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Effect of Forest Litter on the Regeneration of *Larix sibirica*: Insight from Aqueous Extract and Litter Coverage

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Abstract

The effect of litter on forest regeneration depends on the characteristics of regional climate and also shows community specificity. The influences of plant litter on seed germination and seedling growth of Larch Siberian forest in the Altai Mountains were investigated through two simulated experiments including litter coverage and litter aqueous extracts. In the litter coverage experiment, three litter coverage methods including above (D), below (S) and in the middle (Z) of litter were set with the litter coverage thickness of 0, 1, 2, and 4 cm, while two aqueous extract obtained methods using the air-dried litter and litter ash after fir were used with the concentration of 10%, 40%, 80% and 100% in the present study. Results showed that: the aqueous extracts obtained using the air-dried litter restrained the seed germination, while the aqueous extracts obtained using litter ash improved the seed germination. Compared with other litter concentration, the influences of 100% concentration reach highest. The seed germination rate, seed germination potential and vital index under the treatment of seeds above the litter coverage were highest, which were significantly higher than other treatments. The above-ground biomass was significantly higher and the inhibition index of below-ground bio-mass was significantly lower under the treatment of seed above the litter with thin litter cover-age (S1) compared to other litter coverage treatments. These results indicated that the litter aqueous extract and the litter coverage had a combined effect on the seed germination and seedling growth of Siberian larch forest. Fire disturbance could promote seed germination by modifying the adverse effects of litter aqueous extracts and litter coverage, and thus plays an important role in the regeneration of Siberian larch in the Altai Mountains.

Keywords

Litter Physical Barrier, Fire Burning, Seed Germination, Northwest China

1. Introduction

Litter is the general term of organic material produced by vegetation in different ecosystems, including the shed debris of leaves, branches, shed bark, reproductive organs of dead trees, shrubs and grass (Meentemeyer et al., 1982). Litter production and decomposition were known as the major pathway of carbon and nutrient flux in forest ecosystems (Stoler & Relyea, 2030). Knowing the dynamics of plant litter and the direct and indirect influences of plant litter on the vegetation populations is important for the sustainable use of forest ecosystems (Facelli & Pickett, 1991; Wu et al., 2021).

Decomposition of plant litter can release nutrients and a series of intermediate produces into soils, which can significantly change the physical and chemical environment (Gu et al., 2022). Many previous studies reported that litter could directly or indirectly affect seed germination and seedling growth through different ways during the forest regeneration (Huo et al., 2019; Huang et al., 2015). The releasing nutrients including nitrogen, phosphorus and potassium by litter decomposition increased the nutrient contents in soil, which could be used by plant seedling for growth (Wu et al., 2021; Zheng et al., 2021). The accumulated litter in soil surface intercepts light, reduces the soil evaporation, changes the soil temperatures, and thus alters the microenvironment of vegetation growth (Veen et al., 2019; Facelli & Pickett, 1991). Through creating a physical barrier to plant seed, the cumulative litter intercepts the seeds reaching the soil, and reduces the chance of seed germination and seedling growth (Stoler & Reyea, 2020). In addition, the plant litter can also produce a series of allelochemicals, which could significantly improve or hinder the seedling germination and growth, and thus has an important effect on the forest regeneration (Huo et al., 2019; Muturi et al., 2017). Although some studies have reported that litter had important influences on the seed germination and seedling growth, the related research is still needed to be strengthened because of the complex mechanism.

Soil microbial activity plays an important role in the process of litter decomposition (Stoler & Relyea, 2020; Wu et al., 2021). However, the composition and activity of soil microorganisms are significantly affected by soil temperature and the quality of litter (Nagai et al., 2017). Therefore, different soil temperatures and various litter qualities and species determined the litter decomposition rate in different forest ecosystems, and thus leading to the influences of plant litter on forest regeneration showing significant regional spatial characteristics and community specificity (Krishna & Mohan, 2017; Fanin et al., 2021). In the cold temperate zone of high latitudes, the lower annual average temperature restricts the activity of soil microorganisms and reduces the litter decomposition rate, and then affects the litter thickness in forest and alters the distribution pattern of seed banks (Gao et al., 2022; Rawat et al., 2021). Previous study indicated that increasing litter thickness had negative effects on the seed germination, and appropriate removal of litter is beneficial the regeneration in a Pine and Oak mixed forest (Huo et al., 2019). In addition, the lower nutrient release processes and changes in soil physical - chemical properties and plant allelochemicals resulting from the litter decomposition in the cold temperate zone also lead to the influences of plant litter on forest regeneration different from other regions (Muturi et al., 2017; Liang et al., 2016).

As a Larix tree in the Larch family, *Larix sibirical Ledeb.* is distributed in the eastern Tianshan Mountains and the Altai Mountains in Xinjiang, as well as in Ural Mountains, Eastern Siberia and other cold temperate regions of high latitude (Chertov et al., 2021). In the middle part of the Altai Mountains in China, the widely distributed, typical and important natural vegetation type in the forest ecosystem is the *Lrix sibirica* forest, which play an important role in maintaining regional ecological security and biodiversity (Kharuk et al., 2019). However, the natural regeneration of *Lrix sibirica* faced serious problems, which results in obvious forest degradation (Juricka et al., 2020). Although previous studies had reported the influences of human activities (e.g. tourist activity and grazing) on the forest regeneration of *Lrix sibirica* in the Altai Mountains, the effects of litter including the litter thickness and the allelopathy (aqueous extract of litter) on the forest regeneration were still unknown (Peng et al., 2022; Dulamsuren, 2021). The lack of the related degradation reasons of *Lrix sibirica* hinders the protection and restoration of degraded forest ecosystem in Altai Mountains. Therefore, the objectives of this study were to: 1) compare the influences of different litter thickness on the seed germination and growth of *Lrix sibirica*, and 2) assess the effects of aqueous extract of litter on the seed germination of *Lrix sibirica*.

2. Materials and Methods

2.1. The Seed Collection and Pretreatment

Lrix sibirica seeds were collected in the Habahe forest region (48˚30' - 48˚33'N, 86˚71' - 86˚78'E, **Figure 1**) in Sep. 2021, which was the season of seed ripening. The collecting seeds with full grain and good quality (**Figure 2**) were put in a breathable cloth bag and stored at the room temperature for seed germination experiment. Two pretreatment steps were used to select the appropriate *Lrix sibirica* seeds before starting the seed germination experiment. First, the wash clean seeds by the distilled water were put in a 1000 ml beaker with 40˚C warm distilled water for 3 h, and then removed the floating seeds and picked out the sunken seeds. Second, the chosen seeds in the first step were put another 1000 ml beaker with 0.2% $KMnO_4$. After 30 mins, the soaking seeds were taken out from the $KMnO_4$ solution, and then washed three times by the distilled water for the following seed germination experiment.

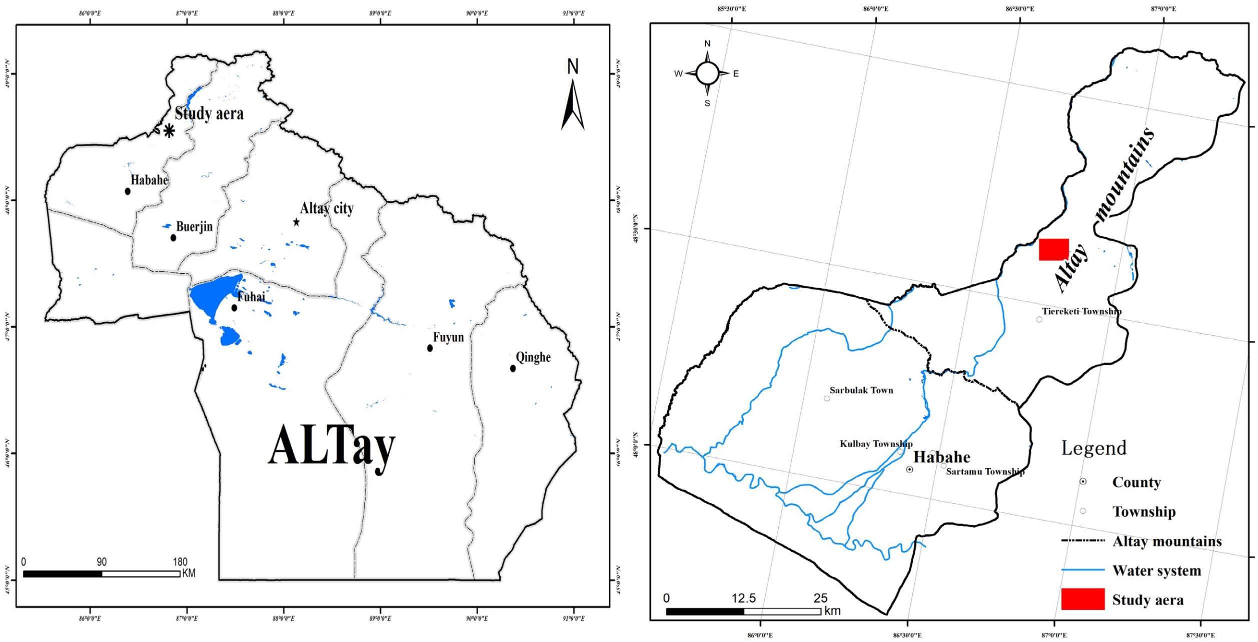

Figure 1. The map of the study area.

Figure 2. The seeds of *Lrix sibirica* used in this study.

2.2. Collection of Plant Litter

In Mar. 2021, five standard plots (50 m × 50 m) were randomly established as the replicates in the Qinghe Forest Park, Altai mountain. In each replicated plot, five sub-plots (1 m × 1 m) in the four directions of south, north, east and west, and the central part were set up to collect the litter. The collected plant litters were immediately taken back to the laboratory and air dried.

2.3. The Litter Aqueous Extract Experiment

Two methods were used to prepare the litter aqueous extract in the present study (Li et al., 2006; Li et al., 2021). 1) 100 g air dried litter was put in a beaker with 1000 ml distilled water that formed a 1:10 mixture. After soaking for 48 h, the mixture of litter and distilled water were filtered with filter paper to obtain the mother liquid. Then, the mother liquid was diluted into four concentration gradients of 10%, 40%, 80% and 100% using distilled water. 2) 500 g air dried litter was fired for 24 h to collect the litter ash. The litter ash was steeped 48 h with 500 ml distilled water, and the mixture was stirred using a glass rod for 10 mins in each 6 h. The soaking mixture were filtered to obtained the mother liquid, and then was diluted into four concentration gradients of 10%, 40%, 80% and 100% using distilled water. The obtained litter aqueous extracts using above two methods were put in brown jar and stored in a refrigerator at 2˚C for the following seed germination experiment.

The seed germination experiment was conducted in a light incubator (PXY-250g-B, Keli company, Anhui, China) with a humidity of 60% (23˚C, 12 h light with light intensity of 250 $\mu mol \cdot (m^2 \cdot s)^{-1}$/20˚C, 12 h dark). 50 seeds were put in a culture dish (9 cm inner diameter) with two filter papers, and then 20 ml litter aqueous extract was added in this culture dish. Nine aqueous extract treatments were selected in the present study including one control (CK, using distilled water instead of aqueous extract, 0%), four aqueous extracts using air dried litter (W1, 10%; W2, 40%; W3, 80%; W4, 100%), and four aqueous extracts using litter ash (H1, 10%; H2, 40%; H3, 80%; H4, 100%). Each treatment was replicated four times. 20 ml similar aqueous extracts as the treatment were added in each treatment every 2 days during the experiment (Li & Ren, 2008).

2.4. The Litter Coverage and Thickness Experiment

Four litter coverage types were used in the present study: 1) CK, seeds were put above the soils and no litter coverage; 2) D, seeds were put between the litter and soils; 3) Z, seeds were put in the center of the plant litter; 4) S, seeds were put above the litter (**Figure 3**). There were three litter thickness levels in each litter coverage treatment. In the litter coverage of D treatment, the litter thicknesses above the seeds were 1 cm (D1), 2 cm (D2), and 4 cm (D4). In the litter coverage of Z treatment, the litter thickness above and below the seeds were all 0.5 cm (Z1), 1 cm (Z2), and 2 cm (Z4). In the litter coverage of S treatment, the litter thicknesses below the seeds were 1 cm (S1), 2 cm (S2), and 4 cm (S4). 50 seeds were put in a germination bed with the litter coverage and thickness treatment. Each treatment was replicated three times, and a total of 30 seed germination beds were used in this study. These 30 seed germination beds were put in a light incubator (PXY-250g-B, Keli company, Anhui, China) with a humidity of 60% (23˚C, 12 h light with light intensity of 250 $\mu mol \cdot (m^2 \cdot s)^{-1}$/20˚C, 12 h dark). After sowing, 100 ml distilled water was supplemented in each treatment every 2 days during the germination experiment.

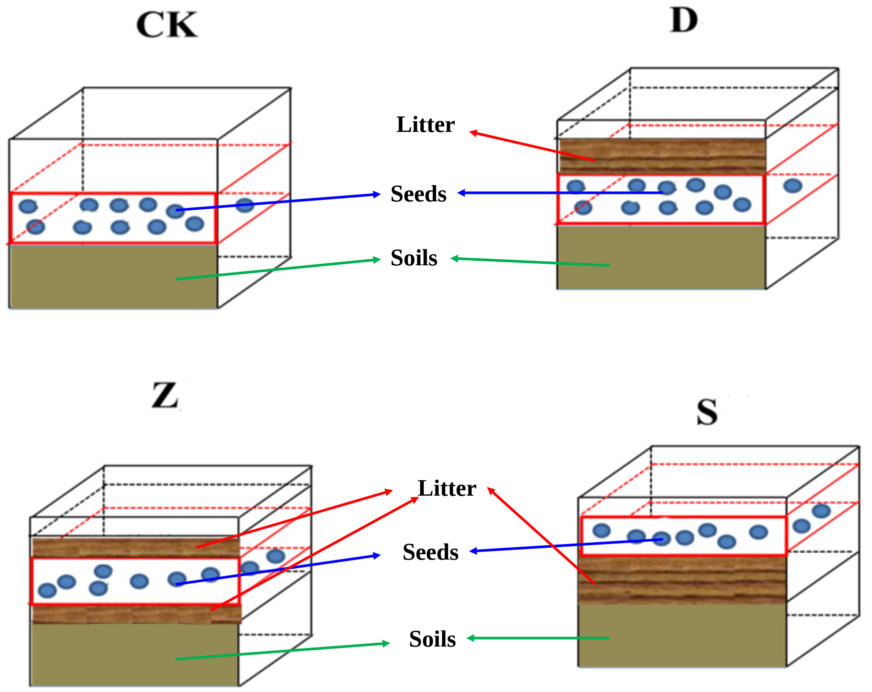

Figure 3. Seed germination experiment demonstration of litter coverage and thickness.

2.5. Determination of Seed Germination and Seedling Growth

The number of germinated seeds was counted every two days. The seed was considered to germination when the radical reached 1/2 length of the seed. Seed germination was considered to be finished when no seed germinated for 7 consecutive days. After completion of the germination experiment, the germination rate, germination potential and seed vital index were calculated using following equations:

$$\mathrm{GR} = \frac{(\text{The number of germinated seeds})}{\text{The total number of seeds}} \times 100\%$$

$$\mathrm{GP} = \left(\frac{\text{The maximum number of germinated seeds per day}}{\text{The total number of seeds}}\right) \times 100\%$$

$$\mathrm{VI} = \mathrm{S} \times \sum (\mathrm{Gt}/\mathrm{Dt})$$

where GR refers to the germination rate; GP refers to the germination potential; VI refers to the vital index; S refers to the average length of germination seed when the completion of the germination experiment; Gt refers to the number of germination seeds; Dt refers to the germination time (Li et al., 2021).

The seedling growth was only measured in the experiment of litter coverage and thickness. The number of seedling was counted in each treatment after 40 days seedling growth period. The seedling growth period was calculated from the end of the germination experiment. In each treatment of litter coverage and thickness, 9 seedlings of *Larix sibirica* were selected to measure the length, fresh weight and dry weight of above-ground and below-ground biomass. The inhibition index was calculated using following equations:

$$\text{IHI} = \frac{\text{The length of control} - \text{The length of treatment}}{\text{The length of control}} \times 100\%$$

where IHI refers to the inhibition index of above-ground or below-ground biomass.

2.6. Data Analysis

All data analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to test the effect of different treatments on seed germination and seedling growth. The differences among the treatments groups was compared using Fisher's least significant difference test at the significance level of $P = 0.05$.

3. Results

3.1. Effect of Litter Aqueous Extract on the Seed Germination

The aqueous extracts obtained using the air-dried litter restrained the seed germination, while the aqueous extracts obtained using litter ash improved the seed germination (**Figure 4**). The inhibiting effect of the aqueous extracts using the air-dried litter increased with the increasing of the solution concentration. The seed germination rates under the solution concentration of 10%, 40%, 80% and 100% were 20.5%, 10.5%, 5.0% and 0.0%, respectively. In contrast, the seed germination rates increased from 21.67% to 34.58% when the concentration of aqueous extract using the litter ash increased from 10% to 100%. The change trends of seed germination potential and seed vital index under the different aqueous extracts using the air-dried litter and litter ash were similar with that of seed germination rate (**Figure 4**). Compared with the CK, the seed germination rate, seed germination potential and seed vital index under the solution concentration of 40%, 80% and 100% were significant lower for the aqueous extracts using the air-dried litter, while they were significant higher for the aqueous extracts using the litter ash.

3.2. Effect of Litter Coverage and Thickness on the Seed Germination

Different litter coverages and thicknesses have significant effects on the seed germination (**Table 1**). Compared with the CK, S treatment significantly increased the germination rate, the germination potential and the vital index, while the treatments of D and Z reduced the germination rate, the germination potential and the vital index. In the treatment of S, the lower litter thickness (S1) has higher germination rate, germination potential and the vital index than the S2 and S4. In the treatment of D, although the differences of germination rate, germination potential and the vital index under the D1, D2, D4 were not significant, these values under the D2 was litter higher than that under D1and D4. In the treatment of Z, the germination rate and germination potential under the Z4 treatment were significantly higher than that under the Z1 and Z2, while the difference between Z1and D2 was not significant.

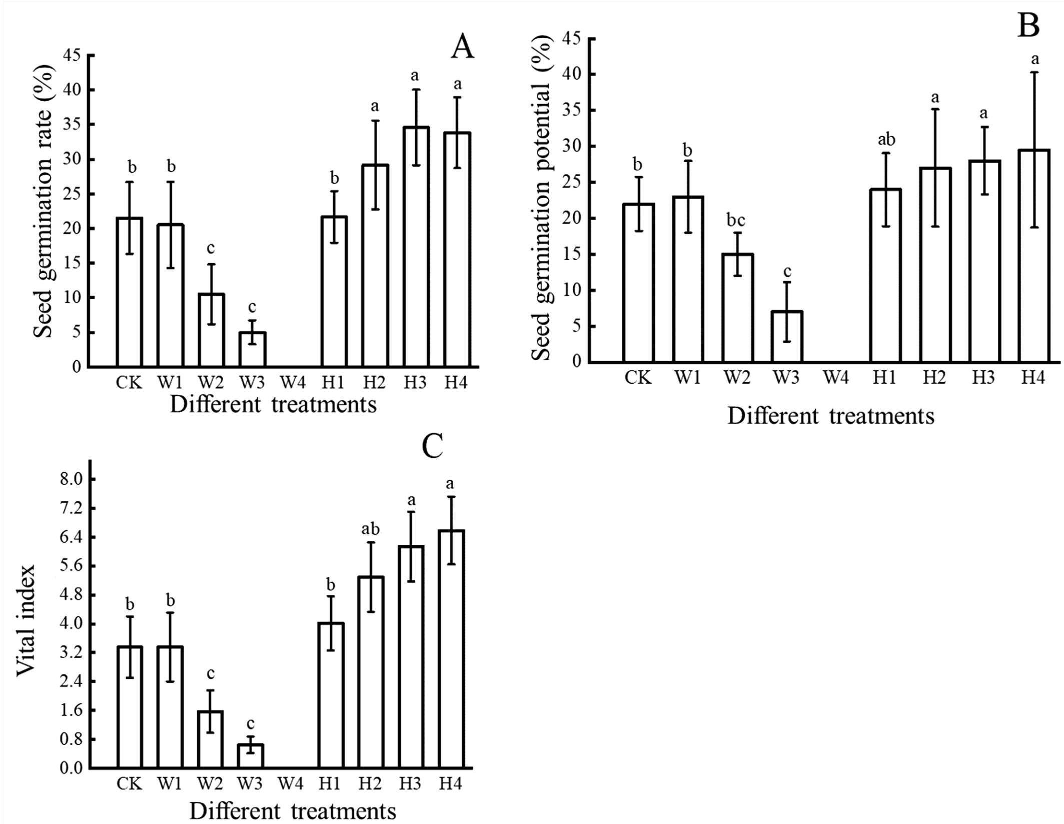

Figure 4. Effects of litter aqueous extracts on the germination of larch seeds. CK means control. W1, W2, W3 and W4 mean the concentration of litter aqueous extracts using air-dried litter were 10%, 40%, 80% and 100%, respectively. H1, H2, H3 and H4 mean the concentration of litter aqueous extracts using litter ash were 10%, 40%, 80% and 100%, respectively. Different lower letters indicate significant differences ($P < 0.05$) among treatments.

Table 1. Seed germination under the different litter coverage and thickness.

Litter thickness/cm	Germination rate/%	Germination potential/%	Vital index
CK	26 ± 3.85b	22 ± 1.73b	49.22 ± 5.3b
D1	2.6 ± 0.88c	2 ± 1.86c	3.33 ± 1.03c
D2	3.4 ± 0.3c	2 ± 0.33c	5.69 ± 1.05c
D4	2 ± 1c	1.2 ± 1.45c	2.6 ± 0.45c
Z1	12.6 ± 1.45bc	10 ± 5bc	20.25 ± 3.56c
Z2	12 ± 1bc	9.2 ± 1.45c	20.39 ± 2.14c
Z4	22 ± 2.3b	19.2 ± 3.84b	38.31 ± 3.26bc
S1	56 ± 5.57a	50 ± 0.33a	128 ± 15.26a
S2	45.4 ± 2.85a	34.6 ± 0.67ab	80.69 ± 2.36a
S4	36.6 ± 5.17ab	29.2 ± 2.67b	67.75 ± 6b

Note: D1, D2 and D4 represent the litter thickness above the seed was 1, 2 and 4 cm, respectively; Z1, Z2 and Z4 represent the seed was put in the middle of the plant litter, where the litter thickness was 1, 2 and 4 cm, respectively; S1, S2 and S4 represent the litter thickness below the seed was 1, 2 and 4 cm, respectively. The lowercase letters within treatments are not significant different at $P < 0.05$.

3.3. Effect of Litter Coverage and Thickness on the Seedling Growth

Different litter coverage and litter thickness had significantly influenced on the seedling growth of *Larix sibirica* (Figure 5). The highest above-ground biomass was found under D1, which was significantly higher than that under other treatments. D4 had the lowest above-ground biomass, and it was significantly lower than that under other treatments except the D2, Z2, and S4. The below-ground biomass under Z4 was significantly higher than other treatments, while the D4 had the lowest value. The D1 inhibited the growth of above-ground and below-ground biomass, while the S1 and S2 improved the growth of above-ground and below-ground biomass. The treatment of D2, D4, Z1, Z2, Z4, and S4 improved the growth of above-ground biomass, but inhibited the growth of below-ground biomass. The higher improvement of above-ground biomass was found under Z2, S1 and S2 than that under D2, D4, Z1, Z4 and S4, while the higher inhibition of below-ground biomass was found under D1, D4, Z1 and Z4 than that under D2, Z2 and S4.

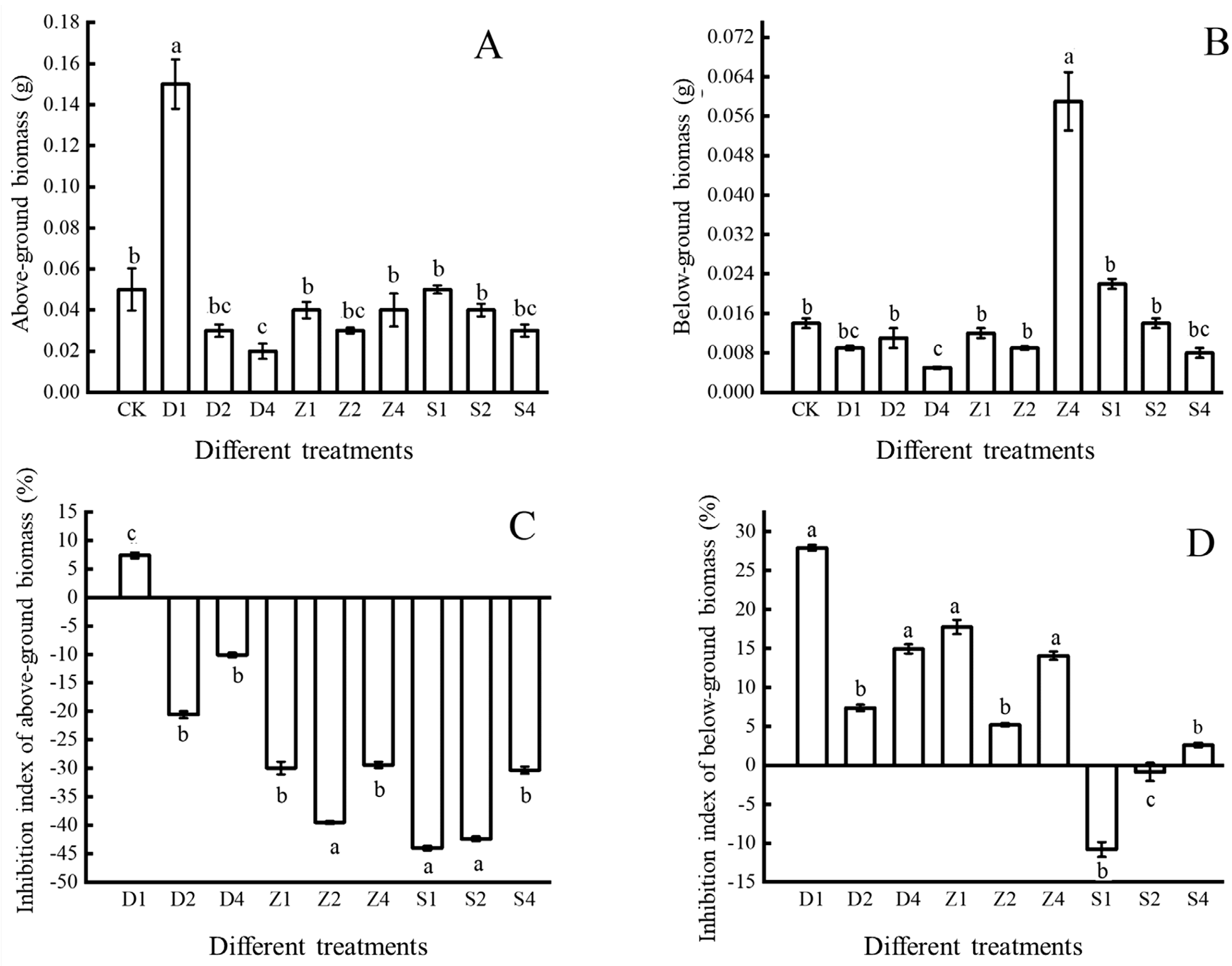

Figure 5. Influence of different litter coverage and thickness on the seedling growth. Different lower letters indicate significant differences ($P < 0.05$) among treatments. See Figure 4 for abbreviation.

4. Discussion

Seed germination and seedling growth are the most vulnerable and sensitive stages of plant life history, which are easily affected by the interference and inhibition of external environmental conditions and thus causing death (Tobe et al., 2000). The litter in the forest ecosystem determines the seed bank in soils, changes the environmental conditions for the early growth of plants, and thus affecting the natural regeneration of forest system (Calviño-Cancela et al., 2018).

Results of the present study showed that litter aqueous extracts had significant influences on the seed germination of *Larix sibirica* (**Figure 4**). The aqueous extracts obtained using the air-dried litter restrained the seed germination, and the inhibiting effects increased with the increasing of contents in the litter aqueous extracts. This was mainly due to the interspecific competition between plants. The abundant litter in forest ecosystem inhibited the seed germination, and produced autotoxic effects on the plant seedling, and thus helping the trees win the competition (Lorenzo et al., 2010). In contrast, the aqueous extracts obtained using litter ash improved the seed germination rate and the vital index, and the improvement on the seed germination increased with the increasing of contents in the litter aqueous extracts. This finding was in line with the results of Wang et al. (2021) in the northeast China. These results indicated that the allelopathic substances contained in the plant litters were transformed and rendered harmless or improved the growth and the seed germination when the litters were properly disturbed by fire. The fire accelerated the decomposition of plant litter and released a lot of soil nutrients needed for plant growth, and thus enhanced the seed germination and plant growth (Athina et al., 2008; Stevens-Rumann & Morgan, 2019). In addition, fire disturbance could change the vegetation coverage and composition of specifics, soil conditions and microclimate, and thus creating a favorable and suitable habitat for seed germination in forest ecosystem, which is beneficial to forest regeneration (Hille & Ouden, 2004; Bontrager et al., 2019; Hammond et al., 2019). These results suggested that we can carry out appropriate auxiliary recovery by artificially intervention after forest fire to improve the regeneration and recovery of larch forest in southwest China, such as artificially sowing seeds in some burned areas. However, there are many uncertain factors of fire distribution in the natural environment, so it is necessary to be careful in the concrete implementation of fire distribution in forest ecosystem.

In addition to influence the seed germination through litter aqueous extracts, the seed germination can also be affected by the litter coverage and thickness. Our results showed that the seed putting above the plant litter improved the seed germination rate, seed germination potential and vital index, and the effects was obvious when the litter thickness was less than 4 cm. The improvement of plant litter on seed germination was mainly related to the soil seed banks. The thick litter cover or the dense litter cover creates a physical barrier that prevents the seed from contacting the soil layer, and thus limiting the seed germination (Stevens-Rumann & Morgan, 2019; Hille & Ouden, 2004). In addition, the thick litter cover reduces the solar radiation, and blockes the gas exchange and material flow between the soil surface and the external environment, and further restricting the seed germination (Chen et al., 2018). These reasons were all supported by the results in the present study (**Table 1**).

The growth of plant seedlings has a certain plasticity. The seedlings themselves changed with the changing of environment, and the litter thickness played an important role in this process (Xu et al., 2022). Results of the present study indicated that the above-ground and below-ground biomass showed a trend of first rising and then falling with the increasing of litter thickness. That is, the thin cover of plant litter had a slight promotion effect on the growth of larch seedlings, but had a significant inhibition effect when the litter cover was thick. These results were consistent with the findings of Xu et al. (2022), Zhao et al. (2019) and Ruprecht et al. (2010) in the study of seedling growth of Chinese fire forest. The improvement of seedling growth under the thin litter cover was mainly due to the reduction in the variations of soil moisture and temperature, which provided a better warm and humid environment for the growth of seedlings. In contrast to the thin litter cover, the thick litter cover formed physical barrier, inhibiting light and photosynthetic respiration, and then limiting the growth of seedlings.

5. Conclusion

The two joint of litter aqueous extracts and litter coverage type and thickness influenced the regeneration of larch community. Litter aqueous extracts obtained from the air-dried litter restrained the seed germination. However, the aqueous extracts obtained using litter ash after fire improved the seed germination, because fire changed the litters' chemical composition. Our results indicated that the thin litter cover had a slight promotion effect on the growth of larch seedlings, while the thick litter cover significantly inhibited the growth of larch seedlings. Compared with other treatments, treatment of litters with a thickness of 1 - 2 cm below the seed layer is most favorable for seed germination. The different effects of litter thickness on the seed germination were due to the physical barriers resulting from the litter. In conclusion, litter of Siberian larch is self-toxic, and thick litter cover had negative effect on the seed germination. The fire burning can reverse these adverse effects of litter. Therefore, we can artificially sow larch seeds on the burned land, effectively improve seed germination and seedling growth, and artificially promote the natural regeneration of the Siberian larch community.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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