Litter Removal Increases Plant Carbon Inputs to Soil in *Pinus Massoniana* Plantation

Chengfu Zhang
Guizhou Academy of Sciences
Yinmei Cai
Guizhou University
Tao Zhang
Guizhou University
Tengbing He
Guizhou University
Jie Li
Chinese Academy of Sciences
Xinying Li
Guizhou University
Qingxia Zhao (qxzhao@gzu.edu.cn)
Guizhou University

Research Article

Keywords: Litter, Root litter, Root exudates, C input

Posted Date: March 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1461079/v1

Abstract

Aims Plants are the main source of soil organic carbon (C) in forest ecosystems; they input photosynthetically assimilated C into the soil through litter, root litter, and root exudates. Variations in plant net primary production caused by global environmental changes are likely to drive shifts in leaf litterfall inputs to soils. However, the effects of these changes on plant aboveground and underground C input remain largely unknown.

Methods We conducted a two-year litter manipulation (litter removal, addition, and control) experiment in a *Pinus massoniana* plantation and studied its impacts on plant C input via litter, fine root, and root exudates.

Results The results showed that litter removal significantly increased litterfall in summer and autumn and reduced root C exudation rates in spring, but had no influence on the C input of fine root. Whereas litter addition did not significantly affect the C input of litter, fine root, or root exudates. The annual C input of litter, fine root, and root exudates in control plots were 348.28 g C m^{-2},42.39 g C m^{-2}, and 17.44 g C m^{-2}, accounting for 85.34%, 10.39%, and 4.27% of the total C input, respectively. Litter removal increased plant annual total C input by 24.55% owing to reducing root exudate C input by 30.50% and increasing litter C input by 31.12%.

Conclusions Increasing the aboveground C input and decreasing underground C input under litter removal is a strategy to maximize forest growth in short term. The increased plant C input under litter removal mitigated the influences of litter alteration caused by global change. This was of great significance for understanding plant growth strategy and forecasting plant growth dynamic under global change.

Introduction

Litter is an essential source of soil C pools and nutrient (Chen et al. 2021; Liu et al. 2019). Approximately 50 Gt of organic C returns the soil every year via litter decomposition (Cotrufo et al. 2013), accounting for 70% of the annual global C circulation (Spain, 1984). In recent decades, forest litter and net primary productivity are altered by global environmental changes, such as increasing atmospheric carbon dioxide concentrations (Song et al. 2019), warming (Li et al. 2017; Quan et al. 2019) (Huang et al. 2016), and atmospheric nitrogen deposition (Du et al. 2019; He et al. 2021).Variation in litter inputs to soil would impact the amount of C and nutrients entering the soil system and thus the amount of C and nutrients internally cycled in forest plantations (Huang and Spohn 2015; Sayer et al. 2011; Sayer et al. 2020; Xu et al. 2021). This impact can feedback to tree growth and exert profound effects on plant productivity and litter production. However, how plant-derived C input respond to litterfall alteration, whether plant increase or reduce its C input to exacerbate or mitigate the influences of litter alteration was unclear?

Plant-derived C input into the soil through aboveground and underground pathways (Kuzyakov and Domanski 2000; Santos et al. 2016). The main aboveground C input is litter decomposition, which inputs large amounts of soluble organic C and nutrients into the soil (Rubino et al. 2010). Whereas crucial underground C input pathways are the mortality and decomposition of plant roots (Hu et al. 2016). Root-derived C inputs influence soil organic matter (SOM) dynamics by promoting soil C formation (Clemmensen et al. 2013; Sokol and Bradford 2019), stabilization (Jackson et al. 2017), and turnover (Meier et al. 2017; Yuan et al. 2018), driving more soil C sequestration than litter (Hu et al. 2016; Liu et al. 2019; Rasse et al. 2005). The turnover of fine root, roots with diameters of < 2 mm (Matamala et al. 2003; Silver et al. 2005; Vogt et al. 1996), accounts for 6.2–88.7% of litter input and its contribution to soil C is 18–58% higher than that of litter (Zhang and Wu 2001). In addition, recent underground ecological studies have found that root exudates is also an important underground C input pathway of plants. Root exudates contain C-containing substances, such as sugar, organic acids, and aldehydes, accounting for approximately 5–21% of plant total C assimilation (Badri and Vivanco 2009; Haichar et al. 2014; Gill et al. 2016; Nguyen 2003). Root exudates can mediate soil microbial communities and stimulate SOM decomposition (Kuzyakov and Domanski 2000), which has a profound impact on soil C turnover and cycling processes (Haichar et al.

2014). However, the relative C input contributions of litter, fine root and root exudates processes have not yet been thoroughly investigated.

The effects of litter manipulation on litter nutrient input have been previously reported. For example, a five-year litter manipulation study in lowland semi-evergreen tropical forests showed that litter addition (LA) increased litterfall, whereas litter removal (LR) did not influence forest nutrient cycling (Sayer and Tanner 2010). A large-scale litter manipulation experiment in a wet tropical forest revealed that LA significantly increased leaf litter production but not litter nutrient concentrations (Wood et al. 2009). However, studies on the responses of plant underground C inputs to litter manipulation are scarce. LR or LA might affect the production and turnover of fine root due to the altered soil microclimate and nutrient status (Rodtassana and Tanner 2018; Sayer et al. 2006). A 4-year field experiment showed that soils with higher fertility sequestered more shoot-, root -, and AMF-derived C (Huang et al. 2021). Furthermore, root C exudation is easily affected by the changes of microclimate, nutrients, or plant productivity caused by LA or LR (Facelli and Pickett 1991; Phillips et al. 2009; Phillips et al. 2011; Sayer 2006; Yin et al. 2013). In conclusion, previous studies most focus on single C input processes under litter treatments, but collaborative responses of litter, roots, and root exudate C input pathways are unknown.

Pinus massoniana is the most widely distributed afforested tree species in subtropical regions and plays an important role in stabilizing forest ecological functions. Therefore, we carried out a litter manipulation experiment (LR, control (CT), and LA) in a *P. massoniana plantation*. After 2 years' litter manipulation, we systematically studied the responses of C inputs by the litter, fine root, and root exudate to litter manipulation. We hypothesized that litter manipulation affects plant C input and that there may be different responses between aboveground and underground C inputs.

Materials And Methods

Site description

This study was conducted in a *P. massoniana* plantation in southern Guiyang, Guizhou Province, China (106°398"E, 26°2815"N, alt.1145 m). The study area has a subtropical monsoon climate, and the main landforms are mountainous and hilly. The mean annual temperature, mean annual sunshine duration, relative humidity, and annual average rainfall are 14.9°C, 1354 h, 75.5%, and 1178.3 mm, respectively. At the time of study, the *P. massoniana* plantation was approximately 35-year-old and no artificial logging took place during the entire growth period. The canopy density was 0.8 with a tree density of 853 stem $\cdot hm^{-2}$. The average tree diameter and height were 32.2 cm and 28.32 m. The dominant understory species were *Ligustrum lucidum* Ait., *Viburnum dilatatum* Thunb., *Rhapis excelsa* (Thunb.) Henry ex Rehd., and *Hedera nepalensis* K. Koch var. *sinensis* (Tobl.) Rehd. According to the Chinese classification system, the soil type is yellow soil and was developed from red clay in the quaternary. The soil properties for 2021 are summarized in Table 1.

Table 1
Soil properties of P. massoniana plantation in 2021.LR, litter removal; CT, control; LA, litter addition. Different lowercase letters indicate significant differences among the litter treatments ($p < 0.05$).

treatment	The annual mean Moisture of 5cm soil depth (%)	The annual mean Temp of 5 cm soil depth (°C)	pH	Soil organic carbon ($g \cdot kg^{-1}$)	Total N concentration ($g \cdot kg^{-1}$)	C:N	Microbial biomass carbon (MBC) ($mg \cdot kg^{-1}$)	Microbial biomass nitrogen (MBN) ($mg \cdot kg^{-1}$)	MBC:MBN
LR	29.07± 0.15a	14.97± 0.05a	4.86± 0.04a	19.07± 1.58a	2.14± 0.28a	9.90± 0.89a	381.53± 41.84a	54.06± 5.49a	7.30± 0.89a
CT	28.45± 0.12a	15.24± 0.06a	4.81± 0.04a	18.75± 2.01a	2.15± 0.09a	8.87± 0.90a	316.41± 54.25a	54.19± 14.80a	6.54± 0.97a
LA	29.79± 0.13a	15.26± 0.06a	4.95± 0.11a	21.73± 0.29a	2.09± 0.28a	10.81± 1.31a	344.85± 41.02a	47.65± 5.75a	7.36± 0.69a

Experimental design

Our experiment began in October 2018 using randomized block design. Three similar blocks in the *P. massoniana* plantation were established and three litter treatments: LR, CT, and LA, were randomly arranged in each block. Litterfall on the forest floor in LR plots was removed monthly by hand and transferred to LA plots. The LR and LA plots were paired in space. Each plot was 10 × 10 m, was enclosed with a rope, and had a 10 m buffer zone.

Litter sampling

In May 2020, 17 months after the plots were established, three litter traps were randomly placed in each plot to monitor litterfall. Each trap was placed 0.2 m aboveground level and had a collection area of 0.25 m^2 (0.5 m × 0.5 m). Litter was then collected monthly from June 2020 to May 2021. When large leaves lay across the trap, the portion of the leaf lying within the frame area was collected and the remainder was discarded. The collected litter was dried to a constant weight at 65 °C and weighed. The litter was then ground with a plant crusher (FW100, Taisite, China) and the C content was measured using the potassium dichromate external heating method. One year was divided into four seasons according to the temperature and precipitation condition in this region: (1) spring (March to May); (2) summer (June to August); (3) autumn (September to November); (4) winter (December to February). Litter C input is calculated as:

$$\text{litter C input} = Li \times Ci$$

where Li is litterfall ($g \cdot m^{-2}$) and Ci is litter C content ($g \cdot kg^{-1}$).

Fine root sampling

Fine root (< 2 mm diameter) were collected using a 5-cm diameter soil core sampler in July, October, and December 2020 and April 2021, representing summer, autumn, winter, and spring, respectively. Three 40 cm deep soil cores were randomly collected in each plot. Roots were removed from the cores with tweezers and washed with deionized water. Roots with a diameter < 2 mm were classed as fine root and were dried to constant weight at 65 °C and weighed.

Fine root biomass (FRB) is calculated as:

$$\text{FRB} = \text{Fine root dry weight}(g) \times 10^4/[\pi(d/2)^2]$$

where d is the diameter of the soil core sampler (5 cm).

Annual fine root mortality (FRM) is calculated according to the calculation methods of Vogt (Vogt et al. 1998)and Mei (Mei, 206):

$$\text{FRM} = FRB \times T$$

where *FRB* is the annual average fine root biomass, *T* is the annual turnover rate of fine root (T = 1.05 was used in this study according to the turnover rate in *P. massoniana* plantation (Wang et al. 2012)).

Fine root C input is calculated as:

$$\text{Fine root C input} = FRM \times \text{fine root C content}$$

Root exudation measurements

Two target trees with similar growth conditions in each plot were randomly selected for root exudates collection. Root exudates were collected using an in-situ collection device (Phillips et al. 2008) in July, October, December and 2020, and April 2021, representing summer, autumn, winter, and spring, respectively. Three terminal fine roots (2 mm average diameter with laterals) in every target tree were excavated from the topsoil (0–10 cm). The excavated roots were washed thrice with deionized water and twice with a nutrient solution (0.2 mM K_2SO_4, 0.3 mM $CaCl_2{\cdot}2H_2O$, 0.1 mM KH_2PO_4, and 0.2 mM $MgSO_4{\cdot}7H_2O$). The clean roots were placed into 30 mL syringe with sterile 1-mm-diameter glass beads to make up the volume. Glass wool was placed at the bottom to prevent the glass beads from clogging syringe barrel. 15 ml of nutrient solution was injected into the syringe to meet the growth and activity demands of the fine root. The syringe was then covered with aluminum foil and placed in the original soil environment. After a 24-h equilibration period, the solutions in the syringe were flushed using a vacuum pump to remove any soluble C. Then, 15 ml of nutrient solution was injected to continue cultivation as a 'trap solution' for the root exudates. After a 24-h incubation period, the trap solutions containing exudates were collected by a vacuum pump. Root exudates were collected three times from each syringe via the same method and cultivation lasted for three days. Each plot also contained six syringes without roots as blanks. The collected solutions were mixed for three days and transferred to brown bottles. Then, they were filtered through a filter (0.22 μm) and stored in a refrigerator (-20°C) for preservation. The C in the root exudation was analyzed using a total organic carbon analyzer (Vario, Germany). For root exudation collection, root samples in the syringe were cut and scanned at 400 dpi using an Epson scanner (Seiko Epson Corporation, Japan), and the root surface area and length were analyzed using WinRHIZO Pro 2019a (Regents Instruments Inc., Quebec, Canada). Subsequently, the roots were oven-dried at 65°C and weighed. The root C exudation rate was calculated as the mass of C (μg) flushed from each root system (minus the average C concentration in the control cuvettes) over the 24 h incubation period. The exudation rates I (μg C g^{-1} root biomass h^{-1}), II (μg C cm^{-1} root length h^{-1}), and III (μg C cm^{-2} root area h^{-1}) were calculated by dividing the total amount of C flushed from the root system by the total fine root biomass, root length, and root area, respectively.

Seasonal root exudate C input and annual exudate C input were calculated using the following equation based on mass-specific root exudation rates, fine root biomass, and the number of days in a season and year.

Seasonal root exudate C input = root C exudation rate per root biomass × root biomass × days

Annual root exudate C input = annual root C exudation rate per root biomass × root biomass × days

Statistical analyses

All data were tested for normal distribution and homogeneity of variance before further analyses. Two-way analysis of variance was applied to assess the effects of litter treatments, season, and their interaction on C input. Significant differences between treatments were examined using Tukey's test when variances were equal, whereas Dunnett's test was used for unequal variances. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed using SPSS software v22.0 (Chicago, IL, USA) and diagrams were drawn using Origin 9.0.

Results

Litter C input

Season and litter treatments had significant effects on litterfall and litter C input (Fig. 1a and Fig. 1c) but did not significantly affect litter C content (Fig. 1b). Litterfall and C input showed seasonal variations in CT (summer, spring > autumn, winter), LA (summer, spring > autumn, winter) and LR (autumn, summer > spring, and winter). The differences in litterfall and C input among treatments depended on seasons: in summer and autumn, litterfall and C input were higher in LR than in LA and CT; there was no significant difference among treatments in spring and winter (Fig. 1a and Fig. 1c). The annual litterfall and C input in LR were 32.15% and 31.12% higher than those in CT (Table 2).

Table 2
Annual litterfall and litter C input under litter treatments (mean ± SE, n = 3).LR, litter removal; CT, control; LA, litter addition. Different lowercase letters indicate significant differences among the litter treatments ($p < 0.05$).

Treatment	Annual litterfall(g m^{-2})	Annual litter C input (g C m^{-2})
LR	893.08 ± 38.80a	456.68 ± 13.95a
CT	675.80 ± 32.97b	348.28 ± 12.80b
LA	717.23 ± 26.50b	372.20 ± 16.05b

Fine root C input

Season and litter treatments had no influences on fine root biomass or its C content (Fig. 2a and Fig. 2b). Annual fine root C input due to fine root mortality was not significantly different among treatments (Table 3).

Table 3
Annual fine root mortality and C input under litter treatments (mean ± SE, n = 3).LR, litter removal; CT, control; LA, litter addition. Different lowercase letters indicate significant differences among the litter treatments ($p < 0.05$).

Treatment	Annual fine root mortality(g m^{-2})	Annual fine root C input (g C m^{-2})
LR	95.10 ± 9.68a	39.50 ± 3.56a
CT	103.63 ± 11.12a	42.39 ± 5.02a
LA	97.35 ± 4.60a	38.87 ± 1.62a

Root exudate C input

Root exudation rates showed strong seasonal patterns in all plots. Root exudation rate I was significantly higher in spring and summer than in autumn and winter (Fig. 3a), and root exudation rates II and III were significantly higher in spring than

in the other three seasons (Fig. 3b and Fig. 3c). LR significantly decreased root exudations I, II, and III in spring but had little influence on root exudation in summer, autumn, and winter (Fig. 3). On the contrary, LA had no influences on root exudations I, II, and III in all seasons.

Root exudate C input varied with season and treatments (Table 4). In CT and LA, root exudate C input in spring was significantly higher than that in autumn. In LR, root exudate C input in summer was significantly higher than that in autumn.LR treatment significantly reduced the root exudate C input in spring and winter but did not in summer and autumn. LR treatment reduced annual root exudate C input by 30.50% compared to CT (Table 4). In contrast, LA had no significant effect on root exudate C input.

Table 4

Root exudate C input under litter treatments (g C m^{-2}) (mean ± SE, n = 3).LR, litter removal; CT, control; LA, litter addition. Different capital letters indicate significant differences between the seasons ($p < 0.05$). Different lowercase letters indicate significant differences among the litter treatments ($P < 0.05$).

Treatments	Root exudate C input in spring	Root exudate C input in summer	Root exudate C input in autumn	Root exudate C input in winter	Annual Root exudate C input
LR	3.02 ± 051ABb	3.65 ± 033Aa	1.66 ± 017Ba	2.92 ± 056ABb	12.12 ± 0.77b
CT	6.58 ± 1.54Aa	4.26 ± 0.89ABa	1.77 ± 0.15Ba	4.92 ± 0.85ABa	17.44 ± 2.23a
LA	4.96 ± 0.58 Aab	4.30 ± 1.03 ABa	1.98 ± 0.15 Ba	3.19 ± 1.18 ABab	14.57 ± 0.14ab

C input pattern

In CT plots, litter, fine root and root exudate C input accounted for 85.34% (348.28 g C m^{-2}), 10.39% (42.39 g C m^{-2}), and 4.27% (17.44 g C m^{-2}) of the annual total C input, respectively. The aboveground and underground C inputs were significantly affected by litter treatments (Fig. 4). In LR plots, root exudate C input was 30.50% lower but litter C input was 31.12% higher than that in CT resulting in an increase of annual total C input by 24.55% (Fig. 4). However, LA had no significant influence on plant C input compared to CT.

Discussion

Effects of litter treatments on aboveground C input

Our study indicated that LR treatment increased litterfall by 32.15% but did not alter litter C content, giving rise to a 31.12% increase of litter C input (Fig. 1). These results supported our hypothesis that aboveground C input increases under LR. The higher litterfall under LR might be a result of increased leaf turnover rather than an increase in total leaf production (Wood et al. 2009; Sayer and Tanner 2010). As litter generally consisted of old leaves with weak photosynthetic capacity, the fall of these leaves reduced plant energy consumption and transferred more nutrients to tender and active organs. Therefore, increased litterfall under LR might be a strategy to improve the nutrient utilization efficiency. Moreover, the positive effect of LR on litterfall in this study mainly occurred in summer and autumn. Previous studies have also found that litterfall during the rainy season was more easily affected by litter treatments than the dry season (Sayer and Tanner 2010; Wood et al. 2009). This is becasued litter decompose faster in these seasons due to the higher temperature and precipitation, compensating for soil C loss due to LR. However, since the duration of litter treatments in our study was only 2 years, the increased litterfall of plants under LR may only be a short-term solution. Indeed, previous long-term LR experiments have found that the nutrients in the forest can become depleted under these conditions. For example, after LR for 3–5 years, litterfall was reduced and available P, Ca, Mg, and K in the soil and the growth of trees seriously declined (Sayer, 2006). Therefore, if litterfall can keep increase in a long term under LR needed further observation. On the contrary, the impact of

LA treatment on litter C input was not significant, which was inconsistent with the results that litterfall increased by 22% after LA (Wood et al. 2009). The main reason for this was that soil organic C storage and nutritions was not largely altered during the 2 years (Kuzyakov and Domanski 2000).

Effects of litter treatments on underground C input

Litter treatments did not significantly affect fine root biomass and its C input. This was consistent with findings from the Costa Rican rainforest (Leff et al. 2012). The main reason was that our litter manipulation test lasted only 2 years, which is not enough time for soil nutrients to significantly change (Table 1). Soil nutrients were only slightly altered under short-term litter manipulation treatments (Rodtassana and Tanner 2018). With the extension of treatment time, the effects of litter manipulation on soil nutrient may be strengthened (Tanner et al. 2016). Moreover, the influence of LR on soil nutrients was related to the original nutrition status. In soil with poor-nutrition, LR quickly led to plant growth restriction, eventually causing a decrease in root biomass (Lima et al. 2010; Rodtassana and Tanner 2018). In addition, the responses of old trees to external changes may not alter fine root growth, as this required more energy investment, instead opting for more economical changes, such as root exudates.

The results of this study showed that LR significantly reduced root exudate C by 30.50%. Previous studies found that root C exudate varies with soil nutrition, which was an important mechanism for nutritional acquisition (Degryse et al. 2008). Under low-nutrient conditions, plants usually increased root C exudate, intended to improve microbial activity and the production of extracellular enzymes, thereby stimulating SOM decomposition and nutrient release (Kuzyakov and Bol 2006; Meier et al. 2017; Nobili et al. 2001). The increasing root C exudate can also reduce the stability of the SOM-mineral complex interface through abiotic effects (such as coordination, complexation, and dissolution reactions), remove protected C from soil organics, and release C from the mineral complex for microbial decomposition and utilization (Keiluweit et al. 2015; Yuan et al. 2018). Therefore, declined nutrient availability under long-term LR could increase root exudation to stimulate SOM decomposition (Bengtson et al. 2012). However, this contradicted our results that root exudate C input decreased under LR. The decreased root exudate was mainly caused by the decrease in growth (Haichar et al. 2014; Sayer et al. 2006; Sayer and Tanner 2010). Although soil nutrition did not change in our study, plant growth was still affected by external temperature or microbial activity due to LR. The decreased in root exudation C could retained more potosythetic C for plant growth. Moreover, the lower C exudate reduced the mineralization and decomposition of soil C, and increased the persistence of organic matter (Bengtson et al. 2012; Cotrufo et al. 2013) (Fontaine et al. 2007; Schmidt et al. 2011). Therefore, the lower C exudate in LR may be a plant strategy for keeping growth and reducing C consumption.

Effects of litter treatments on C input pattern

This study quantified the C inputs of litter, fine root, and root exudates into the soil, and found that their annual C inputs accounted for 85.34%, 10.39%, and 4.27%% of the total plant C input, respectively. Although underground C input is only a small part of total plant C input, its contribution to soil C is significant (Hu et al. 2016; Liu et al. 2019). Therefore, underground C input, especially root C exudates, should be considered in future C cycle studies. Moreover, LR increased the annual total C input by 24.55%, as a result of reducing root exudate C input by 30.50% and increasing litter C input by 31.12%. The result support our hypothesis that the responses of aboveground and underground carbon inputs were differences. It suggested that short-term LR reduced the allocation of underground C but increased litterfall input, thereby altered the plant-soil C input pattern. Increasing litterfall and reducing root C input were plant adaptive strategies to reduce C consumption and maximum growth, compensating for the soil C loss due to LR.

Conclusions

This study investigated litter, fine root, and root exudate C inputs under litter treatments in a *P. massoniana* plantation. LR significantly increased plant C input by reducing root exudate C input and increasing litter C input into the soil. However, LA did not significantly affect C input. This suggested that LR affected C input and C cycle processes in forest ecosystems.

The higher litterfall reduced nutrient consumption by the old leaves and contributed to the rapid replenishment of the soil C pool. The lower root exudation rates reduced the investment of plant photosynthetic C and the decomposition of SOM. The increased plant C input under LR mitigated the influences of litter alteration caused by global change. This was of great significance for understanding plant growth strategy and forecasting plant growth dynamic under global change.

Abbreviations

CT: control; LA: litter addition; LR: litter removal; SOM: Soil organic matter;

Declarations

Authors' contributions

Qingxia Zhao and Tengbing He conceived the ideas and designed methodology. Yinmei Cai and Xinying Li carried out the laboratory analyses and conducted the field work. Tao Zhang collected the data. Chengfu Zhang contributed to the analysis and interpretation of data and wrote the main manuscript text. Jie Li helped in formal analysis, visualization, writing—review. All authors contributed critically to the drafts and gave final approval for publication.

Funding

This study was financially supported by the National Natural Science Foundation of China (No. 32060339) and Guizhou forestry scientific research project (J[2018]06).

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

References

1. Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32:666–681.
2. Bengtson P, Barker J, Grayston SJ (2012) Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. Ecol Evol 2(8): 1843–1852
3. Chen L, Fang K, Wei B, Qin S, Feng X, Hu T, Ji C, Yang Y (2021) Soil carbon persistence governed by plant input and mineral protection at regional and global scales. Ecol Lett 24: 1018–1028
4. Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J, Finlay RD, Wardle DA, Lindahl BD (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science 339(6127): 1615–1618
5. Cotrufo MF, Wallenstein MD, Boot CM, Denef K, Paul E(2013) The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Glob Change Biol 19(4): 988–995
6. Degryse F, Verma VK, Smolders E (2008) Mobilization of Cu and Zn by root exudates of dicotyledonous plants in resin-buffered solutions and in soil .Plant Soil 306:69–84
7. Du E, Fenn ME, Vries WD, Ok YS (2019) Atmospheric nitrogen deposition to global forests: Status, impacts and management options. Environ Pollut 250: 1044–1048
8. Facelli JM, Pickett STA (1991) Plant litter: Its dynamics and effects on plant community structure. Bot Rev 57: 1–32

9. Fontaine S, Barot S, Barré P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450: 277–280
10. Gill AL, Finzi AC, Penuelas J (2016) Belowground carbon flux links biogeochemical cycles and resource-use efficiency at the global scale. Ecol Lett 19(12): 1419–1428
11. Haichar FEZ, Santaella C, Heulin T, Achouak W (2014) Root exudates mediated interactions belowground. Soil Biol Biochem 77: 69–80
12. He W, Yuan Y, Zhang Z, Xiao J, Liu Q, Laiho R, Yin H(2021)Effect of N addition on root exudation and associated microbial N transformation under Sibiraea `angustata in an alpine shrubland. Plant Soil 460:469–481
13. Hu YL, Zeng DH, Ma XQ, Chang SX (2016) Root rather than leaf litter input drives soil carbon sequestration after afforestation on a marginal cropland. For Ecol Manag 2016,362: 38–45
14. Huang J, Liu W, Yang S, Yang L, Peng Z, Deng M, Xu Shan, Zhang B, Ahirwal J, Liu L (2021) Plant carbon inputs through shoot, root, and mycorrhizal pathways affect soil organic carbon turnover differently. Soil Biol Biochem 160:108322
15. Huang L, He B, Chen A, Wang H, Liu J, Lű A, Chen Z (2016) Drought dominates the interannual variability in global terrestrial net primary production by controlling semi-arid ecosystems. Sci Rep 6: 24639
16. Huang W, Spohn M (2015) Effects of long-term litter manipulation on soil carbon, nitrogen, and phosphorus in a temperate deciduous forest. Soil Biol Biochem 83:12–18
17. Jackson RB, Lajtha K, Crow SE, Hugelius G, Kramer MG, Piñeiro G (2017) The Ecology of Soil Carbon: Pools, Vulnerabilities, and Biotic and Abiotic Controls. Annu Rev Ecol Evol S 48:419–445
18. Keiluweit M , Bougoure J J, Nico P S, Pett-Ridge J, Weber PK, Kleber M (2015) Mineral protection of soil carbon counteracted by root exudates. Nat Clim Chang 5:588–595
19. Kuzyakov Y, Bol R (2006) Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. Soil Biol Biochem 38(4): 747–758
20. Kuzyakov Y, Domanski G (2000) Carbon input by plants into the soil. Review. J Plant Nutr Soil Sc 163:421–431
21. Leff JW, Wieder WR, Taylor PG, Townsend AR, Nemergut DR, Grandy AS, Cleveland CC (2012) Experimental litterfall manipulation drives large and rapid changes in soil carbon cycling in a wet tropical forest. Glob Change Biol 18(9): 2969–2979
22. Li G, Han H, Du Y, Hui D, Xia J, Niu S, Li X, Wan S (2017) Effects of warming and increased precipitation on net ecosystem productivity: A long-term manipulative experiment in a semiarid grassland. Agr Forest Meteorol 232(15):359–366
23. Lima TTS, Miranda IS, Vasconcelos SS, Vasconcelos SS(2010) Effects of water and nutrient availability on fine root growth in eastern Amazonian forest regrowth, Brazil. New Phytol 187(3)622–630
24. Liu X, Lin TC, Vadeboncoeur MA, Yang Z, Yang Y (2019) Root litter inputs exert greater influence over soil C than does aboveground litter in a subtropical natural forest. Plant Soil 444(1/2):489–499
25. Matamala R, Gonzàlez-Meler MA, Jastrow JD, Norby RJ, Schlesinger WH (2003) Impacts of fine root turnover on forest NPP and soil C sequestration potential. Science 302 (5649):1385–1387
26. Mei L (2006) Fine root turnover and carbon allocation in Manchurian Ash and Davurian Larch plantations. Dissertation, Northeast Forestry University
27. Meier IC, Finzi AC, Phillips RP (2017) Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. Soil Biol Biochem 106: 119–128
28. Nguyen C (2003) Rhizodeposition of organic C by plants : mechanisms and controls. Agronomie 23:(5-6):375–396
29. Nobili MD, Contin M, Mondini C, Brookes PC (2001) Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biol Biochem 33: 1163–1170

30. Phillips RP, Bernhardt ES, Schlesinger WH (2009) Elevated CO2 increases root exudation from loblolly pine (Pinus taeda) seedlings as an N-mediated response. Tree Physiol 29(12):1513–1523
31. Phillips RP, Erlitz Y, Bier R, Bernhardt ES (2008) New approach for capturing soluble root exudates in forest soils. Funct Ecol 22: 990–999
32. Phillips RP, Finzi AC, Bernhardt ES (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol Lett 14:187–194
33. Quan Q, Tian D, Luo Y, Zhang F, Crowther TW, ZHU K,Chen HYH, Zhou Q, Niu S (2019) Water scaling of ecosystem carbon cycle feedback to climate warming. Sci Adv 5(8): eaav1131.
34. Rasse DP, Rumpel C, Dignac M-F (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269: 341–356
35. Rodtassana C, Tanner EVJ(2018) Litter removal in a tropical rain forest reduces fine root biomass and production but litter addition has few effects. Ecology 99(3): 735–742
36. Rubino M, Dungait JAJ, Evershed RP, Bertolini T, Angelis PD, D'Onofrio A, Lagomarsino A, Lubritto C, Merola A, Terrasi F, Cotrufo MF(2010) Carbon input belowground is the major C flux contributing to leaf litter mass loss: Evidences from a 13C labelled-leaf litter experiment. Soil Biol Biochem 42(7): 1009–1016
37. Santos F, Nadelhoffer K, Bird JA (2016) Rapid fine root C and N mineralization in a northern temperate forest soil. Biogeochemistry 128:187–200
38. Sayer EJ (2006) Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. Biol Rev Camb Philos Soc 81(1):1–31
39. Sayer EJ, Baxendale C, Birkett AJ, Bréchet LM, Castro B, Kerdraon-Byrne D, Lopez-Sangil L, Rodtassana C (2020) Altered litter inputs modify carbon and nitrogen storage in soil organic matter in a lowland tropical forest. Biogeochemistry156(1):1–16
40. Sayer EJ, Heard MS, Grant HK, Marthews TR, Tanner EVJ (2011) Soil carbon release enhanced by increased tropical forest litterfall. Nat Clim Chang 1:304–307
41. Sayer EJ, Tanner EVJ (2010) Experimental investigation of the importance of litterfall in lowland semi-evergreen tropical forest nutrient cycling. J Ecol 98(5):1052–1062
42. Sayer EJ, Tanner EVJ, Cheesman AW (2006) Increased litterfall changes fine root distribution in a moist tropical forest. Plant Soil 281:5–13
43. Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DAC, Nannipieri P, Rasse DP, Weiner S, Trumbore SE (2011) Persistence of soil organic matter as an ecosystem property. Nature 478: 49–56
44. Silver WL, Thompson AW, Mcgroddy ME, Varner RK, Dias JD, Silva H, Crill PM, Keller M (2005) Fine root dynamics and trace gas fluxes in two lowland tropical forest soils. Glob Change Biol 11:290–306
45. Sokol NW, Bradford MA (2019) Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. Nat Geosci 12:46–53
46. Song J, Wan S, Piao S et al (2019) A meta-analysis of 1,119 manipulative experiments on terrestrial carbon-cycling responses to global change. Nat Ecol Evol 3: 1309–1320
47. Spain AV(1984) Litterfall and the standing crop of litter in three tropical Australian rainforests. J Ecol 72(3):947–961
48. Tanner EVJ, Sheldrake MWA, Turner BL (2016) Changes in soil carbon and nutrients following 6 years of litter removal and addition in a tropical semi-evergreen rain forest. Biogeosciences 13: 6183–6190
49. Vogt KA, Vogt DJ, Bloomfield J (1998) Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant Soil 200:71–89

50. Vogt KA, Vogt DJ, Palmiotto PA, Boon P, O'Hara J, Asbjornsen H (1996) Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. Plant Soil 187: 159–219
51. Wang R, Cheng R, Xiao W, Feng X, Liu Ze, Ge X, WANG X, Zhang W (2012) Fine root production and turnover in *Pinus massoniana* plantation in Three Gorges Reservoir Area of China. J Appl Ecol 23(9) : 2346–2352
52. Wood TE, Lawrence D, Clark DA, Chazdon RL (2009) Rain forest nutrient cycling and productivity in response to large-scale litter manipulation. Ecology 90(1):109–121
53. Xu S, Sayer EJ, Eisenhauer N, Lu X, Wang J, Liu C (2021) Aboveground litter inputs determine carbon storage across soil profiles: a meta-analysis. Plant Soil 462:429–444
54. Yin H, Li Y, Xiao J, Xu Z, Cheng X, Liu Q (2013) Enhanced root exudation stimulates soil nitrogen transformations in a subalpine coniferous forest under experimental warming. Glob Change Biol 19(7): 2158–2167
55. Yuan Y, Zhao W, Zhang Z, Xiao J, Li D, Liu Q, Yin H (2018) Impacts of oxalic acid and glucose additions on N transformation in microcosms via artificial roots. Soil Biol Biochem 121: 16–23
56. Zhang X, Wu K(2001) Fine-root production and turnover for forest ecosystems. Scientia Silvae Sinicae 37(3): 126-138

Figures

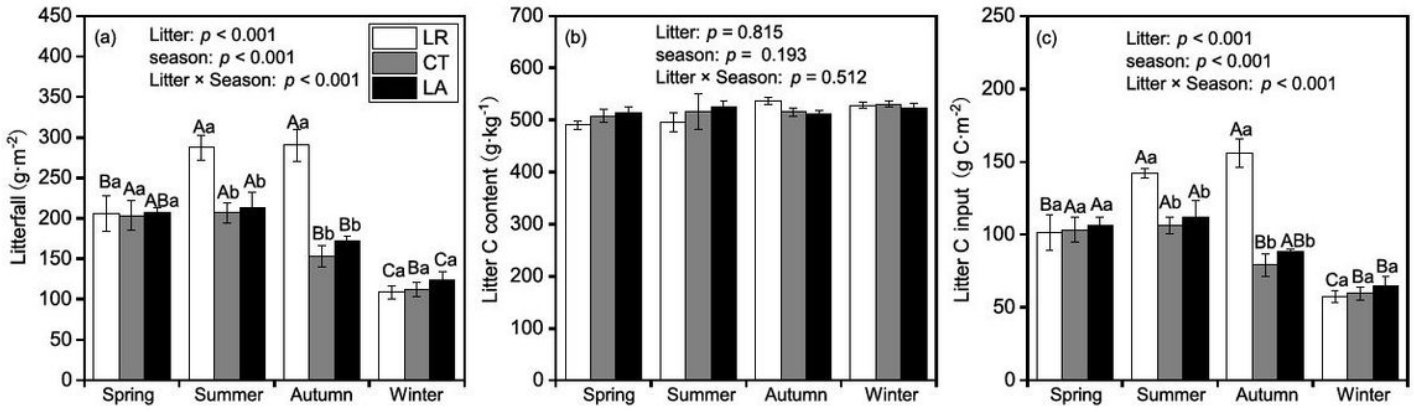

Figure 1

Seasonal variation in (a) litterfall, (b) litter C content, and (c) litter C input under litter treatments (mean ± SE, n = 3). LR, litter removal; CT, control; LA, litter addition. Different capital letters indicate significant differences between the seasons (P <0.05). Different lowercase letters indicate significant differences among the litter treatments (P <0.05).

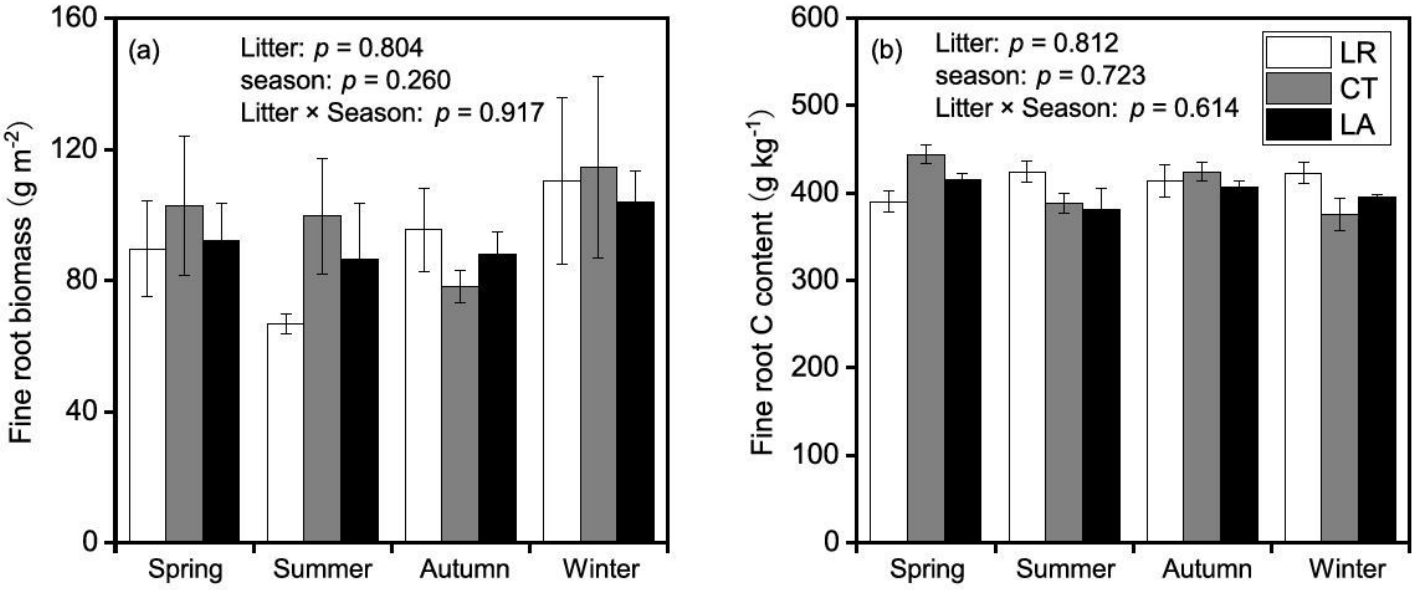

Figure 2

Seasonal variation in (a) fine root biomass and (b) C content under litter treatments (mean ± SE, n = 3). LR, litter removal: CT, control; LA, litter addition.

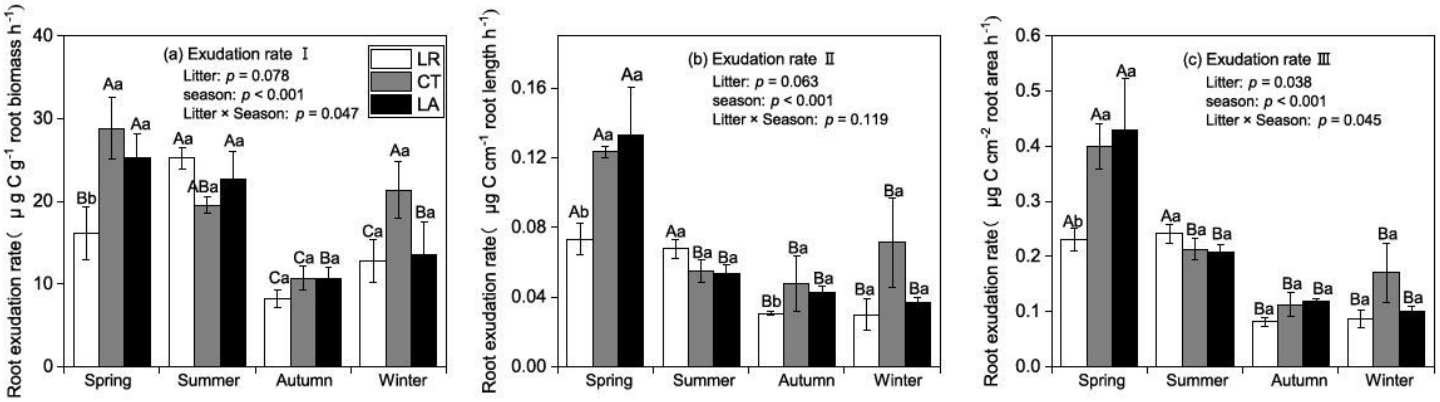

Figure 3

Seasonal variation in root exudation of *Pinus massoniana* under litter treatments (mean ± SE, n = 3). (a) Root exudation I (μg C g^{-1} root biomass h^{-1}), (b) Root exudation II (μg C cm^{-1} root $length^{-1}$), (c) Root exudation III (μg C cm^{-2} root area h^{-1}). LR, litter removal; CT, control; LA, litter addition. Different capital letters indicate significant differences between the seasons ($p < 0.05$). Different lowercase letters indicate significant differences among the litter treatments ($p < 0.05$).

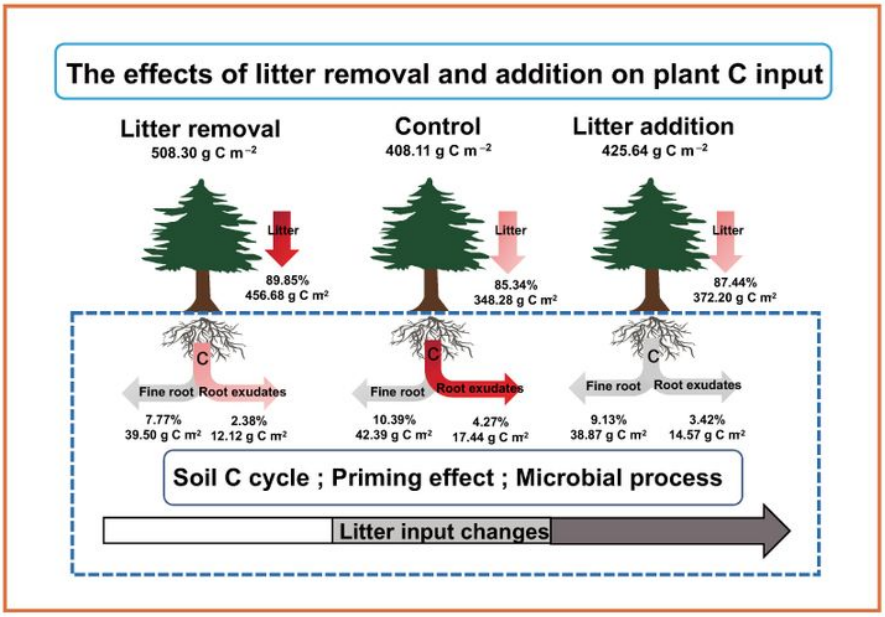

Figure 4

Effects of litter treatments on plant C input. The dark red arrows indicate significant increases of C input among the litter treatments ($p < 0.05$), the light red arrows indicate significant decreases of C input among the litter treatments ($p < 0.05$), and the light gray arrows indicate no significant differences of C input among the litter treatments ($p > 0.05$).