Effect of litter type on soil microbial parameters and dissolved organic carbon in a laboratory microcosm experiment

W. Li[1], K.W. Pan[1], N. Wu[1,2], J.C. Wang[1], Y.J. Wang[1], L. Zhang[1]

[1]*Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization, CAS and Ecological Restoration and Biodiversity Conservation, Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, P.R. China*
[2]*International Centre for Integrated Mountain Development, Kathmandu, Nepal*

ABSTRACT

A laboratory microcosm experiment was conducted to evaluate the effects of the four single-species (*Pinus tabulaeformis* (Pt), *Pinus radiata* (Pr), *Cercidiphyllum japonicum* (Cj), and *Ostryopsis davidiana* (Od) litters from southwestern China and mixed pine-broadleaf (Pt + Cj, Pr + Cj, Pt + Od, Pr + Od) litters on soil microbial activities and dissolved organic carbon (DOC). Microcosms with the local typical soil and litterbags containing the eight litter types were incubated with 60% water field capacity for 84 days at 20°C. The results showed that the dynamics of soil microbial parameters and DOC were influenced by the litter types with different initial chemical quality. Due to their initial poor nutrient contents, the Pt and Pr litter treatments always showed lower soil microbial activities and DOC at each sampling compared with the Cj and Od litter treatments. However, compared with the single-species pine litter treatments, the inclusion of broadleaf Cj or Od litter into pine litter significantly increased soil microbial activities, and the concentrations of soil DOC during the whole incubation process. The current work thus provided a good implication for plantation management that it should be appropriate to consider Cj as an ameliorative species or retain Od in the pine plantations to improve soil conditions.

Keywords: soil labile organic carbon; soil dehydrogenase activity; soil β-glucosidase activity; single-species and mixed-species litter

Understanding the effect of litter type on soil biochemical processes is crucial for developing effective forest site management (Mukhopadhyay and Joy 2010). In Minjiang River Valley of Sichuan, Southwestern China, the pure pine plantations, such as indigenous *Pinus tabulaeformis* (Pt) and introduced *P. radiata* (Pr) plantation, are now being faced with the problems of soil degradation, mainly due to the slow decomposition and nutrient release of pine needle litter (Li et al. 2009, Pang and Bao 2011). It is hence urgent to find some solutions for promoting the decomposition rate of needle litter and ultimately restoring soil fertility in these pine plantations.

Some studies (Hu et al. 2006) have shown that the nutrient availability and soil quality of coniferous stands could be improved by mixing broadleaf litters. Such practices may therefore be considered carrying out to restore soil fertility of pine plantations at our study site. However, the litter mixing experiments showed that not all the litter mixing would speed up the processes of litter decomposition and nutrient release, and that the negative and neutral effects were also often found

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(Gartner and Cardon 2004). It is not clear either if litter mixing from different conifers and broadleaf species changed the subsequent soil biochemical processes and soil properties, compared to the pure pine litter treatments (Gartner and Cardon 2004).

In a previous study (Li et al. 2009), we investigated the influences of mixing the local broadleaf tree (*Cercidiphyllum japonicum* Sieb. et Zucc., Cj) litter or shrub (*Ostryopsis davidiana* Decne., Od) litter into the two pine (Pt and Pr) litter on decomposition and N dynamics. In the present study, we further concentrated on evaluating the effects of the four single-species litters and mixed broadleaf-conifers litters on soil microbial parameters and dissolved organic carbon (DOC) using the microcosm method. The microcosm method was validated to provide a good access for studying the effect of litter type on soil properties during the early stage of decomposition (Taylor and Parkinson 1988, Vargas et al. 2006, Wang et al. 2010). Since soil microbial biomass, enzyme activities, and DOC were suggested as early indicators of biological changes, and even important indicators of soil quality (Liang et al. 1998, Hu et al. 2006, Wang et al. 2013), this study should provide some valuable information for the soil fertility management of the local pine plantations.

MATERIAL AND METHODS

Sampling preparation and microcosm design. The sampling sites located at the Maoxian Ecological Station of Chinese Academy of Sciences (31°41'N, 103°53'E), Minjiang River Valley, Sichuan province, China. We used the same microcosms described in Li et al. (2009) for studying the effects of litter mixture on soil microbial parameters and DOC. Details of the climatic properties, the afforestation background and the sampling processes were also described previously in Li et al. (2009).

Briefly, we selected four sample plots, Pt, Pr, Cj plantation and shrubland dominated by Od, for litter collection. The sites of the three plantations had ever been shrubland dominated by Od. The two pine plantations were simultaneously planted in the adjacent slope land in 1992. As one of the main shrub vegetation distributing in the Minjiang River Valley, Od is also a main understory species in the pine plantations. Previously published initial chemical traits (Li et al. 2009) showed that the Pt and Pr litter had higher C/N ratio, lignin/N ratio and soluble phenol, but lower soluble C than the Cj and Od litter. The data suggested that the two pine litters had the lower quality than the two broadleaf litters.

To get the homogeneous soil for the microcosm experiment, soil sampling was wholly collected from the surface (0–5 cm) of the above shrubland dominated by Od. The soil is classified as Calcic Luvisols according to FAO-UNESCO (1988), which is one of typical soil types in this area. The soil had a textural composition of 15.6% sand, 64.5% silt and 19.9% clay, mean organic C content of 21.9 mg/g, total N content of 1.5 mg/g, and bulk density of 0.91 g/cm^3.

We arranged 9 treatments for the microcosm experiment, including four single-species litters (1 g Pt, 1 g Pr, 1 g Cj, and 1 g Od), four mixed-species litters (0.5 g Pt + 0.5 g Cj, 0.5 g Pr + 0.5 g Cj, 0.5 g Pt + 0.5 g Od and 0.5 g Pr + 0.5 g Od) and a control (no litter addition). And hence, 81 microcosms (9 treatments × 3 replications × 3 sampling times) were prepared by filling every 250-mL polyethylene beaker with 100 g (oven dry equivalent) air-dried soil. For the litter-amended treatments, circular litterbags (1 mm mesh) containing 1 g (oven dry weight) leaf litter were placed on the soil surface. In order to minimize the effect of litter size on the decomposition, the plant litter was fragmented into pieces of approximately 0.01 m long (pine needle litter) or 1 cm^2 (broadleaf litter) before put into litterbags. The microcosms were incubated with 60% water field capacity for 84 days at 20°C. After 14, 42 and 84 days of incubation, a total of 27 microcosms, three replicates of 9 treatments, were respectively harvested and soil from each microcosm was removed and thoroughly mixed for the following analyses.

Analytical procedures

Soil microbial parameters

Microbial carbon dioxide (CO_2) respiration. Another 27 incubation jars (three replicates of each treatment) containing the nine treatments were prepared for determining C-CO_2 evolution (Hassink 1994). CO_2 was captured in 20 mL of 1 mol/L NaOH contained in a beaker suspended in each jar. The jars were closed and put into the same controlled chamber with the above 81 microcosms. Meanwhile, three empty jars were used as blanks. The beakers were removed periodically, and the CO_2 evolved was analyzed for remaining NaOH with 0.5 mol/L HCl after adding 10 mL 1 mol/L $BaCl_2$. After the beakers were removed every time, glass jars were flushed with compressed air to allow replenishment of O_2 and water to be added to maintain the 60% field capacity. Beakers containing the fresh NaOH were then added.

Soil microbial biomass carbon (MBC). MBC was determined by the fumigation extraction method using a correction factor (k_{EC}) of 0.38 according to Vance et al. (1987).

Soil enzyme activities. The activities of the enzymes, intracellular dehydrogenase and extracellular β-glucosidase, were measured according to the methods given by Guan (1986). Detailed method of determining soil dehydrogenase activity was described by Hu et al. (2006). Dehydrogenase activity was expressed in μg triphenyl formazan (TPF) released/g dry soil/h. β-glucosidase was determined using salicin as substrate, and the saligenin released from the substrate reacted with 2,6-dibromchinon-4-chlorimide to form a blue indophenol dye, which was determined colorimetrically at 578 nm. β-glucosidase activity was expressed as μg saligenin released/g dry soil/h.

Soil KCl-extractable dissolved organic carbon

The soil of each sorted microcosm at 14, 42 and 84 days of incubation, was extracted with 2 mol/L KCl (5 g soil in 25 mL solution) in the polypropylene tube on a reciprocating shaker at 200 rev/min for 30 min. After shaking, soil KCl-extractable solution was obtained by centrifugation and filtered through 0.45-μm cellulose acetate membrane filters. DOC concentration was then determined on a TOC-500 total organic carbon analyzer (Shimadzu, Kyoto, Japan).

Statistical analysis. ANOVA was used to test the significance of differences in soil microbial parameters and soil DOC at 14, 42 and 84 days of incubation. Multiple comparisons were performed by the Duncan's Multiple Range Test. All statistical analyses were performed using SPSS 11.5 (SPSS Inc., Chicago, USA). The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of litter type on CO_2 evolution. C-CO_2 evolution of each treatment during the period of 0–14 days was the largest, accounting for 41.7% to 49.5% of total C-CO_2 evolution (Figure 1). The total CO_2 efflux of the no litter-amended treatment was 278.3 mg C-CO_2/kg soil, which was significantly lower than those of the litter-amended treatments. Among the litter-amended treatments, the two single-species pine litter treatments had the lowest cumulative CO_2 released for each incubation period, which was probably due to the low soluble C content in the two pine litters. As reported by Sall et al. (2003), C-CO_2 evolution was positively correlated with the soluble C content of the litters, especially significantly in the first 15 days of incubation. Compared with the single-species Pt litter

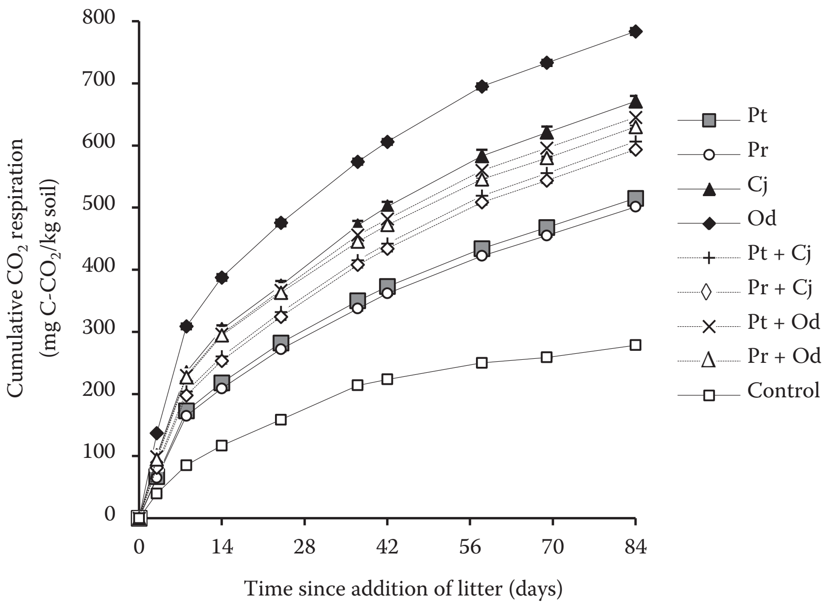

Figure 1. Cumulative CO_2 released (mg C-CO_2/kg soil) from different treatments during an 84-day incubation period. Data points represent mean ± SE with three replicates. Pt – *Pinus tabulaeformis*; Pr – *Pinus radiata*; Cj – *Cercidiphyllum japonicum*; Od – *Ostryopsis davidiana*

treatment, the inclusion of broadleaf Cj or Od litter into the Pt litter significantly increased the total C-CO_2 evolution by 17.8% and 25.3%, respectively ($P < 0.05$). Compared with the single-species Pr litter treatment, the inclusion of broadleaf Cj or Od litter into the Pr litter significantly increased the total C-CO_2 evolution by 18.4% and 25.7%, respectively ($P < 0.05$). The results suggested that the inclusion of broadleaf litters into the pine needle litters provided more diversified substrates for microbial activity and C mineralization.

Effect of litter type on soil microbial biomass carbon. Soil MBC was highest at 14 days of incubation, ranging from 105.4 mg C/kg in the control to 189.0 mg C/kg in the Od microcosm (Figure 2). The result was similar to the report by Schwendener et al. (2005), who also found that the peak of MBC occurred at 14 days after the litters were amended in a field study. Earlier studies (Jensen 1997) pointed out that the soil microbial biomass pool and its activity would increase when litters were amended to the soil and this was reflected in higher microbial growth and greater respiratory output from the soil. However, present findings showed that not all the litter types amended to the soil would increase MBC along with time. Although the Pt and Pr treatments had higher soil MBC than the control at day 14 and 42, no differences in soil MBC between them were found at day 84 (Figure 2). This could be explained by the depletion of soluble carbohydrates and the presence of polyphenols with the litter decomposition, which could inhibit microbial growth (Sall et al. 2003). Low soluble C contents and high soluble phenols in the two pine litters induced low concentrations of soil MBC and caused soil MBC to decrease rapidly. Compared with the single-species Pt litter treatment, the Pt + Cj treatment significantly increased soil MBC by 15.2, 16.8 and 26.0%, and the Pt + Od treatment significantly increased it by 22.2, 18.0 and 43.0% at day 14, 42 and 84, respectively ($P < 0.05$). Compared with the single-species Pr litter treatment, the Pr + Cj treatment significantly increased soil MBC by 10.6, 18.4 and 25.7%, and the Pr + Od treatment significantly increased it by 14.7, 20.6 and 30.5% at day 14, 42 and 84, respectively ($P < 0.05$). The increasing substrate availability underlying the mixed leaf litters may contribute to the increase of soil MBC, which was also observed in the other studies on soil microbial properties responding to the litter mixtures (Hu et al. 2006).

Effect of litter type on soil enzyme activities. With the same changing tendency of soil MBC, the two measured soil enzymes (intracellular dehydrogenase and extracellular β-glucosidase) activities both showed high values at day 14 and then declined in the following 10 weeks (Figure 3). The results were in accordance with the findings of Stark et al. (2008), who reported that soil MBC and enzyme activities together declined with time in a lupin residue amended incubation experiment,

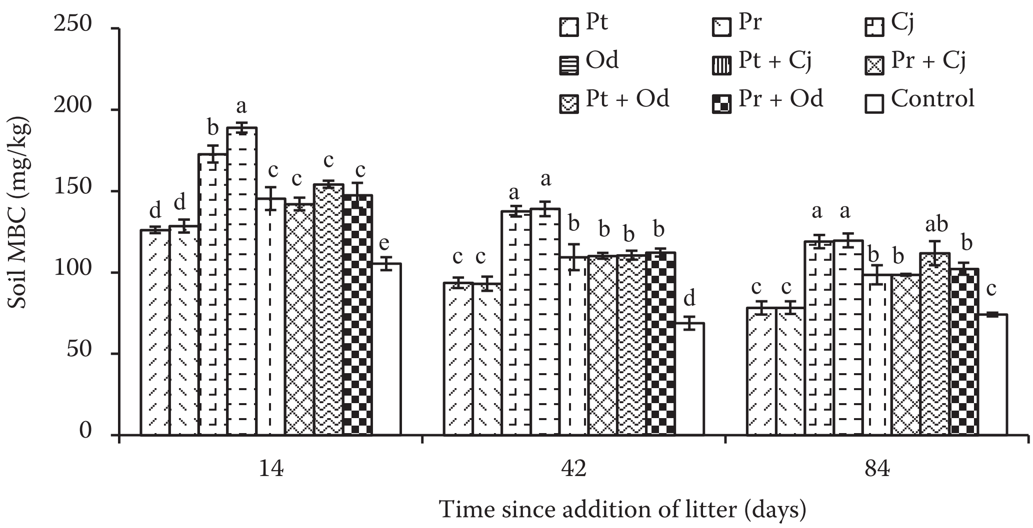

Figure 2. Soil microbial biomass carbon (MBC) in litter-amended and no-litter amended (control) microcosms at 14, 42 and 84 days of incubation (the values are mean ± SE with three replicates for each treatment). Different letters indicate significant differences among treatments ($P < 0.05$) at day 14, 42 and 84, respectively. Pt – *Pinus tabulaeformis*; Pr – *Pinus radiata*; Cj – *Cercidiphyllum japonicum*; Od – *Ostryopsis davidiana*

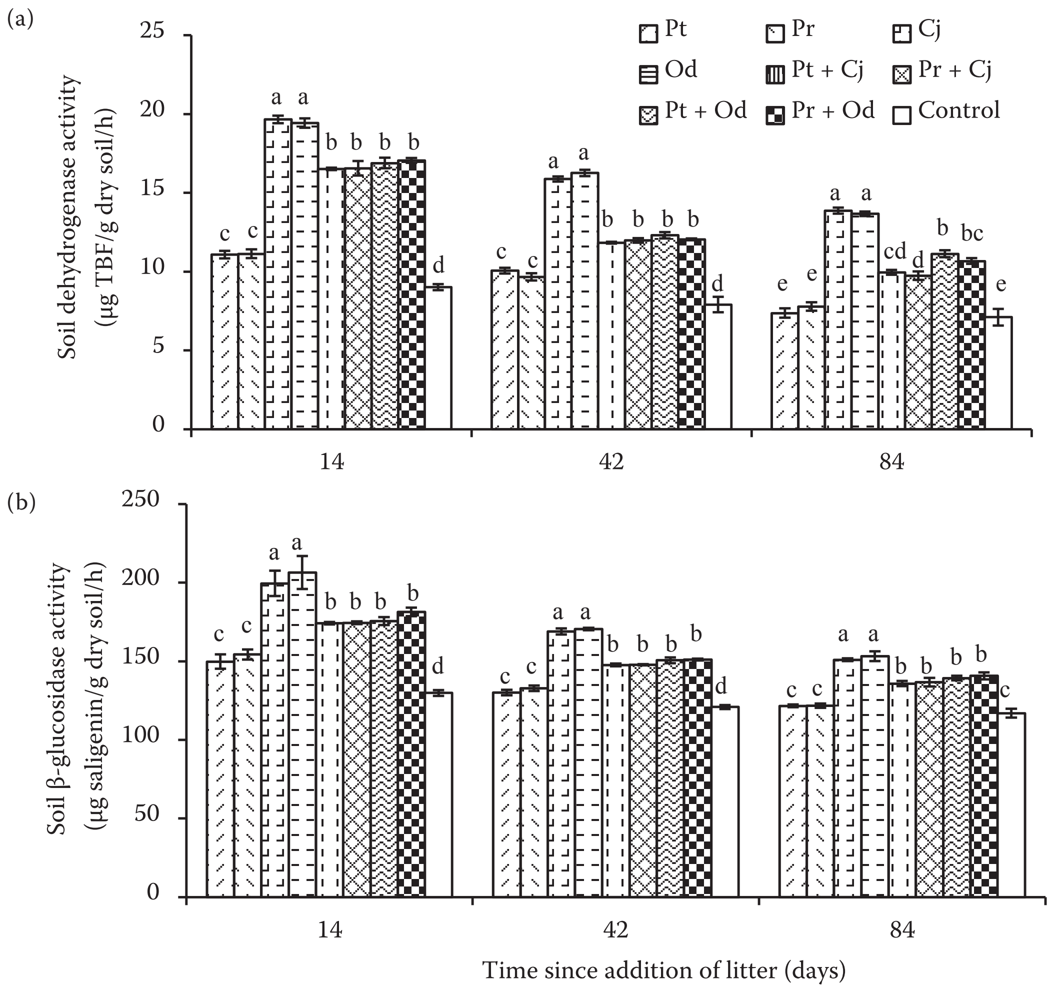

Figure 3. Soil dehydrogenase activity (a) and soil β-glucosidase activity (b) in litter-amended and no-litter amended (control) microcosms at 14, 42 and 84 days of incubation (the values are mean ± SE with three replicates for each treatment). Different letters indicate significant differences among treatments ($P < 0.05$) at day 14, 42 and 84, respectively. Pt – *Pinus tabulaeformis*; Pr – *Pinus radiata*; Cj – *Cercidiphyllum japonicum*; Od – *Ostryopsis davidiana*

due to highly positive correlations between enzyme activities and MBC. Despite the same changing tendency of the two enzyme activities among all the litter amended treatments, the present results always showed the lower soil dehydrogenase and β-glucosidase activities in the two pine litter amended treatments than in the two broadleaf litter amended treatments. Similarly, Mukhopadhyay and Joy (2010) observed that the high-quality *Cassia* and *Dalbergia* litters much more increased soil enzyme activity than the low-quality *Shorea* and *Acacia* litters. The available substrates varied in different litter types could determine the variance of soil enzyme activities among the litter-amended treatments (Kourtev et al. 2002, Sall et al. 2003). Since the available substrates for microbial activities were more abundant in the mixed litters than in the two pine litters, soil dehydrogenase and β-glucosidase activities in the mixed broadleaf-pine litters treatments were significantly higher than those in the pure pine litters treatments during the whole incubation process (Figure 3).

Effect of litter type on soil dissolved organic carbon. Soil DOC concentrations in the litter-amended treatments were significantly higher than those in the control at each sampling (Figure 4). This agreed with the report of Poll et al. (2008) in a rye residue addition experiment, that the increased DOC concentration of the soil below the litter is due to the diffusion of soluble C released from the litter. These data might prove again that recent amended litter was a direct source of dissolved organic matter in soil (Don and Kalbitz 2005). Soil DOC in the Cj and Od microcosms gradually declined, while those of the Pt and Pr microcosms had almost no changes at day 42 and day 84 of incubation. This

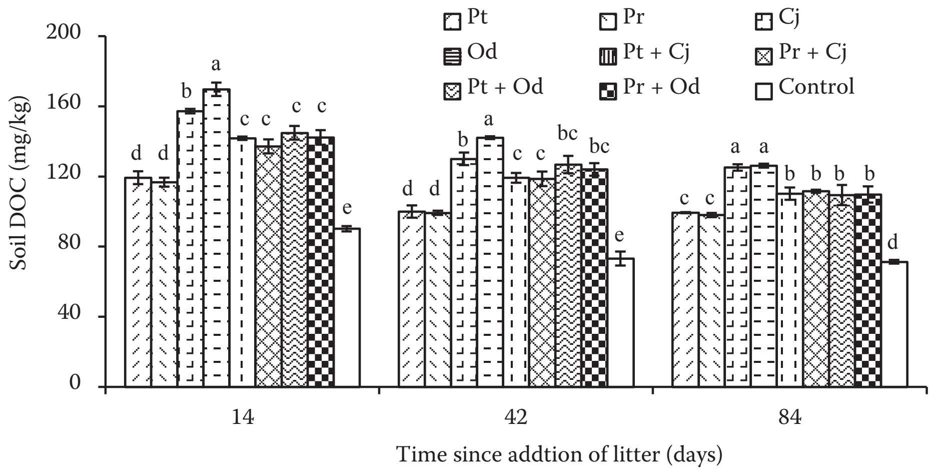

Figure 4. Soil dissolved organic carbon (DOC) in litter-amended and no-litter amended (control) microcosms at 14, 42 and 84 days of incubation (the values are mean ± SE with three replicates for each treatment). Different letters indicate significant differences among treatments ($P < 0.05$) at day 14, 42 and 84, respectively. Pt – *Pinus tabulaeformis*; Pr – *Pinus radiata*; Cj – *Cercidiphyllum japonicum*; Od – *Ostryopsis davidiana*

might be due to the quality of soil DOC varied in the different litter-amended treatments. Don and Kalbitz (2005) observed an increasing degree of aromaticity and complexity for DOC, and hence the low bioavailability of DOC from pine needles with the needles decomposition. No changes of soil DOC in the two pine litters treatments at day 42 and day 84 could be due to the bioavailability of those parts from the needles becoming low with the needles decomposition. Incubation experiment still showed the lower soil DOC concentration in the Pt and Pr treatments than in the Cj and Od treatments. However, compared with the pure pine litter treatments, the inclusion of broadleaved litters into the pine needles significantly enhance soil DOC concentration. The causes could be due to high soluble C content in the broadleaved litters contributing to the increase of soil DOC concentration, and meanwhile the increasing microbial activity in the mixed-litter treatments promoting DOC production from the soil (Kalbitz et al. 2000).

In conclusion, our study clearly demonstrated that the litter types influenced not only the dynamics of soil microbial parameters but also the dynamics of soil DOC. The lower initial quality of litter determined that the two pine litter treatments had lower soil microbial activities and DOC than the Cj and Od litter treatments. However, the changes of soil microbial properties and soil DOC in the mixed leaf litters treatments suggested that the inclusion of broadleaf litters into pine needle litter could influence soil quality through supplying diversified substrates and promoting soil nutrient availability. Therefore, associated with our previous findings that the presence of Cj or Od litter increased the decomposition of pine litter and soil N availability (Li et al. 2009), the present results further verified that it would be advantageous to use Cj as an ameliorative species or retain Od in the pine plantations to improve soil conditions, in terms of utilizing the interaction of mixed-species litters.

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Corresponding author:

Prof. Dr. Kai-Wen Pan, Chinese Academy of Sciences, Chengdu Institute of Biology, Chengdu 610 041, P.R. China
phone: + 86 28 8289 0522, e-mail: pankw@cib.ac.cn