

Effects of Decomposing Leaf Litter of *Eucalyptus grandis* on the Growth and Photosynthetic Characteristics of *Lolium perenne*

Yiqiao Li¹, Tingxing Hu¹, Xiaoyu Duan², Fanming Zeng¹, Hong Chen¹ & Xiuhua Wu¹

¹ Key Laboratory of Forestry Ecological Engineering of Sichuan Province, College of Forestry, Sichuan Agricultural University, China

² College of Landscape Architecture, Sichuan Agricultural University, Sichuan, China

Correspondence: Tingxing Hu, Key Laboratory of Forestry Ecological Engineering of Sichuan Province, College of Forestry, Sichuan Agricultural University, China. Tel: 86-835-288-2335. E-mail: hutx001@yahoo.com.cn

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Abstract

The essence of grass growth is a complex system that is driven by light energy. The accumulation of 90% of the dry matter comes from the production of photosynthesis. In this study, we investigated the effect of decomposing leaf litter of *Eucalyptus grandis* on the growth and photosynthetic characteristics of *Lolium perenne* by pot experiment. Four treatments with different amounts of leaf litter were designed, including A1 (30 g/pot), A2 (60 g/pot), A3 (90 g/pot) and control (0 g/pot; CK), and the blank experiment groups with distilled fallen leaves were set as the above. The growth indices (including shoot biomass, root biomass and leaf area) were measured, and the photosynthetic characteristics were tested during 9:00-11:00 am on a sunny day. The test results showed that increasing amounts of *E. grandis* leaf litter significantly inhibited the accumulation of shoot and root biomass, growth of leaf area, synthesis of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), photosynthetic rate and gas exchange parameters (G_s , C_i and Tr) of *L. perenne* seedlings ($P < 0.05$). The parameters of photosynthetic rate in response to CO_2 concentration and light intensity showed a remarkable downward trend except for the CO_2 compensation point and were significantly difference from CK, namely, $CK > A1 > A2 > A3$ ($P < 0.05$). The blank experimental showed that soil chemical and physical properties have not been changed by leaf litter decomposition. This demonstrated that the allelochemicals from *E. grandis* leaf litter decomposition can negatively affect the photosynthetic capacity and decrease the accumulation of biomass, and eventually inhibit the growth of *L. perenne*.

Keywords: allelopathy, *Eucalyptus grandis*, leaf litter, *Lolium perenne*, growth, photosynthesis

1. Introduction

Eucalyptus grandis was first discovered in Australia and the adjacent islands. It is a tall straight-trunked tree that grows extremely fast in warm and humid climates. Because of its great environmental adaptability, excellent form and rapid growth, it is the most widely planted industrial eucalypt (Jairus et al., 2011). Since this species was introduced to China in the early 1990s, extensive plantations have teemed in the southern part of China, especially in Sichuan province (Zhang et al., 2008). Although *Eucalyptus* (*E. grandis*) plantation brought great economic benefits, the negative effects on ecological diversity and ecosystems degradation has also resulted and drawn our great attention (Campoe et al., 2013). According to reports, the leaf litter of *E. grandis* contains at least eight kinds of chemical substance, such as alkanes, aromatic hydrocarbons, esters etc (Goya et al., 2008). Among these compounds, the phenols, terpene and esters are the major allelochemicals, which can influence the growth of undergrowth (Yu et al., 2009). Therefore, ecological diversity would be affected by *E. Grandis*, which would degrade the ecosystem.

Recently, with the interconnection between forestry and agrostology (branch of botany that deals with the study of grasses), and the requirement that there be comprehensive treatment of the ecological environment, people are paying more and more attention to forest-grass compound management. This can make full use of resources in the forest, increase vegetation coverage of the understory and balance the ecological, economic and social benefits, and is an important management mode in plantation (Wang et al., 2003).

Perennial Ryegrass (Lolium perenne), which belongs to the *Lolium* genus in the *Gramineae* family, is an annual or perennial herb with better traits of tenderness, nutrition and taste. It is also an important pasture and green manure grass (Lee et al., 2010). The mixed planting between *L. perenne* and *E. grandis* probably can mitigate the ecological problems in *E. grandis* monoculture, and has an important effect on the utilization of forestland. Generally, leaf litter of *E. grandis* is plowed into the soil while conducting the forest-grass management mode, so the grass may be affected by the allelochemicals from leaf litter decomposition.

Currently, there are few studies on the allelopathic effect of *E. grandis* on the undergrowth plants using the method of simulation experiment in the greenhouse. Consequently, it is important to discuss the mechanism of allelopathy of *E. grandis* on *L. perenne*. The objective of this experiment was to determine the effect of decomposing *E. grandis* leaf litter on the growth and photosynthetic characteristics of *L. perenne* in a natural state, and provide research and reasonable suggestions on allelopathy, provide a management strategy for *E. grandis* plantation and its compound system with grass as well.

2. Method

2.1 Site for Experiment

The experiment was performed in a big plastic shed at the Forestry Station of Sichuan Agricultural University (101° 56' 2"-103° 22' 35" E, 28° 51' 18"-30° 56' 30"N, 600-750 m a.s.l.), Ya'an, Sichuan province, where is a typical *E. grandis* plantation region in the southwest China. Mean annual precipitation, annual temperature and relative air humidity in this area is 1800 mm, 16.2 °C, and 79%, respectively. The annual cumulative temperature (≥ 10 °C) is 5231°C, annual sunshine is 1039.6 h, and frost-free period is 298 d.

2.2 Research Design

The soil texture is typical sandy loam with the pH of 7.85 and the organic matter content is 24.38 g/kg. The soil fertility status is as follows: total N 0.66 g/kg; total P 0.67 g/kg; total K 5.57 g/kg; available P 14.73 mg/kg and available K 15.15 mg/kg. The test soil was sterilized by carbendazim after sieving with 2 mm mesh. Polyethylene pots with capacity of 12 kg soil (29 cm in diameter of opening, 17 cm in diameter of bottom and 22 cm in height) were employed for planting. Fresh fallen leaves were randomly collected from the 10 year old *E. grandis* plantation in the experiment site, and cut into small pieces of about 1 cm² after natural withering, then weighed and very good mixed with disinfected soil. The healthy seeds of *L. perenne* were disinfected with 0.05% KMnO₄ for 20 min, and soaked in the warm water for 2 h after washing.

Three treatments were applied according to the annual amount of leaf fall in pure matured *E. grandis* plantation (9000 kg/hm²), including A1 (30 g/pot), A2 (60 g/pot), A3 (90 g/pot) and a control group was also set (0 g/pot). 30 seeds were planted in each pot with 10 replicates in every treatment on 10th March 2012. The soil moisture was kept at 80% of the field capacity. The growth indices were measured on 15th May 2012.

In order to observe the effect of addition of leaf litter on the soil permeability and aeration, the blank experiment without allelochemicals was set. The fallen leaves, the allelochemicals of which have been removed by distillation, were mixed with soils, and then 4 treatments (0, 30, 60, 90 g/pot) were applied as above. The growth traits (leaf area and dry matter mass) and net photosynthetic rate were measured on 15th May 2012.

2.3 Indices Measuring

The photosynthetic characteristics of *L. perenne* leaf were monitored using portable photosynthesis analyzer (Li 6400, Li-Cor Inc., USA). The concentration of carbon dioxide was controlled at 400 $\mu\text{mol CO}_2/\text{mol}$, temperature was 25°C and light intensity was 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Then the net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s) and intercellular CO₂ concentration were measured during 9:00-11:00 am on 17th May 2012 (a sunny day), with six replications in each test.

Pigment content was measured by the method of equally mixing ethanol and acetone using the leaves which have been used for measuring of photosynthetic parameters, including chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids (Car) (Li et al., 2000). The ripe leaves collected from same part of different treatments were used for measuring the leaf area using a portable laser leaf area meter (Li-3100, Li-Cor, USA). The total bud lengths and roots of 10 seedlings for each treatment were measured and repeated for triplication. The shoots and roots from seedlings were dried in an oven at 105°C for 30 min to deactivate enzymes and then dried to constant mass at 80°C for determination of dry mass (overground part and underground part), and calculate the root-shoot ratio (shoot biomass/root biomass).

2.4 Statistical Analysis

Statistics analyses were performed using SPSS 18.0 software. One-way ANOVA was used to compare the effect of treatments. Least Significant Difference (LSD) method was used for multiple comparison tests, the p-value for significance was set at $P < 0.05$. The program of correlation analysis is used to identify and measure the associations among different traits.

The Photosyn Assistant 1.1.2 was employed to fit the light response curve, and light-CO₂ response curve was fitted by rectangular hyperbolic model.

Response index (RI) of allelopathy was calculated using the formula by Williamson and Richardson (1988):

$$RI = 1 - C/T \quad (T \geq C) \quad \text{and} \quad RI = T/C - 1 \quad (T < C)$$

Where, C is the control response and T is the treatment response. The range of RI is from -1 to +1, and with positive values indicating stimulation by the treatments, whereas the negative values indicating inhibition by them, relative to the controls.

3. Results

3.1 Effect of Decomposing Leaf Litter of *E. grandis* on the Biomass and Leaf Area of *L. perenne* Seedlings

The biomass (shoot and root) and the leaf area of *L. perenne* declined significantly with the increase of leaf litter of *E. grandis* in soil of the three different treatments, shown as CK > A1 > A2 > A3 ($P < 0.05$), namely, the CK group without litter had the best production in biomass and there was a drop as the amount of litter increased. Whereas the response index of allelopathy went up along with the leaf litter increasing in shoot biomass, root biomass and leaf area, and the indices in A3 treatment reached -0.77, -0.87 and -0.72, respectively (Table 1).

Table 1. Effects of leaf litter decomposition of *E. grandis* on the biomass and leaf area of *L. perenne* seedlings

Treatment	Shoot biomass (g/10 plants)	RI	Root biomass (g/10 plants)	RI	Leaf area (cm ² /10 plants)	RI	Root/Shoot
CK	5.73 ± 0.12 ^a	\	1.97 ± 0.14 ^a	\	5.12 ± 0.47 ^a	\	0.34 ± 0.03 ^a
A1	4.89 ± 0.11 ^b	-0.48	1.42 ± 0.09 ^b	-0.58	3.98 ± 0.41 ^b	-0.11	0.28 ± 0.04 ^{ab}
A2	2.75 ± 0.05 ^c	-0.68	0.69 ± 0.04 ^c	-0.72	2.76 ± 0.39 ^c	-0.38	0.25 ± 0.02 ^b
A3	1.67 ± 0.03 ^d	-0.77	0.37 ± 0.02 ^d	-0.87	1.16 ± 0.25 ^d	-0.72	0.22 ± 0.03 ^{bc}

^{a,b,c,d} Mean within a column with no common superscripts are significantly different ($P < 0.05$)

3.2 Effect of Decomposing Leaf Litter of *E. grandis* on Pigment Content and Photosynthetic Characteristics of *L. perenne* Seedlings

The photosynthetic pigment contents were significantly negative affected by decomposing leaf litter of *E. grandis* ($P < 0.05$), and the degree of the effects increase with the increment of leaf litter amount (Table 2). The content of Chl a, Chl b and Chl (a + b) in A1 dropped by 6.89%, 26.3% and 10.8%, respectively, compared to CK, and the magnitude of the declines were 57.1%, 59.6% and 57.1% in the A3 treatment. The changes in the content of Car were same as the chlorophyll in the four treatments. The Car of A3 group was lower than that of CK group by 47.9% ($P < 0.05$), and response index of allelopathy was -0.54 in A3.

Table 2. Effect of decomposing leaf litter of *E. grandis* on pigment content of *L. perenne* seedlings

Treatment	Chl a (mg/g FM)	RI	Chl b (mg/g FM)	RI	Chl (a+b) (mg/g FM)	RI	Car (mg/g FM)	RI
CK	2.03 ± 0.18 ^a	\	0.57 ± 0.14 ^a	\	2.59 ± 0.17 ^a	\	0.48 ± 0.03 ^a	\
A1	1.89 ± 0.11 ^b	-0.18	0.42 ± 0.09 ^b	-0.18	2.31 ± 0.13 ^b	-0.18	0.41 ± 0.04 ^b	-0.17
A2	1.25 ± 0.05 ^c	-0.37	0.33 ± 0.04 ^c	-0.39	1.57 ± 0.05 ^c	-0.38	0.34 ± 0.02 ^c	-0.33
A3	0.87 ± 0.03 ^d	-0.58	0.23 ± 0.02 ^d	-0.54	1.11 ± 0.03 ^d	-0.58	0.25 ± 0.02 ^d	-0.54

^{a,b,c,d} Mean within a column with no common superscripts are significantly different ($P < 0.05$)

Chl a = chlorophyll a; Chl b = chlorophyll b; Car = carotenoids.

The photosynthetic characteristics including net photosynthetic rate, stomatal conductance and transpiration rate in leaf of *L. perenne* displayed decreasing trend with increasing of leaf litter of *E. grandis* in soil (Table 3), and the differences were significant among each treatment. Whereas the change of intercellular carbon dioxide concentration tended to rise, and marked by $A3 > A2 > A1 > CK$ ($P < 0.05$), the range of *RI* was from 0.07 to 0.11 in this trait.

Table 3. Effects of decomposing leaf litter of *E. grandis* on photosynthetic rate and gas exchange parameters of *L. perenne* seedlings

Treatment	<i>Pn</i>		<i>Gs</i>		<i>Ci</i>		<i>Tr</i>	
	$\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	<i>RI</i>	$\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	<i>RI</i>	$\text{mmol CO}_2 \cdot \text{mol}^{-1}$	<i>RI</i>	$\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	<i>RI</i>
ck	13.8 ± 0.76^a	\	0.33 ± 0.03^a	\	283.7 ± 12.2^c	\	4.85 ± 0.23^a	\
A1	8.98 ± 0.56^b	-0.21	0.17 ± 0.02^b	-0.38	290.9 ± 13.4^b	0.07	4.23 ± 0.18^a	-0.11
A2	7.06 ± 0.45^c	-0.32	0.15 ± 0.01^b	-0.49	295.3 ± 11.2^b	0.19	3.15 ± 0.18^b	-0.24
A3	5.18 ± 0.41^d	-0.57	0.12 ± 0.01^c	-0.052	304.1 ± 14.5^a	0.11	2.92 ± 0.11^c	-0.29

^{a,b,c,d} Mean within a column with no common superscripts are significantly different ($P < 0.05$).

3.3 Correlation between the Growth Indices and Photosynthesis Parameters of *L. perenne* Leaves

The correlation between the growth indices and photosynthesis parameters of *L. perenne* leaves were shown in the Table 4. The relation among shoot biomass, root biomass and leaf area were significantly positive ($P < 0.05$) or extremely significantly positive ($P < 0.01$). Also, there were significantly or extremely significantly positive correlations between photosynthetic pigment contents in leaf and shoot biomass, root biomass, leaf area, *Pn*, *Ci* and *Tr* ($P < 0.05$, $P < 0.01$). The *Ci* was negatively related to all indices.

Table 4. The correlation between the growth indices and photosynthesis parameters of *L. perenne* leaves

Items	Shoot biomass	Root biomass	Leaf area	Chl (a+b)	Car	<i>Pn</i>	<i>Ci</i>	<i>Gs</i>	<i>Tr</i>
Shoot biomass	1								
Root biomass	0.986**	1							
Leaf area	0.812	0.885*	1						
Chl (a+b)	0.902*	0.956**	0.972**	1					
Car	0.861*	0.921*	0.986**	0.998**	1				
<i>Pn</i>	0.853	0.899*	0.923*	0.971**	0.972**	1			
<i>Ci</i>	-0.801	-0.861*	-0.852	-0.902*	-0.908*	-0.981**	1		
<i>Gs</i>	0.991**	0.982**	0.801	0.898*	0.853	0.844	-0.827	1	
<i>Tr</i>	0.823	0.888*	0.938*	0.918*	0.901*	0.813	-0.699	0.805	1

* mean that the correlation is significant ($P < 0.05$), ** mean that the correlation is highly significant ($P < 0.01$).

3.4 The Response of Photosynthetic Rate of *L. perenne* Leaf on the CO_2 and Light in the Different Treatments

Overall, the *Pn* went up with the increase in CO_2 concentration in the different treatments. Specifically, the *Pn* picked up quickly at the lower CO_2 concentration ($0 \sim 400 \mu\text{mol} \cdot \text{mol}^{-1}$), but then the increase trends in four groups of *Pn* were gradually at the higher CO_2 concentration (Figure 1).

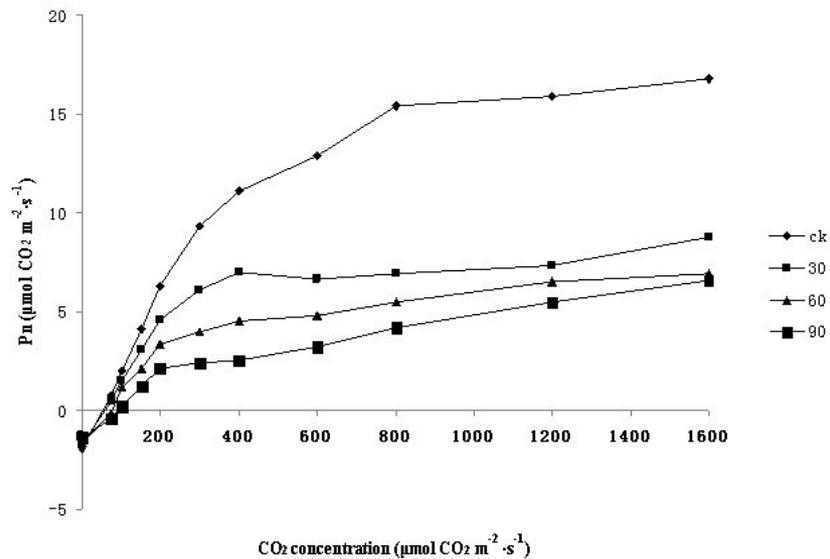


Figure 1. CO₂ response curve of photosynthesis in *L. perenne* leaves in different leaf litter treatments

Parameters of photosynthetic rate of *L. perenne* leaf in response to CO₂ concentration in each experiment group were shown in Table 5. There were significant falls in the RuBP carboxylation efficiency (CE), P_{n max}, CO₂ saturation point (CSP) and Photorespiration rate (*R_p*) from CK to A3, whereas the CO₂ compensation point (CCP) went up significantly with the increase of leaf litter of *E. grandis* ($P < 0.05$).

Table 5. Parameters of photosynthetic rate of *L. perenne* leaves in response to light intensity in different leaf litter treatments

Treatment	AQY ($\mu\text{mol CO}_2$ $\text{m}^{-2}\cdot\text{s}^{-1}$)	<i>P_n max</i> ($\mu\text{mol CO}_2$ $\text{m}^{-2}\cdot\text{s}^{-1}$)	LSP ($\mu\text{mol photons}$ $\text{m}^{-2}\cdot\text{s}^{-1}$)	LCP ($\mu\text{mol photons}$ $\text{m}^{-2}\cdot\text{s}^{-1}$)	<i>R_d</i> ($\mu\text{mol CO}_2$ $\text{m}^{-2}\cdot\text{s}^{-1}$)
ck	0.039 ± 0.009 ^a	17.6 ± 3.45 ^a	528 ± 76.2 ^a	16.7 ± 4.12 ^a	1.21 ± 0.19 ^a
A1	0.031 ± 0.010 ^b	7.59 ± 2.13 ^b	375 ± 57.3 ^b	10.1 ± 3.78 ^b	0.72 ± 0.17 ^b
A2	0.026 ± 0.009 ^c	3.43 ± 1.08 ^c	217 ± 32.9 ^c	3.71 ± 0.98 ^c	0.36 ± 0.09 ^c
A3	0.023 ± 0.008 ^d	3.19 ± 1.12 ^c	207 ± 33.6 ^c	3.02 ± 0.87 ^d	0.31 ± 0.07 ^c

^{a,b,c,d} Mean within a column with no common superscripts are significantly different ($P < 0.05$)

AQY = apparent quantum yield, LSP = light saturation point and LCP = light compensation point.

The *P_n* went up as the increase of the photosynthetically available radiation (PAR) in the four groups of treatments (Figure 2). Especially, the *P_n* of *L. perenne* leaf rose rapidly at lower light intensity (0~200 $\mu\text{mol mol}^{-2} \text{s}^{-1}$). However, changing trends were stable when the PAR was above 800 $\mu\text{mol mol}^{-2} \text{s}^{-1}$ in three treat groups.

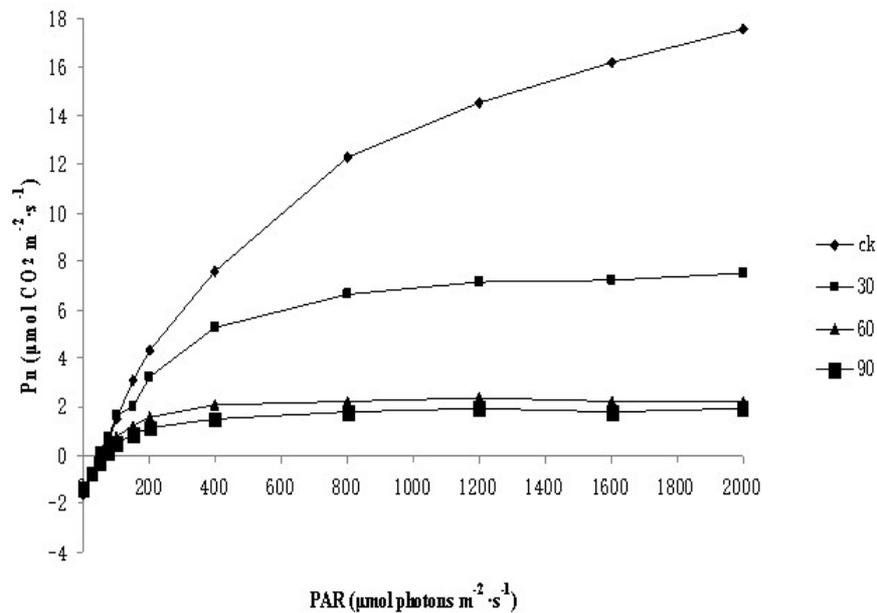


Figure 2. Light response curve of photosynthesis in *L. perenne* leaves in different leaf litter treatments

The values of apparent quantum yield (AQY), $P_{n\max}$, light saturation point (LSP), light compensation point (LCP) and R_d were shown in Table 6. The allelochemicals from decomposing leaf litter had significant effects on these five indices ($P < 0.05$). For AQY, $P_{n\max}$, LSP and R_d there was no obvious difference between A3 and A4, but these two groups were significantly lower than A2 and CK ($P < 0.05$), meanwhile, the A2 was dramatically lower than CK ($P < 0.05$). For LCP, the significant difference was observed among four groups, marked by CK > A1 > A2 > A3 ($P < 0.05$).

Table 6. Parameters of photosynthetic rate of *L. perenne* leaves in response to CO₂ concentrate in different leaf litter treatments

Treatment	CE ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$)	$P_{n\max}$ ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$)	CSP ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$)	CCP ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$)	R_p ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$)
ck	0.068 ± 0.013^a	22.6 ± 3.98^a	628 ± 101^a	45.2 ± 5.43^d	2.83 ± 0.45^a
A1	0.059 ± 0.011^b	12.7 ± 2.78^b	475 ± 107^b	52.3 ± 5.12^c	2.22 ± 0.51^b
A2	0.042 ± 0.012^c	7.15 ± 2.81^c	412 ± 99.2^c	64.1 ± 5.71^b	1.79 ± 0.44^c
A3	0.035 ± 0.008^d	6.34 ± 2.45^c	368 ± 89.1^d	81.9 ± 7.62^a	1.32 ± 0.31^d

^{a,b,c,d} Means within a column with no common superscripts are significantly different ($P < 0.05$).

CE = RuBP carboxylation efficiency, CSP = CO₂ saturation point and CCP = CO₂ compensation point.

3.5 Effect of Distilled Leaf Litter of *E. grandis* on Growth Indices and P_n of *L. perenne* Seedlings

The effect of distilled leaf litter of *E. grandis* on growth indices of *L. perenne* seedlings were also tested in this study. From the Table 7, there were no significant differences observed in the leaf area, shoot biomass, root biomass and photosynthetic among the different experimental groups ($P > 0.05$). Similarly, no significant differences were observed in the index of photosynthetic rate between the control group and each treatment group ($P > 0.05$).

Table 7. Effect of distilled leaf litter of *E. grandis* on growth indices and P_n of *L. perenne* seedlings

Treatment	Leaf area (cm ² / 10 plant)	Shoot biomass (g/ 10 plants)	Root biomass (g/ 10 plants)	Photosynthetic ($\mu\text{mol CO}_2 \text{ m}^{-2}\cdot\text{s}^{-1}$)
ck	5.22 \pm 0.56 ^a	6.22 \pm 0.56 ^a	1.32 \pm 0.09 ^a	16.25 \pm 1.28 ^a
A1	5.14 \pm 0.41 ^a	6.14 \pm 0.41 ^a	1.31 \pm 0.18 ^a	16.18 \pm 1.34 ^a
A2	5.19 \pm 0.54 ^a	5.99 \pm 0.54 ^a	1.28 \pm .008 ^a	16.22 \pm 1.22 ^a
A3	5.33 \pm 0.32 ^a	6.33 \pm 0.32 ^a	1.33 \pm 0.11 ^a	16.42 \pm 1.37 ^a

^{a,b,c,d} Means within a column with no common superscripts are significantly different ($P < 0.05$)

4. Discussion

Although grass production is a complex biological process, its essence is the accumulation of dry matter via carbon assimilation driven by light energy (Knox et al., 2010). Commonly, about 90% to 95% of the biological yield of grass comes from photosynthetic product. Therefore, photosynthesis is a vital factor to determine the production of crop (Wang et al., 2010). Photosynthesis is a complex physiological process, which is influenced by the self-factors such as chlorophyll content, leaf thickness and mature grade of leaf, as well as correlated nearly with external factors such as light intensity, temperature, relative humidity, soil moisture balance (Cheng et al., 2009). Photosynthesis can be influenced by several factors, but all groups were treated by the same method and planted at one environment condition in our trail, therefore we considered the decomposition of leaf litter was the only factor to affect the photosynthesis of *L. perenne* seedling in different treat groups of our study.

It has been proven that leaf litter can release chemicals, which influence directly or indirectly on the growth and distribution of plants and microbes during the process of its decomposing. In this research, the ratio of root and shoot of the receptor were decreased with the increase of leaf litter of *E. grandis*, and root/shoot in A3 and A2 was significant lower than that of CK ($P < 0.05$), meanwhile, which in A3 was remarkable lower than A1 ($P < 0.05$). It showed that the effect of allelopathy on root may more sensitive, and was consistent with the report that allelochemicals firstly acted on membranes of plants' root cells, and influenced the functions of cell membranes through affecting the membrane potential, activity and permeability, ultimately influenced the photosynthesis by a series of biological mechanisms (Lang et al., 2009).

Photosynthetic pigments, especially the chlorophyll is the foundation of organic nutrient in grass, and play important roles in accepting and converting light energy into biochemical energy (Kana et al., 2008). Chl a is a major part of complex in the center of reaction, and serves the function in energy conversion, while Chl b is an important component of light-harvesting complex protein, and mainly applies in harvesting and transferring of light (Krause et al., 1991). Theoretically, the decrease of Chl a and Chl b will undoubtedly reduce the photosynthesis. Several studies have proven that chlorophyll can be affected by allelochemicals in different kinds of plants (Einhellig et al., 1979; Yang et al., 2004; Yang et al., 2002). In our current study, we demonstrated that the accumulation of photosynthetic pigments in leaves of *L. perenne* was inhibited by the leaf litter of *E. grandis*. This could ultimately lead to the decrease in photosynthesis of *L. perenne*.

In the process of photosynthesis, the spread of CO₂ from air to photosynthetic part in the chloroplast was affect by many factors, including CO₂ conductance, stomatal conductance, mosephyll conductance, intercellular CO₂ concentration and so on (Hanba et al., 2003). Stomata, as the most important gas transport channels in plant leaves, directly controls the access of CO₂ and leaves transpiration. Wong et al. (1994) found that there is a feedback between G_s and P_n that the G_s increase in favor of photosynthesis in mesophyll cells, whereas the G_s decrease under unfavorable circumstances. Pattersion (1981) showed that cinnamic acid, benzoic acid, salicylic acid and several of allelochemicals can not only limit the increase of dry matter, leaf area and plant weight in *Glycine max*, but also inhibited the P_n and G_s of leaves. Mersie and Singh (1993) reported that the allelochemicals such as ferulic acid and vanillic acid can cause a significant decline in P_n of *Calathea leopardina* leaves with decrease of G_s . In our research, the decomposition of *E. grandis* leaf litter negatively affected the T_r and G_s of *L. perenne* seedlings, and ultimately resulted in decrease of P_n in A1, A2 and A3 treatments. This illustrated that allelochemicals released from decomposition of leaf litter could destroy photosynthesis, impeded the growth of *L. perenne* seedlings finally.

In a certain environmental condition, the $P_{n\ max}$ represents the maximum photosynthetic capacity of leaf (Lu et al., 2001). Apparent quantum yield (AQY) reflects the utilization of the light energy, especially in weak light (Kisch, 2010). CO₂ saturation point (CSP) indicates that when the concentration of CO₂ is low in the air, the photosynthetic rate goes up with the increasing of concentration of CO₂, and the rise of photosynthetic rate will stop at a certain concentration of CO₂ (Cao et al., 2011). In this study, the $P_{n\ max}$, AQY and LSP significantly decreased with the increase of the amount of leaf litter, which indicated that allelochemicals may reduce the response of *L. perenne* seedlings to the changing of concentrate of CO₂ and light.

There was no significant effect on the leaf area, shoot biomass, root biomass and photosynthetic when the distilled leaf litters of *E. grandis* were added into the soil in each treatment. This demonstrated that the soil physical properties, especially soil aeration and water permeability were not been obviously changed after the addition of leaf litter into the soil and in the process of leaf litter decomposition, because allelochemicals have been removed from the leaf litter by distilling, and further showed that the differences in growth of *L. perenne* seedlings were mainly caused by allelochemicals which were released from the decomposition of original leaf litter of *E. Grandis*.

5. Conclusion

In conclusion, allelochemicals released from decomposing *E. Grandis* leaf litter can inhibit the opening of stomata on leaves, and decrease the content of synthesis of photosynthetic pigments and the response to CO₂ concentration and light intensity in *L. perenne*. These can reduce the photosynthetic capacity of leaves, and eventually inhibiting the growth of height and leaf area and the accumulation of biomass of *L. perenne*. Our results can provide basis research and reasonable suggestion on allelopathy, and provide a management strategy for *E. grandis* plantation and its compound system with grass as well. However, the main limitation of our study was the small number of samples, and further large-scale research should be conducted to check the effects of this allelopathy of *E. Grandis* on the growth of *L. perenne*.

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