ALLELOPATHIC EFFECT OF CASTANOPSIS KAWAKAMII FOREST LITTER ON SEED GERMINATION OF SMALL PHILIPPINE ACACIA (ACACIA CONFUSA)

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Abstract. Nutrient limitation constrains the regeneration of *Castanopsis kawakamii* seedlings in subtropical forests. Small Philippine acacia (*Acacia confusa*), a common tree species in mixed forests, is known for its nitrogen fixation capabilities. Therefore, we attempted to intercrop *A. confusa* in a *C. kawakamii* natural forest. However, this forest contained abundant litter, harboring ample tannins and total phenols. Whether the allelopathy of litter could constrain the seed germination of *A. confusa* seed was unknown. Therefore, we aimed to explore the influence of forest litter extracts on the seed germination and radicle growth of *A. confusa*. The results indicate that low concentration litter extracts promote whereas high concentrations inhibit *A. confusa* seeds. The stock solution produced the strongest inhibition, whereas the 1:160 litter extract concentration had promoted the germination rate and radicle growth. The *A. confusa* radicle increase was remarkable in the first eight days, and tended to decrease from day 9 to 15. Overall, the allelopathic effect of litter is not a factor limiting the mixing of *C. kawakamii* and *A. confusa*. This could provide an alternative method to improving soil nutrition, thus promoting the regeneration of *C. kawakamii* seedlings.

Keywords: germination rate, germination potential, germination index, radicle length, mixed forest

Introduction

Castanopsis kawakamii is a unique subtropical evergreen broad-leaved species. It has survived as an endangered tertiary relict plant, only found in Fujian, Taiwan, Jiangxi, Guangdong, and Guangxi provinces in China (Buanjan et al., 2018). Currently, natural C. kawakamii forests are characterized by limited seed germination and seedling growth due to the low total nitrogen and total phosphorus in the soil (Su et al., 2012). As crucial aspects of a forest ecosystem, the physical and chemical properties and nutrients in the soil are significant to seedling development (Hou et al., 2011). However, previous studies mainly focused on community ecology (He et al., 2019); forest gap regeneration (He et al., 2018); natural forest, its plantation litter, and soil biochemistry cycle (Ma et al., 2015); soil microbes, biochemical characteristics (Ma et al., 2014); and soil-litter

stoichiometry to protect this rare species (Feng et al., 2017). Little research has focused on improving soil nutrition to promote species regeneration in this forest. Thus, in this study, we aimed to replenish soil nutrients to accelerate the regeneration of *C. kawakamii* seedlings.

Consistent with the importance of rhizobia-mediated N partitioning, inclusion of legumes is instrumental for generating positive diversity-productivity relationships (Wang et al., 2019a). With the existence of legumes and rich nitrogen in the litter layers, *Acacia confusa* has powerful fixation ability. Previous reports showed that one hectare of *A. confusa* forest can provide 3500 kg of litter per year, which is equivalent to 500 kg of carbamide after litter decomposition (Hu et al., 2015; Tung et al., 2015; Liu, 2011). As a common tree species in mixed forests, *A. confusa* is cultivated in tropical and subtropical regions in southwestern and southeastern provinces in China. It has a fast growth rate, wide adaptability, high biomass, and good water retention. Mixed with *Castanopsis* plants, such as *C. hainanensis*, *C. carlesii*, and *C. hystrix*, *A. confusa* could help *Castanopsis* plants to grow better. *A. confusa* also does not cause damage to *Castanopsis* plants (Liu, 2011), providing an alternative method for managing *A. confusa* in natural *C. kawakamii* forest to replenish and enrich the nutrient contents and to improve the establishment and regeneration of the target seedlings.

The amount of litter and the contents of tannin and total phenol in the C. kawakamii natural forest are relatively high (Feng et al., 2017; Yang et al., 2003). The seed germination and radicle growth of C. kawakamii are significantly inhibited under the action of a stock solution of litter (Wang et al., 2019b). The allelochemicals produced by the litter may also affect the germination of A. confusa seeds. Therefore, we hypothesized that: (1) different concentrations of litter extracts have different effects on A. confusa seeds, and high concentration of litter extracts inhibit the seed germination and radicle growth; (2) under the treatment of litter, A. confusa radicles grow considerably in the early period and growth decreases later; and (3) if the litter extract promotes the germination of A. confusa seeds, soil N content may be increased to improve the regeneration of C. kawakamii's seedlings; if the litter extract inhibits germination, it indicates that A. confusa may not suitable as a mixed tree species in C. kawakamii natural forest. Finding other methods to improve the soil N content in this forest is necessary. Therefore, in this study, the extracts of C. kawakamii natural forest litter were used as allelopathic substances to explore the allelopathic effects of litter extracts on the germination of A. confusa seeds. The objective of our study was to provide a scientific basis for intercropping A. confusa species to improve soil nutrition, thereby accelerating the regeneration of *C. kawakamii* seedlings in this forest.

Materials and methods

Study sites and natural history

The *C. kawakamii* Nature Reserve is located in Sanming, Fujian Province, China, at 26°07'–26°12' N and 117°24'–117°29' E. The elevation varies between 180 and 604 m. The climate is medium subtropical monsoon, with an annual average temperature of 19.5 °C, annual precipitation around 1500 mm, and annual relative humidity of 79%. The soil type in this forest is mainly ferric acrisols with abundant humus. The research area has 700 ha of *C. kawakamii*, in which the average age of the population is about 130 years. The arbor layer is dominated by *C. kawakamii*, *Pinus massoniana*, and *Schima superba*. Accordingly, the population density, average tree height, diameter at

breast height, and canopy density are estimated to be 100 individuals/hm², 21.5 m, 32.4 cm, and 0.8, respectively. The shrub layer is mainly composed of *Litsea subcoriacea*, *Vaccinium carlesii*, and *Syzygium buxifolium*; and the herbaceous layers are represented by *Dicranopteris pedata*, *Gahnia tristis*, and *Alpinia oblongifolia* (Liu et al., 2011; Ma et al., 2015).

Experiment design

The litter baskets were arranged in the east, south, west, north, and middle of the fixed plots in the *C. kawakamii* natural forest, and were collected monthly from Sept. 2016 to Sept. 2017. We set up a total of 10 plots and the size of each plot was designed to be about 100 m^2 and the litter baskets were $1.0 \times 1.0 \text{ m}$. The litter basket arrangement is shown in *Figure 1*. The collected litter was divided into four types: leaves, nutshells, branches, and bark. Then, we mixed the litter of each of the four types in accordance with their proportion. In the next step, we weighed 1 kg of each type. We collected 5 kg of *A. confusa* seeds from the same family for experimental materials.

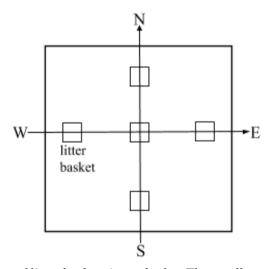


Figure 1. The location of litter baskets in each plot. The small square box represents the location of the litter basket

Preparation of litter extracts

Each kilogram of collected litter sample was washed three times with distilled water to remove surface-attached soil and humus. Next, the samples were crushed and sieved after drying in an oven. After sieving, 10 g of litter were placed into a 50 mL centrifuge tube and 15 mL of 70% ethanol was added. The mixture was leached twice at 80 °C for 1 h in a water bath. After free cooling, the samples were placed into a centrifuge at 4 °C for 5 min at a rotation speed at 8000 rpm. To obtain a stock solution (with three replicates), we filtered the extracts twice and then diluted the extracts to 50 mL with distilled water. As the extraction of litter in nature is repetitive and diverse, the range of extract types and contents is relatively wide. Ethanol was applied as an effective extraction agent to ensure polarized allelochemicals could be maximally extracted out of the litter.

The stock solution was then placed on a rotary evaporator to evaporate the ethanol. The average litter quality and annual precipitation of the *C. kawakamii* natural forest

fixed plot from 2010 to 2017 were 11,010 kg/hm² and 1500 mm/year. Referring to the litter conversion (Yuan et al., 2010), 1:160 is the natural leaching concentration of the litter of natural *C. kawakamii* forest. Considering the rain leaching process, repeated extraction, and microbial decomposition, five concentrations were set as treatments: 1:20, 1:40, 1:80, 1:160, and 1:320. Therefore, the stock solution was diluted again with distilled water with five different concentrations. The dilution rates are listed as follows: 1:20 litter extracts (stock solution was diluted 4 times with distilled water), 1:40 litter extracts (stock solution was diluted 8 times with distilled water), 1:80 litter extracts (stock solution was diluted 16 times with distilled water), 1:160 litter extracts (stock solution was diluted 32 times with distilled water), and 1:320 litter extracts (stock solution was diluted 64 times with distilled water), and then we stored the extracts in a 4 °C refrigerator until use. During the extraction, the ethanol evaporation was incomplete. As a consequence, we also created a blank control group as a contrast that was exempted from the treatment: the same procedure was repeated but without the litter.

Seed germination experiment

The selected and same-sized *A. confusa* seeds were disinfected with 0.5% potassium permanganate for 30 min, then washed with distilled water 3 times before arranging them evenly in a petri dish with *C. kawakamii* forest soil. The *C. kawakamii* forest soil was sterilized in a high temperature sterilization pot at 120 °C for 20 min. The different concentrations of litter extracts were added into each petri dish, with 100 seeds placed uniformly in each petri dish. We set up a total of 6 treatments including stock solution, 1:20 litter extract, 1:40 litter extract, 1:80 litter extract, 1:160 litter extract, and 1:320 litter extract with 3 replications each with distilled water, and a blank control group as the control. The experiment conditions were cultured with a 12 h photoperiod at 25 °C, 12 h dark cycle at 15 °C, light intensity 50 nmol/cm²/s, and relative humidity 75–80% in the incubators. We added 5 mL litter extract daily into the filter paper of the petri dish to maintain moisture.

We recorded the seed germination 3 days after the experiment. A 2 mm seed radicle was defined as seed germination, and the number of germinated seeds was recorded every day (Aldo et al., 2018). We finished the experiment when no further seed germination occurred for 3 continuous days. The radicle length measurements were recorded when the seed germination reached the peak (Wang et al., 2019b). We measured the radicle length of 20 seeds with a similar radicle length in different petri dishes after recording the seed germination for three days when the seed germination reached the peak. The length of the radicle was also measured daily with an electronic digital caliper with a resolution of 0.01 mm. The radicle daily growth amount was simultaneously calculated by the difference in the value of the radicle length between the current day and the previous day. On the 15th day, we finished the radicle experiment when the radicle growth was generally stable. Finally, the germination rate, germination potential, germination index, allelopathic effect index, and the radicle length of seeds were thereby calculated (Bruce et al., 1988). The formulas are listed below (He et al., 2012; Gabriel et al., 2017).

Germination rate =
$$\frac{\text{number of seeds germinated}}{\text{total number of seeds}} \times 100\%$$
 (Eq.1)

Germination potential=
$$\frac{\text{number of seeds that were normally germinated to the highest}}{\text{total number of seeds}} \times 100\% \quad (Eq.2)$$

Germination index =
$$\Sigma \frac{Gt}{Dt}$$
 (Eq.3)

where Dt refers to the number of germination days, and Gt refers to the number of germinated seeds in that Dt day.

Allelopathy index:

$$I = 1 - \frac{c}{\tau} \qquad \text{(when } T \ge C\text{)}$$

$$I = \frac{\tau}{c} - 1 \qquad \text{(when } T < C\text{)}$$
(Eq.4)

where C refers to the control value, T refers to the treatment value, while I < 0 represents the inhibition effect, I > 0 represents the promotion effect, and I = 0 represents the neutral effect.

Data analysis

The experimental data were calculated and plotted using Excel 2016 (Microsoft, Redmond, Washington, USA). The statistical ANOVA was analyzed using SPSS 21.0 (IBM, Almonk, New York, USA). We first calculated the effect of different concentrations of litter extracts on the germination indices, radicle length, radicle growth, and allelopathy index of *A. confusa* seeds. Then, the variance analysis of these data was performed, and Tukey's test was applied in multiple comparisons to determine the significant difference among various treatments. The data were considered to be significantly different at P < 0.05.

Results

Effects of different extract concentrations on germination of A. confusa seeds

The effects of various concentrations of litter extracts on seed germination of A. confusa is shown in Tables 1 and 2. A. confusa seeds demonstrated different sensitivities to different concentrations of litter extract, which generally promoted seed germination at low concentrations and inhibited it at high concentrations. We found a negative correlation between litter extract concentration and germination potential, germination rate, and germination index. The germination rate was highest with a litter concentration of 1:160, which promoted the seed germination rate. The seed germination rate was significantly lower than those of the other treatments at a concentration of 1:20, demonstrating the most inhibition of the seed germination rate. The seed germination rates under high concentration treatments (including stock solution, 1:20, and 1:40) were significantly lower than under low concentration treatments (1:160 and 1:320). High concentrations of litter extract inhibited the seed germination rate, whereas low concentrations had a promotion effect. The germination potentials for high concentration (including stock solution and 1:20) flitter extract treatments were significantly lower than for low concentration (1:80 and 1:160) treatments. The germination potential and index were the lowest for the stock solution, which revealed negative effects on the seed germination potential and index. The

germination indexes for the high concentration (including stock solution and 1:20) treatments were significantly lower than for the low concentration litter extract treatments (including 1:80, 1:160, and 1:320). No significant differences in germination rate, potential, or index were found between distilled water and the control group. We found that when the concentration of litter extracts exceeded the dilution ratio of 1:40, the seed germination was restrained. The 1:80 dilution rate was considered the turning point for seed germination; the optimum dilution for seed germination was found to be 1:160. The 1:160 litter extract was the most appropriate concentration for the germination of *A. confusa* seeds.

Table 1. Effects of different concentrations of litter extracts on seed germination of A. confusa

Leaching solution	Germination rate/%	Allelopathy index	Germination potential/%	Allelopathy index	Germination index	Allelopathy index
Distilled water	63.00±1bcd	0.00	50.00±3a	0.00	81.32±2bc	0.00
Control group	61.60±1.44bcd	0.03	47.50±3abc	-0.05	79.16±2.07cd	-0.05
Stock solution	60.00±1cd	-0.05	13.50±4.5d	-0.73	56.06±2.36f	-0.33
1:20	57.00±0e	-0.10	46.00±0c	-0.08	73.77±0e	-0.12
1:40	60.50±1.5cd	-0.07	46.50±2.5bc	-0.07	75.98±1.5cd	-0.09
1:80	63.50±0.5abc	0.05	52.50±0.5ab	0.05	84.26±0abc	0.01
1:160	66.00±2a	0.09	54.50±3a	0.09	88.61±1.28a	0.06
1:320	64.00±1ab	0.02	51.00±0abc	0.02	81.46±1.76bc	-0.02

The purpose of the control group is to test whether the ethanol solution added to the litter extracts affect the seed germination of A. confusa. Data (mean \pm SD) marked with different letters in plots were significantly different under p level as 0.05 in the same column. The same below

Table 2. Effects of treatments on seed germination of A. confusa were tested using one-way ANOVA

		df	F	P
	Between groups	7		
Germination rate	Within groups	16	15.815	<0.001***
	Total	23		
	Between groups	7		
Germination potential	Within groups	16	98.107	<0.001***
	Total	23		
	Between groups	7		
Germination index	Within groups	16	122.883	<0.001***
	Total	23		

Effects of different concentrations of extracts on radicles of A. confusa seeds

Difference in radicle length between different concentrations of extracts

Figure 2 and Table 3 show the effects of various concentrations of litter extract on the radicle length of A. confusa seeds. Similar to seed germination, the different concentrations of litter extracts tended to promote radicle growth at low concentration and inhibit radicle growth at high concentration. The inhibition effect was aggravated with increasing concentration, among which the most notable effect was observed with the

stock solution. The 1:40 to 1:160 litter extracts demonstrated a promotion effect, that was the strongest at 1:160, whereas the facilitation effect gradually weakened when the concentration was 1:320. The growth in radicle length in the early stage was not obvious for the different concentrations of litter extracts. The difference in radicle length between various concentration of litter extracts and distilled water increased with time. Significant differences in radicle length were detected among the stock solution, distilled water, and 1:160 concentration litter extract. At the end of the experiment, the average radicle lengths for 1:160 litter extract, distilled water, and stock solution were 36.09, 30.06, and 19.59 mm, respectively. No significant differences in radicle length were found between the distilled water and control groups.

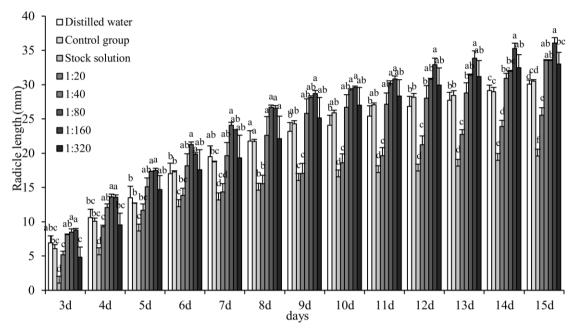


Figure 2. Effects of different concentrations of litter extracts over time on the radicle length of A. confusa seeds. The different letters (from a to d) represent the difference in radicle length among the different concentrations of the same day. Data (mean \pm SD) marked with different letters in plots were significantly different under p level as 0.05 in the same column. Compared with the control group, the treatment group were larger than the control group indicates that the radicle growth is promoted, and vice versa.

We found that *A. confusa* radicle elongation was inhibited under the stock solution and 1:20 litter extract; at 1:40, 1:80, and 1:160 litter extracts, we observed promotion effects, although it was not conspicuous at the concentration of 1:320 (*Table 3*). The stock solution had the strongest inhibiting effects on seed radicle growth. The best promotion effect was detected for the 1:160 litter extract which showed a relative high promotion effect in the early period, then later decreased. Consequently, the 1:160 litter extract remained the optimum concentration for the growth of *A. confusa* radicle length.

The results of the two-way ANOVA analysis of variance were presented in *Table 4*. There was a significant difference in the length of *A. confusa* radicle treated with time or different concentrations of litter extracts. But the difference in interaction between time and different treatments is not significant.

Table 3. Allelopathic index of A. confusa radicle length in different concentrations of litter extracts

Day	D3	D4	D5	D6	D 7	D8	D9	D10	D11	D12	D13	D14	D15
Distilled water	0	0	0	0	0	0	0	0	0	0	0	0	0
Control group	-0.12	-0.05	-0.06	0.02	-0.04	0.00	0.05	0.07	0.06	0.05	0.03	-0.01	0.02
Stock solution	-0.85	-0.51	-0.36	-0.28	-0.32	-0.33	-0.31	-0.31	-0.32	-0.35	-0.35	-0.35	-0.35
1:20	-0.25	-0.13	-0.13	-0.19	-0.26	-0.29	-0.26	-0.23	-0.23	-0.21	-0.18	-0.18	-0.15
1:40	0.15	0.12	0.10	0.06	0.01	0.04	0.10	0.10	0.06	0.04	0.04	0.06	0.10
1:80	0.19	0.22	0.22	0.20	0.19	0.18	0.18	0.18	0.16	0.13	0.12	0.09	0.10
1:160	0.21	0.22	0.23	0.14	0.17	0.18	0.19	0.19	0.18	0.19	0.18	0.17	0.17
1:320	-0.30	-0.10	0.08	0.03	-0.01	0.02	0.08	0.11	0.10	0.10	0.11	0.10	0.09

Table 4. The two-way ANOVA analysis of two treatments on the radicle length of A. confusa

	df	F	p
Day	12	274.867	<0.001***
Leaching solution	7	134.259	<0.001***
Day: Leaching solution	84	1.244	0.108
Residuals	208		

Differences in radicle growth of A. confusa seeds in different time periods

The effects of litter extracts on the seed radicle growth is shown in *Figure 3*. It shows that the seed radicle growth of A. confusa decreased with time, and the growth trends of various concentrations were different. The growth of the seed radicle was greater in the distilled water group at the period from day 4 to 9, and the growth was stable after the 10th day. The radicle growth increased the most on the fifth day and the least on the 11th day. For the stock solution of litter extracts, the seed radicle growth during day 5 to 7 was significantly higher than on other days; the seed radicle grew the most on the fifth day and the least on the 13th day. The 1:20 litter extract group demonstrated the most seed radicle growth on the fifth day and the least on the eighth day; the seed radicle growth during the first eight days was relatively high compared to that on the following days. At the 1:40 concentration, the radicle growth was relatively high during day 4 to 10 (except for the eighth day); the maximum growth occurred on the fourth day, with the least growth on day 11 to 14, and the radicle growth gradually increased in the later stage. At 1:80 and 1:160 concentrations, the seed radicle growth showed a general downward trend, reaching the maximum on the fourth day, and stabilized after decreasing sharply on day 10 to 15. The seed radicle growth at the 1:320 concentration reached a maximum on the sixth day, then declined from day 7 to 16, and the least growth was observed on the 16th day. The different concentrations of litter extracts inhibited the seed radicle growth less in the early period, where the radicle growth was relatively fast, whereas the various concentrations of litter extract had different effects on seed radicle growth in the later stages. Compared with the distilled water group and the control group, the high litter extract concentrations inhibited the radicle growth of A. confusa, which was promoted at low concentrations.

The *A. confusa* seed radicle growth was inhibited on day 7 to 9 and day 12 to 15, and promoted on day 5 to 6 and 10 to 11 in general (*Table 5*). The inhibition effect was obvious in the stock solution and the 1:20 concentration litter extract. The promotion effect appeared in the 1:80, 1:160, and 1:320 litter extract concentrations. The different days and treatments, along with their interaction, significantly affected the radicle growth of *A. confusa* (*Table 6*). Overall, the 1:160 litter extract concentration was the optimum for the increase in *A. confusa* radicle length.

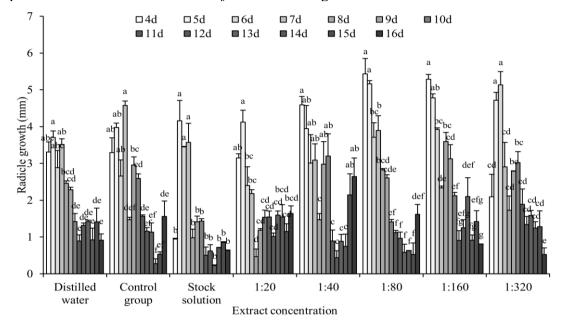


Figure 3. The seed radicle growth of A. confusa in different time periods. the different letters (from a to g) indicate the difference in radicle growth among different days under the same concentration. The amount of radicle growth refers to the length of the radicle from the next day minus the length of the radicle of the previous day. Data (mean \pm SD) marked with different letters in plots were significantly different under p level as 0.05 in the same column

Table 5. Allelopathic index of the seed radicle growth of A. confusa in different concentrations of litter extracts

Day	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16
Distilled water	0	0	0	0	0	0	0	0	0	0	0	0	0
Control group	-0.01	0.07	-0.08	0.23	-0.40	0.23	0.45	0.43	-0.12	-0.20	-0.70	-0.62	0.41
Stock solution	-0.72	0.11	0.16	0.02	-0.60	-0.38	0.01	0.44	-0.53	-0.84	-0.23	-0.39	-0.31
1:20	-0.05	0.10	-0.17	-0.38	-0.81	-0.48	0.08	0.42	-0.23	0.11	0.40	-0.19	0.44
1:40	0.28	0.06	0.04	-0.12	-0.40	0.23	0.56	0.00	-0.67	-0.38	-0.19	0.34	0.65
1:80	0.39	0.28	0.22	0.10	0.13	0.12	-0.01	0.20	-0.27	-0.59	-0.32	-0.63	0.44
1:160	0.37	0.23	0.26	-0.34	0.32	0.27	0.33	0.01	-0.05	0.33	0.00	0.01	-0.11
1:320	-0.37	0.22	0.44	-0.17	-0.30	0.18	0.53	0.53	0.02	0.11	0.26	-0.09	-0.43

Day 4 represents the allelopathy index of the radicle growth amount obtained on the fourth day minus the third day, the same as Day 5 to Day 16.

Table 6. The two-way ANOVA analysis of two treatments on the radicle growth of A. confusa

	df	F	р
Day	12	94.677	<0.001***
Leaching solution	7	12.662	<0.001***
Day: Leaching solution	84	4.759	<0.001***
Residuals	208		

Discussion

With increasing litter extract concentration, the germination potential, germination rate, and germination index of A. confusa decreased. The stock solution and the 1:20 litter extracts concentrations had strongest inhibitory effect on seed germination. The germination potential and germination index of the seeds in the stock solution were the lowest, primarily due to the restraining effect of the allelochemicals in the stock solution on the speed of seed germination. The germination rate in the 1:20 litter extraction concentration group was the lowest and the negative effect of allelochemicals on the seeds were most pronounced. This may be caused by the amount of harmful ingredients in the allelochemicals, which were much more than the amount of beneficial components. Under normal circumstances, the production and elimination of free radicles in plant cells maintains a dynamic equilibrium; under stress conditions, this balance is affected and readily destroyed. When free radicals accumulate to a certain extent, membrane lipid peroxidation occurs, which inhibits seed germination (Shibaeva et al., 2018; Marusa and Ishii-Iwamoto., 2011). At the 1:160 concentration, the three seed germination indices were the highest, and the promotion effect was the most obvious. We speculate that this concentration is the optimum concentration of seed germination, and the beneficial components in the allelochemicals accumulate the most. Within this concentration range, the ion transporters in the cell and various ion channel proteins are involved in cell ion homeostasis, increasing seed germination rate and germination speed (Steven et al., 2003; Zhang et al., 2016; Bachleda et al., 2017; Kilic et al., 2016). The 1:320 litter extract concentration also promoted the germination of seeds, but the overall effect was not significant. The reason for this may be that the concentration was lower and similar to distilled water, and the allelochemicals content was relatively low; thus, the promoting effect was not obvious. In general, compared with the distilled water group, no germination index was significant for the lowconcentration litter extracts, whereas high-concentration litter extracts significantly inhibited the germination indices of A. confusa seeds. When the allelochemicals content is limited, the plant itself produces stress-resistant immunity. At this time, allelopathic chemicals have little effect on the plant. When the residual allelochemicals increase, accumulate, and increase to a certain amount or exceed the critical content, they are harmful to the plant and thus delay seed germination (Liu et al., 2016; Wei et al., 2018).

With the increase in litter extract concentration, the seed radicle growth of *A. confusa* showed an obvious hysteresis effect. The litter extracts had a significant inhibiting effect on seed radicle growth compared with distilled water, mainly because the seed radicle growth was inhibited by the stock solution and the 1:20 concentration. High litter extract concentrations may have physiologically disturbed the plant, inducing intensive anaerobic respiration, which would therefore weaken the plant's ability to scavenge free radicals, resulting in a decrease in its physiological index (Steven et al., 2003; Zhang et al., 2016; Bachleda et al., 2017; Kilic et al., 2016). The hormone content

and enzyme activity in the seeds of A. confusa may have led to the inhibitory effect of litter extracts on seed germination and radicle growth within a certain concentration range (Hunter et al., 2013). When the litter extract concentration was 1:160, the promotion of radicle growth was the most obvious, and the content of beneficial allelochemicals was the highest. We speculate that the concentration of nutrients in this treatment was high, the breathing was strong, the high energy bond in ATP was active, the mitochondria inner membrane activity was strong, the rates of electron transfers and oxidative phosphorylation were fast, and the cell division speed of the apical meristem was accelerated, which effectively promote radicle elongation (Steven et al., 2003; Zhang et al., 2016; Bachleda et al., 2017; Kilic et al., 2016). The 1:320 litter extract also promoted the growth of seed radicle length, but the effect was weakened, which may be due to the litter extract dilution containing less effective allelochemicals. Previous studies have reported low allelopathic effect at low concentration and inhibition at high concentration with: water extracts from corn leaves on the seeds of Scutellaria baicalensis, litter extracts on seed germination and seedling growth of Cunninghamia lanceolate, and litter leaves and soil extracts on the seed germination and seedling growth of P. koraiensis (Chen et al., 2016; Peng et al., 2012; Zhuang et al., 2017). These results are consistent with our findings.

By analyzing the growth in the radicle over time, we discovered that the radicle growth of *A. confusa* treated with litter extract was relatively high in the early stage, and the growth the middle and late stages tended to decline. The reason for this may be that a certain amount of allelopathic substances accumulated in the litter, hindering the water absorption of the seed; inhibiting the respiration, enzyme activity, and radicle growth; and changing the osmotic pressure, thereby constraining the elongation of the radicle (Wei et al., 2018). When the litter extract concentrations were 1:40 and 1:80, the seed radicle growth of *A. confusa* increased gradually in the later stage, which may be due to the increase in peroxidation products in the plant after the stress due to allelopathic substances. When these allelopathic substances reach a certain amount and the stress mechanism is constrained by the plant, its own protective enzyme system could thereby scavenge the free radicals generated. However, this adaptive response is effective, as the degree of damage was relatively low and reversable, whereas the radicle increase was limited at any higher concentration of litter extract (Marusa and Ishii-Iwamoto, 2011).

The low litter extract concentration in nature can promote the germination and radicle growth of *A. confusa* to some extent. So, it is feasible to mix *A. confusa* in *C. kawakamii* forests. However, when mixing *A. confusa*, we should try to select a location where the amount of litter is low and the topography is relatively flat to mitigate the adverse effects of allelopathy. To afforest a successful mixed forest, further studies are required. Firstly, the effects of extracts from *C. kawakamii* natural forest on *A. confusa* should be studied from the perspective of the physiological activity of the radicle. Secondly, the different effects of various types and contents of litter allelochemicals at different forest ages and stages on seeds should be thoroughly considered during the afforestation of mixed forests (Hunter et al., 2013). Allelopathic effect on the plant are complex, having important impacts on the growth of plant seedlings (Kimura et al., 2015; Anne et al., 2019; Chu et al., 2014), thus affecting the colonization and renewal of the population. In the future, research should be continued on the growth response of *C. kawakamii* and *A. confusa* seedlings to litter extract.

Conclusions

In this study, we examined the effects of different concentrations of *C. kawakamii* litter extract on the germination rate, germination potential, germination rate index, and radicle growth of *A. confusa*. Low concentrations promoted growth, whereas higher concentrations inhibited growth. The stock solution had the most obvious inhibiting effect on seed germination and radicle growth, whereas the 1:160 litter extract concentration displayed the strongest promotion. The natural leaching concentration of litter in the wild is about 1:160, which could actually promote the seed germination and radicle growth of *A. confusa*. Therefore, the allelopathic effect of forest litter is not a limiting factor when mixing *C. kawakamii* and *A. confusa* in a forest, providing an alternative method to improve soil nutrition in this forest, and finally promote the regeneration of *C. kawakamii* seedlings in this forest. Further study is needed of the physiological response of *A. confusa* radicle and growth to litter extracts to provide a practical basis to promote *C. kawakamii* regeneration in this forest.

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