte, geophyte, and phanerophyte, whereas more than half of later flowering species comprised chamaephyte. Nearly half of the tested species were dominant or co-dominant in their respective communities. The dry matter content of flower litters in all of the species was ranked from 10% to 60%. Mixed leaf litter of alpine meadows were sampled on the Mt. Kaka (3950 m. a.s.l.), and leaf litters of 13 dominant species were also collected to compare their chemical properties with flower litters. Both types of litters were first spread on blotting paper for air drying. A small portion of each litter was further dried in an oven for 48 h to calculate dry matter content.

Experimental design

Polyvinyl chloride (PVC) pots (15 cm deep, 20 cm diameter at the top, and 12 cm diameter at the bottom) were filled with 2 kg of soils, which were collected in autumn of 2011. The collected soil samples were stored at 4 ℃. The samples were sieved through were sieved through 2 mm mesh and then mixed thoroughly. The soil surface of each treatment was added with 5 g of flower litters or mixed litters (calculated as dry weight) on June 21 (14 species, earlier flowering plants) and Aug 11, 2012 (15 species, later flowering plants). The surface was covered with a thin layer of soil to avoid being blown by wind. Other two additional treatments were conducted without litter addition (control) and with mixed leaf litter addition, respectively. In total, the pot experiment consisted of 33 treatments with three replicates, with a total number of 99 pots. All of the pots were carefully buried 12 cm deep into the field to maintain the same soil temperature in the experimental field. The pots were randomly distributed, and their top edges were approximately 3 cm above the ground to prevent runoff from outside. All of the pots were rearranged every week to create a similar microclimate. After 50 days, each soil sample was collected from three points of each pot in the center and then mixed to avoid the boundary layer effect. Each soil sample from different PVC pots was mixed evenly by sieving through a 2 mm mesh respectively. The samples were stored and marked separately in an ice box prior to chemical determination.

Decomposition rate

A litter bag with a size of 14 cm × 20 cm was used to determine the decomposition rate of different plant litters. The bag was double faced and made from nylon net material with above (4.5 mm × 4.5 mm mesh) and below layers (0.8 mm × 0.8 mm mesh). The above layer with bigger mesh size allowed free access for most micro-arthropods, which dominate the soil fauna of alpine meadow in the eastern Tibetan Plateau, whereas the below layer with smaller mesh size can reduce litter spillage from the litter bags in the process. As representative species, flower litters of *R. przewalskii* and *M. integrifolia* and mixed litter were packed into litter bags with the edges sealed on June 21, 2012. The litterbag experiment was conducted to compare the decomposition rate of flower litters and mixed litter. Each treatment had eight replicates. After 7 weeks (August 8, 2012), litter was obtained from the litter bags and dried in an oven for decomposition calculation. Litter decomposition rates can be determined by the following equation.

$$\mathbf{DR = (P-R)/P \times 100}$$

where *DR* is the decomposition rate, *P* is primary litter mass in the litter bags, and *R* refers to residue litter before determining percentage mass loss.

Chemistry determination of soil and plant

For soil samples, total dissolved N (TN) contents were determined using unsieved fresh moist soil subsamples. Soil subsamples were extracted using 2 M KCl and shaken for 1 h at room temperature (20 ℃), with a soil-to-solution ratio of 1:5 (weight/volume). The extracted solution was filtered through filter paper before further determination (Jones et al., 2004). NH_4^+-N and NO_3^--N were analyzed with the indophenol blue colorimetric (Sah, 1994) and ultraviolet spectrophotometry methods (Norman et al., 1985), respectively. Dissolved organic nitrogen (DON) was calculated by subtracting dissolved inorganic N (NH_4^+-N and NO_3^--N) from TN. Soil solutions were extracted by centrifugal drainage, whereas the exchangeable pool was extracted with 2 M KCl by using the methods reported by Jones et al. (2004). Total phosphorus (TP) and A-P in soils were estimated by extraction with 0.5 M sodium hydroxide

sodium carbonate solution (Dalal, 1973). Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) contents were determined through the chloroform–fumigation direct-extraction technique. Correction factors of 0.54 for N and 0.45 for C were used to convert the chloroform labile N and C to microbial N and C (Brookes et al., 1985). For plant samples, the contents of C and N were determined by dry combustion with a CHNS auto-analyser system (Elementar Analysen systeme, Hanau, Germany) (Brodowski et al., 2006). The content of P was obtained colorimetrically by the chloro molybdophosphoric blue color method after wet digestion in a mixture of HNO3, H2SO4, and HClO4 solution (Institute of Soil Academia Sinica, 1978). Lignin and cellulose were estimated by the method described by Melillo et al (1989).

Data analysis

One-way ANOVA was applied to compare values between the treatments and the control. Post-hoc multiple comparisons were adopted when the groups were three or more. Multivariate ANOVA was conducted to determine the effects of blooming time and different addition of litters and their interactions. To simplify the comparison of soil N and P between control (without flower litter) and the treated (with flower litter), we defined an index **α** as: **α** = Ln (N_2/N_1). $\alpha > 0$, $N_2 > N_1$; $\alpha < 0$, $N_2 < N_1$; $\alpha = 0$, $N_2 = N_1$. N_1 is the control treatment without flower litter, and N_2 indicated nutrition value (N or P) of flower litter treatment. Descriptive analysis was operated to demonstrate α values of different N and P fragments in various species litters addition treatment. The box plots provide the distribution of the values by the medians (central line), the quartiles 25% and 75% (box), and the ranges (whiskers) of ratios. Differences were tested at $P < 0.05$ by using Tukey multiple range test in SPSS 19.0 software package (SPSS Inc., Chicago, IL, USA). The normality of data was tested with one-sample K-S test and Q-Q plot. Otherwise, log-transformation was adopted to meet the normality requirement. Homogeneity of variance test was also utilized during the analysis. In the figures and tables, information is presented as means and standard errors of means. All of the differences were tested at the $P = 0.05$ level.

Results

Flower litter production of dominant species and their biomass allocation

Among 13 dominant species, the flower litters of phenerophyte plants, whose flower litters are comparable with non-flower litters, were calculated through comparison with non-flower litters during the flower litter collection (**Fig. 2 (a)**). The dry weights of flower litters were 10–40 g m^{-2}, whereas their non-flower litters were only 5–25 g m^{-2}. Although neither of the flower litters of *S. angustata* nor *R. capitatum* were significantly different compared with their non-flower litters ($P > 0.05$), the difference between the two remained noticeable, whose values were 28.03 ± 3.56 g m^{-2} versus 13.21 ± 1.49 g m^{-2} for *R. capitatum* and 19.58 ± 3.50 g m^{-2} versus 12.95 ± 0.61 g m^{-2} for *S. angustata*, respectively. The production of flower litters was higher than that of non-flower litters. The other three species significantly produced more flower litters than non-flower litters (*R. przewalskii*: $F = 15.76$, $P < 0.001$; *P. fruticosa*: $F = 4.76$, $P < 0.05$; *S. alpine*: $F = 10.18$, $P < 0.01$). The flower litters of the eight herbaceous species were compared with their individual aboveground biomass (**Fig. 2 (b)**), which ranked from 10% to nearly 40%. This finding indicated that flower litter should be considered to determine the effect of plants on soil nutrition pool during growing season.

Comparison of chemical properties between flower and leaf litters

Total C content was not significantly different between flower and leaf litters (**Fig. 3 (a)**, $F = 1.80$, $P = 0.199$). However, the levels of cellulose, lignin, and structure C of leaf litter were significantly higher than those of flower litter ($F = 6.74$, $P < 0.05$; $F = 5.77$, $P < 0.05$; $F = 10.99$, $P < 0.01$). Hence, flower litter probably contains more non-structure C than leaf litter.

Both N and P contents of flower litters were significantly higher than those of leaf litters (**Fig. 3 (b)**). N in flower litters was nearly doubled to that of leaf litter (23.17 ± 1.52, 11.87 ± 0.77; $F = 45.70$, $P < 0.001$). More than twice the amount of P were also present in flower litters compared with that in leaf litters (2.95 ± 0.25, 1.12 ± 0.12; $F = 43.87$, $P < 0.001$).

For the implication of the ratio of different chemical properties, C/N, N/P, and lignin/N were determined to compare flower and leaf litters. All the three indicators of

leaf litter were significantly higher than those of flower litters (**Fig. 3 (c)**). As parameters used to demonstrate decomposition rate, C/N and lignin/N of leaf litter were nearly double to those of flower litter (39.27 ± 4.16, 19.80 ± 1.39, $F = 37.78$, $P < 0.001$; 21.09 ± 2.25, 12.79 ± 1.15, $F = 7.91$, $P < 0.01$). Furthermore, N/P of flower litter was significantly higher than that of leaf litter (8.42 ± 0.42, 11.60 ± 0.56; $F = 20.62$, $P < 0.001$). These findings indicated that flower litter can supply more P per unit N than leaf litter.

Effects of flower litter on different fragments of soil N pool and P pool

Earlier flowering species exerted positive effects on soil TN, NO_3^--N, and NH_4^+-N (**Fig. 4 (a)**), with the addition of their flower litters according to their size of **α** values. Most parameters were higher than 0, which indicated that $N_2 > N_1$. Flower litter increased soil N pool. All of the minimum **α** values of five indices were also higher than 0 (**Table 2**, 0.42–1.29), which indicated that flower litter addition significantly increased different fragments in soil N pool ($P < 0.001$). Among the later flowering species, except *G.* sino-*ornata* and *L. sinense*, soil N indices were significantly improved with flower litter addition, as demonstrated through **α** values higher than 0 (**Fig. 4 (b)**, **Table 2**). Later flowering species differed from earlier flowering species, with minimum **α** values lower than 0, which resulted from the exceptions of *G.* sino-*ornata* and *L. sinense*. However, all of the mean **α** values were higher than 0, which presented general results after flower litter addition (0.36–1.49). Different fragments of soil N pool were significantly enhanced only after 50 days ($P < 0.001$). Interactions between flowering time and litter addition for NO_3^--N, and NH_4^+-N were significant ($F = 5.043$, $P < 0.05$; $F = 7.947$, $P < 0.01$; $F = 24.143$, $P < 0.05$, respectively) but not for TN ($F = 0.470$, $P = 0.496$). Different flowering times significantly affected NO_3^--N, and NH_4^+-N (**Table 3**, $P < 0.01$) but did not significantly influences TN ($F = 2.80$, $P = 0.10$). As illustrated in **Fig. 4**, litter addition had significant effects on all of the N fragments, which was in accordance with the results in **Table 3**. The interaction of flowering time and litter addition exerted similar effects on different N fragments in soil with flowering time solely.

Flower litters exerted different effects on soil TP and A-P. Soil TP increased in

treatment with early flowering litters (**Fig. 4**, **Table 3**, $F = 8.498$, $P = 0.007$) but not in later flowering litters. The minimum **α** values were lower than 0 (−0.04 and −0.20, respectively). However, A-P of both litter treatments was significantly positively stimulated ($F = 47.39$, $P < 0.001$; $F = 68.82$, $P < 0.001$), whose **α** values were both higher than 0 (0.67–0.13 and 0.06–0.37, respectively). Multifactorial analysis indicated that soil TP was not significantly different between treated with flower litter and control in general (**Table 3**, $F = 1.07$, $P = 0.37$). No significant interaction was evident between flowering time and litter addition treatments on soil TP ($F = 0.01$, $P = 0.93$). Litter addition treatments alone only had a marginal significant effect on soil TP ($F = 3.17$, $P = 0.08$). Moreover, both minimum **α** values were lower than 0, but TP was not significantly different between treatments with later flowering litters and control treatment ($F = 0.97$, $P = 0.33$), which mainly resulted from *G. sino-ornata*, *L. sinense*, and *C. lineare*. Nevertheless, A-P increased significantly after flower litter addition ($F = 43.01$, $P < 0.001$), with a significant interaction between flowering time and litter addition ($F = 6.44$, $P < 0.05$).

Table 2 α values of different N and P fragments in various species litters addition treatment (n = 14 and n = 15 in earlier flowering species and later flowering species, respectively).

Flowering period	Index	Mean	Std. Error	Minimum	Maximum	*F*	*P*
Earlier flowering	TN	1.67	0.06	1.29	2.05	719.05	**0.000**
	NO_3^--N	1.67	0.07	1.08	2.23	563.90	**0.000**
	NH_4^+-N	0.97	0.12	0.42	2.06	68.25	**0.000**
	TP	0.02	0.03	-0.04	0.08	8.498	**0.007**
	A-P	0.31	0.17	0.67	0.13	47.39	**0.000**
Later flowering	TN	1.29	0.21	-0.37	2.40	38.37	**0.000**
	NO_3^--N	1.11	0.18	-0.75	1.55	37.77	**0.000**
	NH_4^+-N	0.36	0.05	-0.09	0.72	60.64	**0.000**
	TP	0.03	0.11	-0.20	0.12	0.97	0.33
	A-P	0.50	0.23	0.06	0.37	68.82	**0.000**

Table 3 Multifactorial analysis of variance for the effects of flowering time, litter addition, and their interactions on different N and P fragments.

Source of variation	TN		NO_3^--N		NH_4^+-N		TP		A-P	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Corrected Model	59.25	**0.00**	59.25	**0.00**	54.07	**0.00**	1.07	0.37	43.01	**0.00**
Flowering time	2.80	0.10	2.80	0.10	24.36	**0.00**	0.02	0.90	6.44	**0.01**
Litter addition treatments	173.47	**0.00**	173.47	**0.00**	117.00	**0.00**	3.17	0.08	114.14	**0.00**
Flowering time × Litter addition treatments	2.80	0.10	2.80	0.10	24.36	**0.00**	0.02	0.90	6.44	**0.01**

Note: ***P*** values for significant effects and interactions are in bold.

Effects of flower litter addition on soil solution N pool and soil MBC and MBN

Soil solution N pool has been improved noticeably from 31.46 mg g^{-1} to 47.35 mg g^{-1} in flower litter treatment compared with the control, particularly in fragment of NO_3^--N, which has been greatly increased (from 30.93 mg g^{-1} to 46.8 mg g^{-1}). (**Table 4**). In mixed leaf litter treatment, there were no obvious variations after litter decomposition, with 32.4 mg g^{-1} NO_3^--N and 0.45 mg g^{-1} NH_4^+-N, respectively. There were notable differences of both MBC and MBN between different treatments. Litter addition not only increased soil microbial biomass C (102.05 mg kg^{-1}, 68.08 mg kg^{-1}, and 46.25 mg kg^{-1} for flower litter, mixed litter, and control, respectively) and MBN (73.02 mg kg^{-1}, 69.29 mg kg^{-1}, 67.13 mg kg^{-1} for flower litter, mixed litter, and control, respectively) but also their C:N ratios (1.40, 0.98, and 0.69 for flower litter, mixed litter, and control, respectively).

Table 4 Comparing median value of soil solution pool and soil microbial biomass between litter addition treated (flower litter and mixed leaf litter) and control.

Treatments	Soil solution N pool (mg g^{-1})		Soil microbial biomass (mg kg^{-1})		
	NO_3^--N	NH_4^+-N	MBC	MBN	MBC/MBN
Flower litter	46.8	0.55	102.05	73.02	1.40
Mixed leaf litter	32.4	0.45	68.08	69.29	0.98
Control	30.93	0.53	46.25	67.13	0.69

Comparison of decomposition rate between flower litter and mixed leaf litter

Two typical plant species, which are widely distributed and easily collected, were assessed to compare the decomposition rate of flower litter and mixed litter. Differences in decomposition rate among flower litter of two species and mixed litter

were supposed to be significant (**Fig. 5**, $F = 130.34$, $P < 0.001$). The flower litters of *R. przewalskii* and *M. integrifolia* decomposed greatly faster than mixed litter. However, within only 50 days, more than 20% of *R. przewalskii* and *M. integrifolia* flower litters decomposed, whereas the decomposition rate for mixed litter was approximately 6% (i.e., the former was nearly three times faster). Moreover, no significant differences were evident in the decomposition rates of the flower litter of *R. przewalskii* and *M. integrifolia* ($P = 0.371$).

Discussion

Plant litter decomposition is a critical step in the formation of soil organic matter, mineralization of organic nutrients, and C balance in terrestrial ecosystems (Austin and Ballaré, 2010). Species-specific variations in plant phenology can affect production of litter fall, which is noticeable during the growing season from the aspect of nutrient cycling although the peak of litter fall happens in autumn. Thus, the early litter fall of alpine plants during the study period from May to August can be a potential nutrient source when nutritional demands increase for rapid growth and development. In particular, the amount of flower fall in study area exceeds the leaf fall during the flowering season. A previous study indicated that reproductive litter production accounted for < 10% of the total litter in January–August and 13%–26% in September–December (Sanches et al., 2008), which was mainly triggered by rainfall variability that directly altered litter production dynamics and indirectly altered forest floor litter. In addition, the flowers are more nutritional than the leaves in terms of nutrients necessary for plant growth (Lee et al., 2011). In this study, summit production of flower litters are booming during special periods for both earlier flowering and later flowering species. Flower biomass of herbaceous plants accounts for 10% to approximately 40% of total aboveground biomass. Moreover, these flower litters produced considerably earlier than other aboveground litters that dropped at the end of growing season. Furthermore, flower litters and non-flower litters (mainly constituted of leaves) of woody plants were 10–40 g m^{-2} and 5–25 g m^{-2}, respectively, which clearly implies that flower litter can be a comparable decomposition substrate

in alpine ecosystems even for phenerophyte plants.

Litter production and decomposition are controlled by biological and physical processes, such as the activity and composition of soil and litter fauna and climate variations (Meentemeyer, 1978; Cornejo et al., 1994; Wieder and Wright, 1995; Aerts, 1997; Cleveland et al., 2004). An integration of index or traits has been recommended to indicate process and rate of litter decomposition. Generally, tissues with high lignin, polyphenol, and wax contents and higher lignin:N and C:N ratios exhibit slow decomposition. The effect of litter quality on decomposition rates was extensively discussed in the literature, and C/N and lignin/N ratios have been commonly accepted as main explanatory factors (Melillo et al., 1982; Berg, 2000). Leaf litter with C/N ratios lower than 30 is known to decompose easily and yield a mull humus type, whereas C/N ratios above 30 result in N immobilization (Heal et al., 1997) and decomposition retardation. In the present study, flower litter had significantly less C/N ratio (19.80 ± 1.39, less than 30) than leaf litter (39.27 ± 4.16, more than 30). Lignin content in flower litters was significantly less than that in leaf litters (211.37 ± 8.63 mg kg^{-1} and 237.88 ± 6.89 mg kg^{-1}, respectively; $F = 5.77$, $P = 0.02$), similar to cellulose (266.93 ± 4.92 mg kg^{-1} and 283.75 ± 4.21 mg kg^{-1}, respectively; $F = 6.74$, $P = 0.01$), which is one of the major cell-wall constituents. All of the results are in accordance with previous studies. Decomposition rate is negatively correlated with the concentration of lignin, which is a group of complex aromatic polymers that serves as a structural barrier impeding microbial access to labile C compounds (Swift et al., 1979; Taylor et al., 1989; Austin and Ballaré, 2010; Talbot and Treseder, 2012). Moreover, greater non-structural carbohydrates existed in flower litters than those in other litters, as indicated by the absence of significant differences of total C content between flower litters and other litters. However, the structural carbohydrates of flower litters were significantly less than that of leaf litters. This finding can be inferred from the contents of lignin and cellulose (**Fig. 3 (a)**). Hence, flower litters can promote nutrients that easily complement soil (Parton et al., 2007) for plants in their whole life history. Decomposition rates of leaf litters have been considered recently from their lignin/N or lignin/cellulose (Talbot and Treseder, 2012; Cornwell

et al., 2008). Furthermore, in the present study, lignin/N was less in flower litters (almost 50% in leaf litters, i.e., 12.79 ± 1.15 and 21.09 ± 2.25, respectively), whereas N/P was higher than that of leaf litters.

A litterbag experiment was adopted and confirmed that the decay rates of flower litters were significantly faster than that of other litters, which is in accordance with the fast decomposition of *R. pseudoacacia* flower from an experiment performed in Korea (Lee et al., 2010). Flower litters contained significantly higher N and P contents than leaf litters (**Fig. 3 (b)**). Plant litter available to the decomposer community encompasses a broad range of issues that differ in chemical and physical properties (Swift et al., 1979). P has to be highlighted because it has been regarded as essential for a long time, which causes a limited attention on mechanisms that drive P limitation and their interactions with the N cycle (Vitousek et al., 2010). Although soil generally contains a large amount of total P, only a small proportion is immediately available for plant uptake from the soil solution. In most soils, the concentration of orthophosphate in solution is low (Richardson et al., 2009). P is derived mainly from rock weathering and related biogeochemical cycle, and ecosystems begin their existence with a fixed complement of P, and even very small losses cannot be readily replenished (Walker and Syers, 1976). The present study indicated that decomposition of flower litter can be one of the beneficial source of soil A-P in alpine ecosystems. Nevertheless, the current study regarding the characteristics and driven mechanism of this source remains at the first stage. Variation in soil physical-chemical properties, vegetation types, and microbial activities can significantly affect chemical compositions and forms and biological availability of soil P directly or indirectly.

Decay rates of different plant organs reflect the diversity that fruits decompose faster than leaves, which in turn decompose faster than woody plant parts (Swift et al., 1979; Kögel–Knabner, 2002). Flower litters decompose rapidly with higher N and P levels supplied to soil, particularly from NO_3^--N in soil solution pool (**Table 4**). Histogram for **α** values of DIN and A-P also presented soil available nutrients positively stimulated by flower litter (**Fig. 6**) for their values distributed at an interval greater

than 0. The high DOC values in flower litter may influence N and P in soil through C substrate supplement for soil microorganisms to enhance N immobilization. Recent empirical studies noted that the changing microbial community composition significantly affects ecosystem processes, such as litter decomposition (Strickland et al., 2009; Ramirez et al., 2012). Shifts from bacterial-dominated to fungal-dominated decomposition happened over short (days to a few months) periods (Poll et al., 2008; McMahon et al., 2005). Although the present study did not present the precise analysis of microbial community, both MBC and MBN differed greatly between different treatments (**Table 4**). Litter addition increased them obviously, which is evident not only in microbial biomass C and N but also in their C:N ratios (1.40, 0.98, and 0.69 for flower litter, mixed litter, and control, respectively). Therefore, microbial community composition varied depending on nutrient supplement from litters. Flower litter contains more than twice MBC (increased from 46.25 to 102.05); hence, microbial biomass and their activities enhance potentially.

Several unexpected species in the experiment reduced soil available nutrients probably because their specific chemical properties, which change as a result of microbial activities and nutrient dynamics (Karmarkar and Tabatabai, 1991), may negatively affect soil microorganism biomass or activities (Wardle et al., 1998, Cipollini et al., 2012). Furthermore, soil microbial communities can be modified through time in response to allelopathic plants with known or potential effects on plant communities (Cipollini et al., 2012, Inderjit and Weiner, 2001). Mineralization and nitrification can be subdued by inhibitory compounds from the exudates of a certain plant species, which come from a negative aspect and mainly result from suppression of related microbes (Cipollini et al., 2012). In another positive perspective, considering "*priming effect*" once flower litter is added in moderate treatments causes strong short-term changes in the turnover of soil organic matter and nutrient release follows litter decomposition (Jenkinson et al., 1985; Kuzyakov et al., 2000; Blagodatskaya and Kuzyakov, 2008). Hence, N and P availability in the soil of alpine ecosystem can be maintained in part by tissue chemistry favorable to microbial decomposition and release of nutrients.

This study provides evidence that plant species, through tissue chemistry, biomass allocation, and phenology, affect local soil properties in alpine ecosystem. Soil has specific susceptibility to decomposition of biochemical compounds in plant tissues, on a spectrum from quickly decomposed labile to relatively recalcitrant. Decomposition rates can be markedly affected by particle size, surface area, and mass characteristics (Angers and Recous, 1997). In addition, physical toughness (lignin, dry matter content, or C content) can be suitable predictors of decomposition across all of the organs. Structural (lignin, DMC) and chemical (N) traits together are proposed to be better predictors for several high-turnover organs than structural traits alone (Freschet et al., 2012). Flower litters have these intuitive benefits chemically and physically, but physical components of litter quality have received little attention in the research on litter quality. Future climate changes in temporal patterns are likely to have important direct and indirect consequences on litter dynamics as well as on phenology and decay process temporally and spatially. In brief, the question of the essentiality and fundamentality of litter decomposition, especially under natural conditions, remains unresolved although the key role of litter quality in decomposition and in ecosystem function is generally clear.

Acknowledgment

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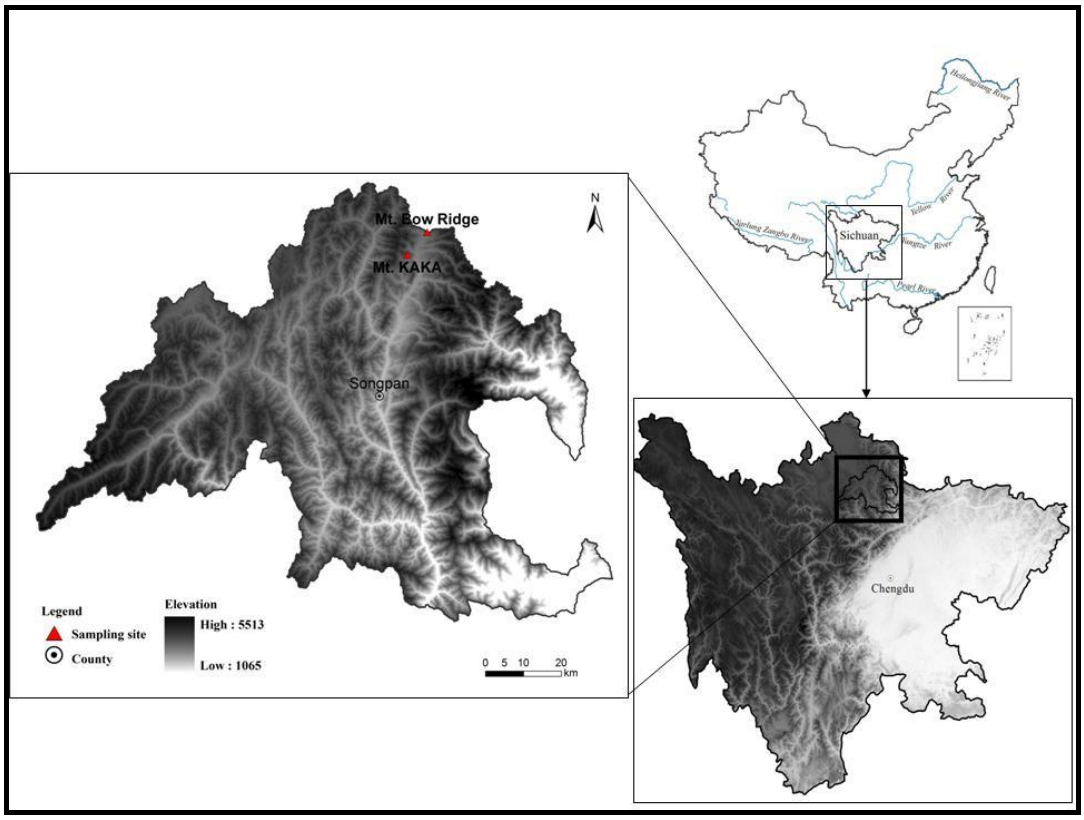
644 **Figures**



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646 **Fig. 1** Location of the study sites.

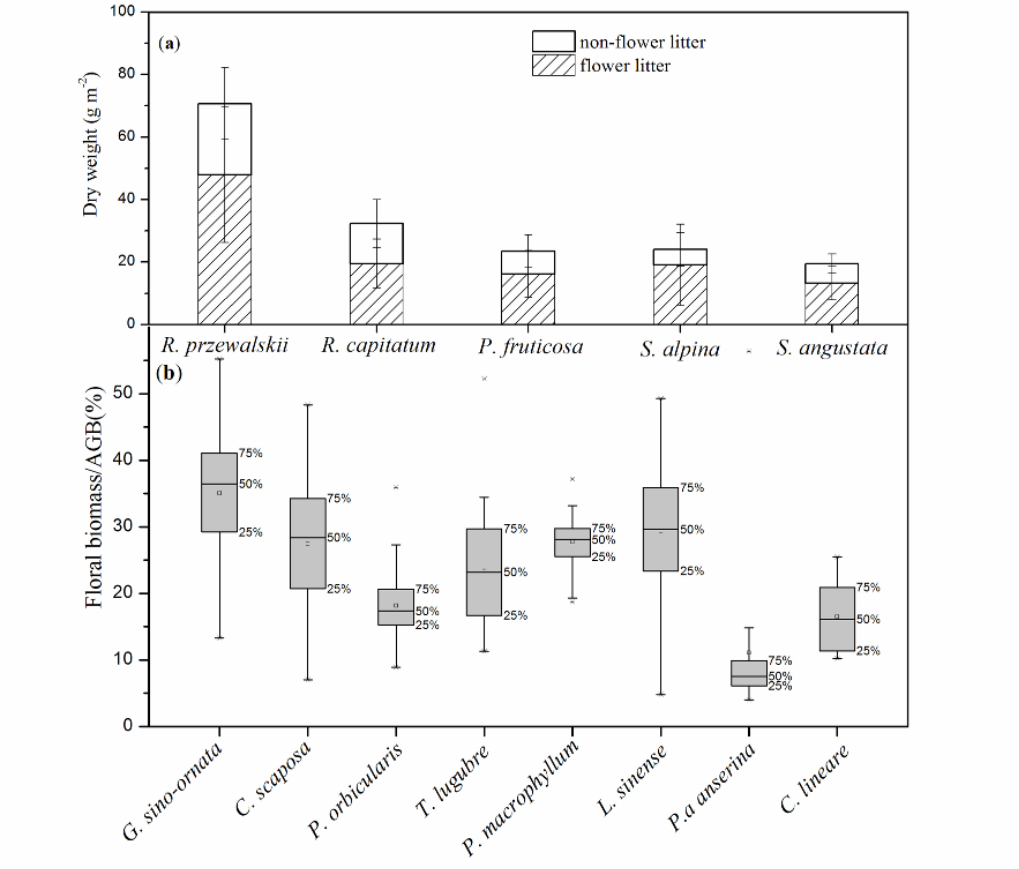

Fig. 2 Production of flower litters and biomass allocation of representative dominant species. (a) Production of flower litters and non-flower litters of shrubs (phaenerophyte) per unit area (m^2); and (b) floral biomasses and their allocation in the aboveground biomass.

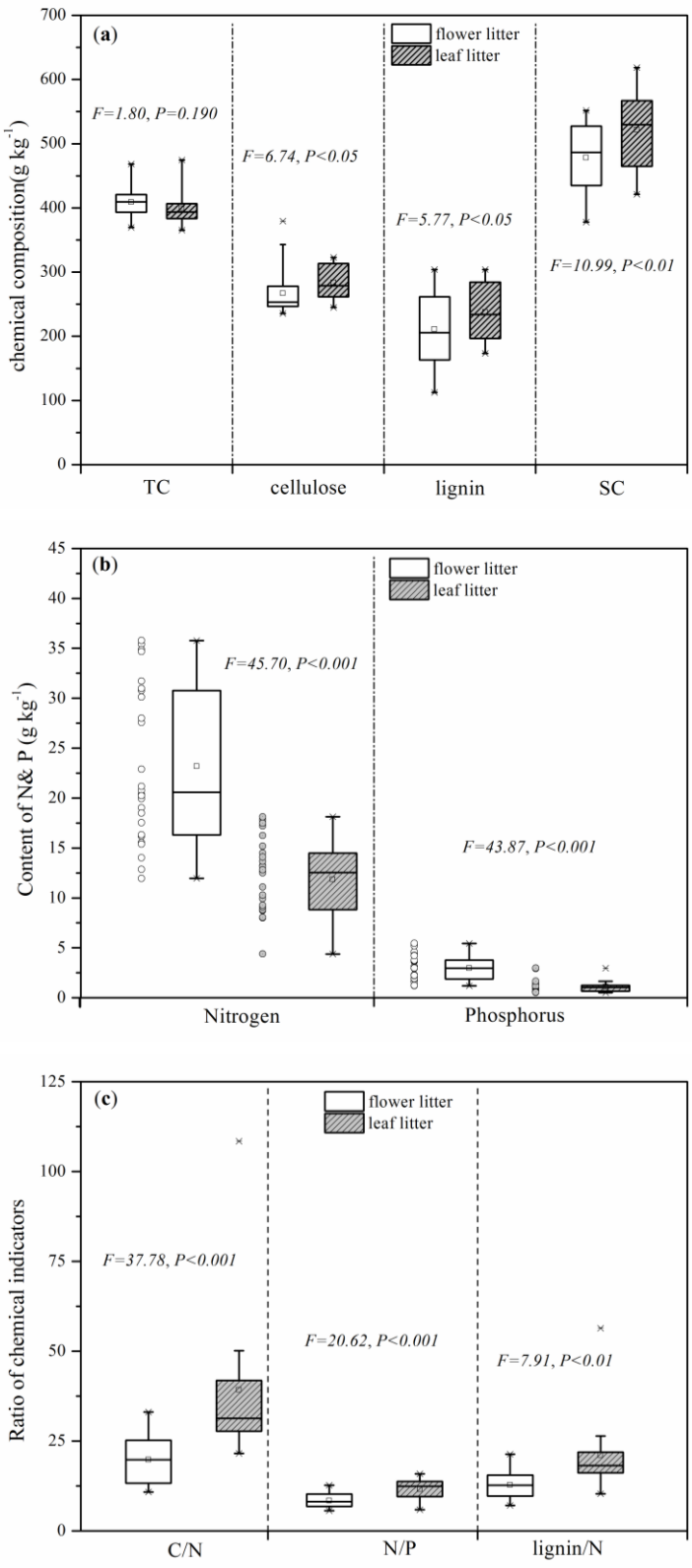

Fig. 3 Chemical composition and their comparison between flower and leaf litters.

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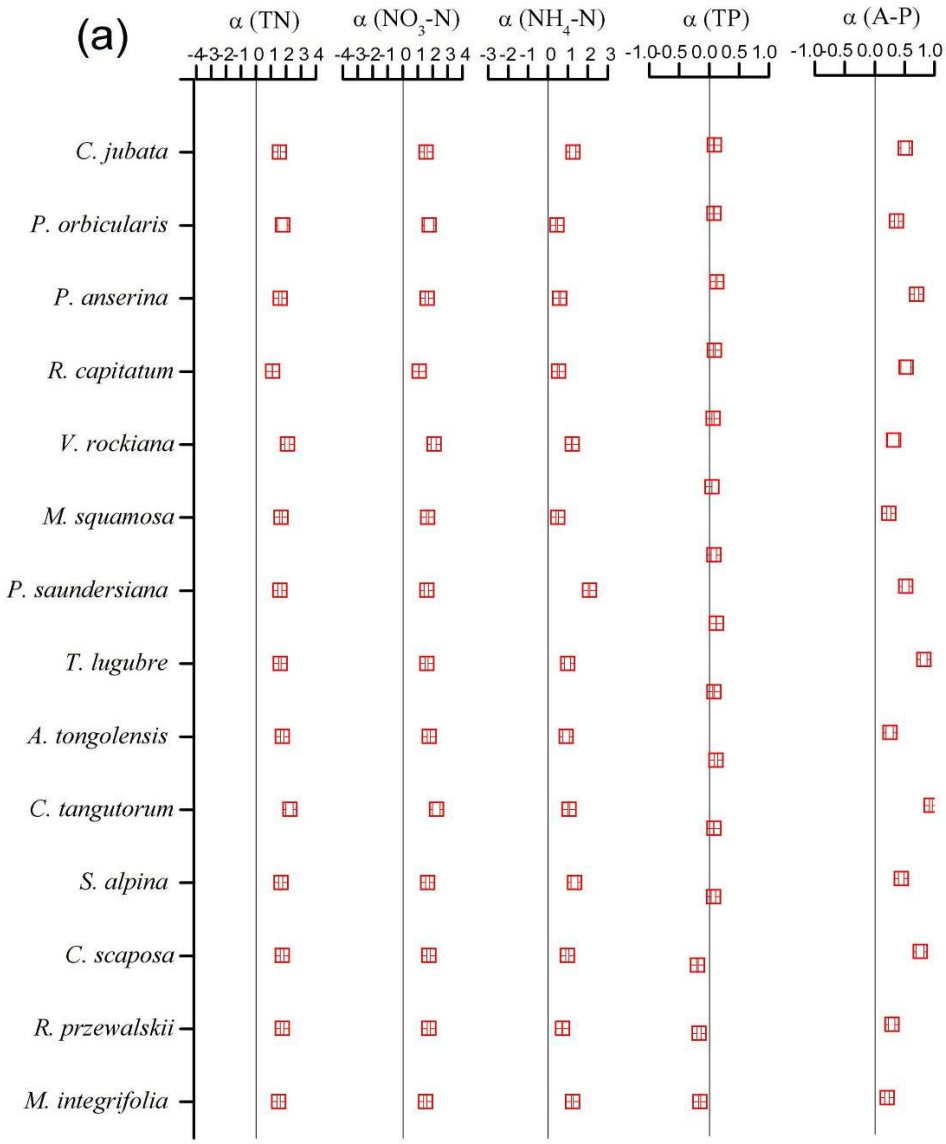
(a)
α (TN)
α (NO3-N)
α (NH4-N)
α (TP)
α (A-P)
-4 -3 -2 -1 0 1 2 3 4
-4 -3 -2 -1 0 1 2 3 4
-3 -2 -1 0 1 2 3
-1.0 -0.5 0.0 0.5 1.0
-1.0 -0.5 0.0 0.5 1.0
C. jubata
P. orbicularis
P. anserina
R. capitatum
V. rockiana
M. squamosa
P. saundersiana
T. lugubre
A. tongolensis
C. tangutorum
S. alpina
C. scaposa
R. przewalskii
M. integrifolia

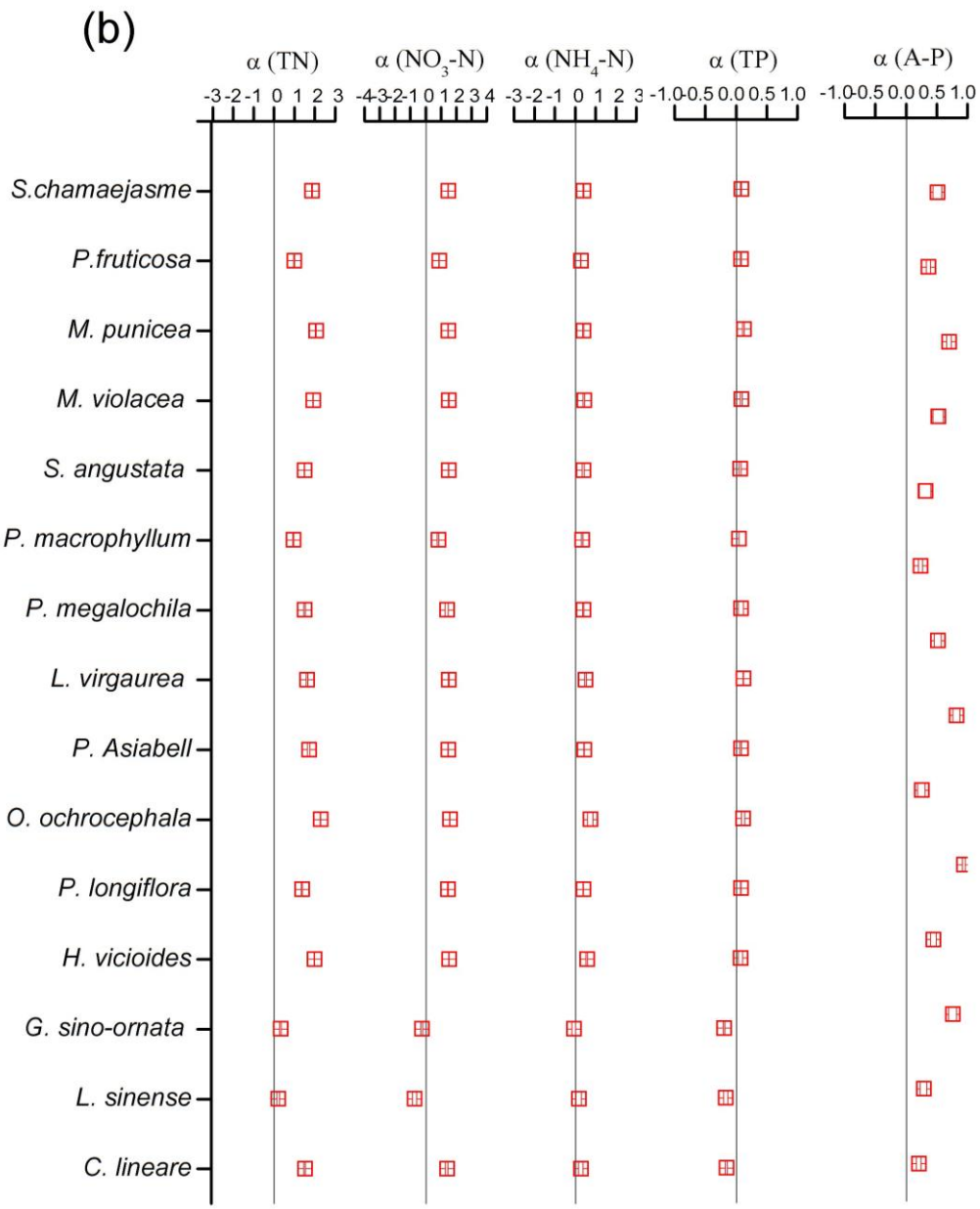

Fig. 4 Variation in soil N pool and P pool after addition of flower litters, (a) earlier flowering species, and (b) later flowering species.

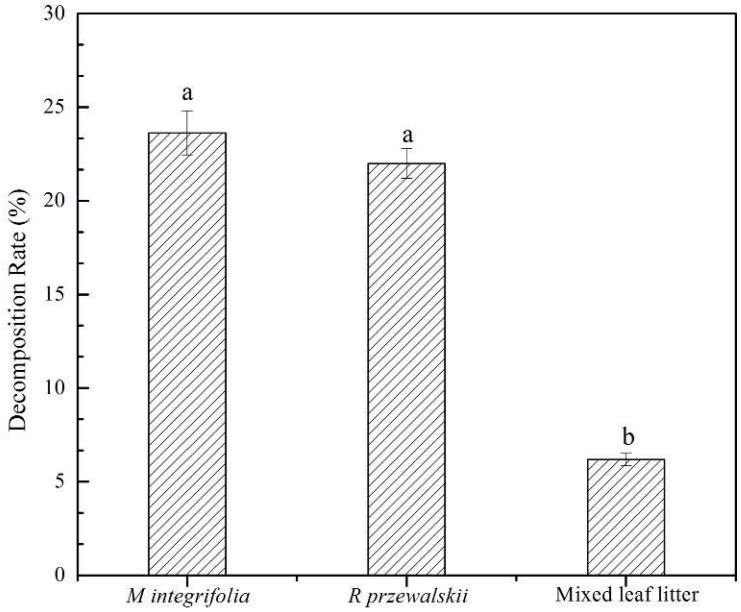

Fig. 5 Percentage of decomposed dry mass of *M. integrifolia* and *R. przewalskii* in a 50-day litter-bag study. *Column* represents mean, and bar indicates Standard Error (n = 8). Different lowercase letters indicate significant differences of decomposition rate between litter materials.

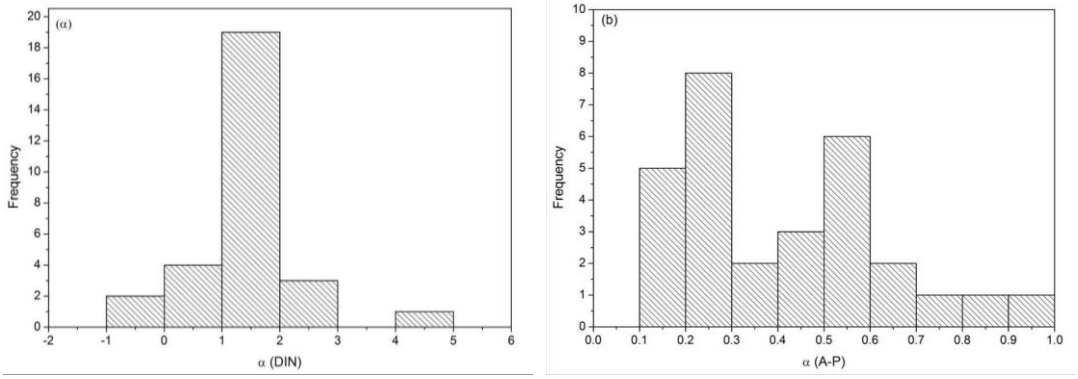

Fig. 6 Variation in soil nutrition pool with flower litters addition. Histogram for **α** values of DIN (a) and A-P (b) indicates the change between treatments and control.