frontiers | Frontiers in Forests and Global Change

TYPE Original Research
PUBLISHED 02 March 2023
DOI 10.3389/ffgc.2023.1136474



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SPECIALTY SECTION
This article was submitted to Planted Forests, a section of the journal Frontiers in Forests and Global Change

RECEIVED 03 January 2023
ACCEPTED 16 February 2023
PUBLISHED 02 March 2023

CITATION
Liu J, Li R, Xu J, Fu S and Wan S (2023) Effect of lime application on soil respiration is modulated by understory vegetation in subtropical *Eucalyptus* L'Hér. plantations. *Front. For. Glob. Change* 6:1136474. doi: 10.3389/ffgc.2023.1136474



# Effect of lime application on soil respiration is modulated by understory vegetation in subtropical *Eucalyptus* L'Hér. plantations

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**Objective:** Lime application and understory plant clearance are two common forest management methods employed to increase forest productivity in subtropical regions. However, the effect of these two management methods, or their combined application, on soil respiration in forest plantation settings is still not fully understood.

**Methods:** Here, a field experiment was conducted to determine how lime application, understory clearance, and their combined application, would impact total soil respiration ($R_s$), autotrophic respiration ($R_a$), and heterotrophic respiration ($R_h$) in a Chinese subtropical *Eucalyptus* plantation. Changes in soil microclimate and microbial community (PLFAs), as well as *Eucalyptus* fine root biomass were also assessed.

**Results:** Lime application stimulated $R_h$, but decreased $R_a$ of understory vegetation (primarily *Dicranopteris dichotoma*), thus consequently inhibiting $R_s$. Understory clearance also reduced $R_s$, primarily due to reductions in soil PLFAs and associated $R_h$, and $R_a$ of understory vegetation. Since the increase in $R_h$ induced by lime application was greater than the decrease in $R_h$ caused by understory clearance, the combined application of lime application and understory clearance decreased $R_s$ primarily by reducing understory root respiration.

**Conclusion:** Our observations suggest that understory plants can modulate the effect of lime application on $R_s$, highlighting the important role of understory vegetation in regulating soil carbon cycling in subtropical *Eucalyptus* plantations. We concluded that the potential interactive effects should be considered in developing management practices that optimize the yield and sustainability of subtropical plantations.

KEYWORDS
**lime application, understory clearance, soil respiration, autotrophic respiration, heterotrophic respiration**

## 1. Introduction

Soil respiration ($R_s$) is the second largest carbon flux between land and the atmosphere (Yang et al., 2022). In terrestrial ecosystems, forests are important regulators of the global carbon balance, with trees storing up to 56% of both above- and below-ground terrestrial carbon, and forest soil respiration accounting for at least 30% of total terrestrial respiration (Wan et al., 2015; Yang et al., 2022). Therefore, even minor alternations in forest soil respiration can significantly change atmospheric carbon dioxide concentration and the global carbon cycle (Valente et al., 2021). Forest soil respiration is affected by forest management practices such as thinning (Sherman and Coleman, 2020), fertilization (Wang et al., 2017), understory removal (Yao et al., 2019), and litter manipulation (Dai et al., 2021). However, since the two main components of $R_s$: autotrophic respiration ($R_a$: from live roots and rhizosphere), and heterotrophic respiration ($R_h$: from microbial decomposition of soil organic matter) are site-specifically related to forest type, soil characteristics, local climate, and other variables, the impact of forest management practices on actual soil respiration are often variable (Mo et al., 2008; Wan et al., 2015). For example, Zhang et al. (2022) explored that thinning increased $R_s$ in subtropical *Cunninghamia lanceolata* plantations. Nonetheless, Pang et al. (2013) found that thinning inhibited $R_s$ in a pine plantation in the eastern Tibetan Plateau. Furthermore, multiple silvicultural practices may be applied simultaneously, potentially producing interactive effects on soil respiration (Sherman and Coleman, 2020). Therefore, a comprehensive assessment of the impact of forest management measures on soil respiration will not only help to mitigate climate warming, but also provide a scientific basis for formulating more rational forest management strategies.

Lime application is a common forestry practice to improve acrisols in humid tropical and subtropical regions (Zhao et al., 2015). In addition to preventing or reversing soil acidification, the lime application can profoundly alter soil microbial communities (Sridhar et al., 2022), although the reported results concerning the effect of lime application on soil respiration have so far been mixed. Shah et al. (1990) and Goupil (2014) reported that lime application increased $R_h$, likely because of enhanced acid intolerant bacterial abundance and fungal abundance, and consequently increased soil respiration. On the contrary, Melvin et al. (2013) reported a reduction in soil respiration by inhibiting soil basal respiration in a broadleaved forest after the application of lime, thereby increasing carbon storage in the forest soil. Meanwhile, Lochon et al. (2019) found that lime application produced a negligible effect on soil respiration because the increase in $R_h$ was counteracted by the decrease in $R_a$. Taken together, it appears that responses of soil respiration to liming are highly dependent on the environment and the balance between $R_a$ and $R_h$. High soil pH tends to favor the growth of acid-intolerant bacteria at the expense of both acid-tolerant bacteria and fungi (Wan et al., 2015), thus alternations of microbial communities in limed soils may at least partially explain changes in soil respiration. Meanwhile, the lime application can increase the availability of some nutrients (i.e., Ca and Mn), while decreasing the availability of some micro nutrients, such as Cu and Zn (Florentino et al., 2021). Therefore, alternations in plant growth under lime application may also explain changes in soil respiration. To date, the effect of lime application has been tested in grassland and agricultural soils (Lochon et al., 2019; Xu et al., 2022). However, there is a lack of information on the response of soil respiration to lime application in forest ecosystems, particularly in *Eucalyptus* plantations grown in strong acidic soil in South China.

*Eucalyptus urophylla* S.T.Blake is a fast-growing, high-yielding tree species cultivated in subtropical and tropical terrestrial regions around the world. In southern China, cultivation has been expanding rapidly in response to increasing demand for fiber and wood (Wan et al., 2019; Gao et al., 2021). In southern Chinese *E. urophylla* monoculture plantations, the native fern *Dicranopteris dichotoma*, tends to create a thick understory blanket (Yang et al., 2021). In practice, understory plants are traditionally removed as they are believed to compete with cultivated trees for both nutrients and water. However, studies have shown that *D. dichotoma* can have profound impacts on soil organisms (Gao et al., 2021), litter decay (Chen et al., 2019), soil microclimate (Wan et al., 2019), and canopy productivity (Wan et al., 2014). Although understory plants may be a substantial driver of both above and below-ground ecosystem function, few studies have quantitatively explored the effect of understory clearance on soil respiration (Wan et al., 2015; Zhao et al., 2022). Even less is understood regarding the potential interactive effects of combined plantation management practices, such as understory clearance and lime application, on soil respiration.

In this study, a field experiment was conducted in a southern Chinese subtropical *E. urophylla* plantation to investigate the individual and combined effect of lime application and understory clearance on soil respiration and its two main components (autotrophic respiration and heterotrophic respiration). We hypothesized that: (1) lime application would decrease soil respiration by inhibiting soil total microbial PLFAs and associated $R_h$ (Melvin et al., 2013; Wan et al., 2019); (2) understory clearance would reduce root respiration of understory vegetation and $R_h$ by reducing labile carbon and nutrient substrates for soil microorganisms, thus consequently decreasing soil respiration (Wan et al., 2021; Xiao et al., 2022); (3) lime application combined with understory clearance would reduce soil respiration by inhibiting both $R_h$ and $R_a$.

## 2. Materials and methods

### 2.1. Field site and experimental design

The field site is located at the Heshan National Field Research Station of Forest Ecosystem (22°34′N,112°50′E), Guangdong Province, Southern China. The overall climate of the area is subtropical, with an annual mean precipitation of 1,700 mm, and an annual mean temperature of 21.7°C. The soil at the field site is an Ultisol developed from sandstone (FAO, 2006). The *Eucalyptus* plantation at the site was re-established after the previously-planted trees (*Pinus elliottii* Engelm.) were clear-cut, and *Eucalyptus* (*Eucalyptus urophylla* S.T.Blake) saplings (1,650 per hectare) were planted with a spacing of 3 × 2 m in 2005. From August to September 2011, three independent *Eucalyptus* plantations, with about 1 ha of area for each, were selected to conduct the experiment. The understory vegetation in these plantations was nearly dominated by *Dicranopteris dichotoma* (a natural perennial fern species widely distributed in southern China), accompanied by a small number of other species, such as *Miscanthus sinensis* Andersson and *Rhodomyrtus tomentosa* (Aiton) Hassk.

Four experimental plots (10 m × 10 m) were established in each plantation, and each plot was applied randomly to one of the four treatments: LA (application of lime), UR (removal of understory), L+UR (application of lime and removal of understory), and CK (control/no treatment). For lime application treatments (LA and L+UR), in order to significantly increase soil pH in the context of heavy rainfall in subtropical regions, the soil surface was treated with lime (CaO) at a rate of 60 kg per 100 $m^2$ per year, with 30 kg applied on 15 December 2011 and the other 30 kg applied on 15 August 2012. For understory removal treatments (UR and L+UR), harvesting hooks were used to manually removal both above and below-ground understory biomass before measurements were conducted (15 December 2011), and any regrowth of the understory plants was removed monthly there after with aid of machete.

## 2.2. Measurement of soil respiration, soil temperature, and soil moisture

LI-8100 Automated Soil $CO_2$ Flux System (LI-COR, Lincoln, NE, USA) was used to measure soil respiration. Five PVC soil collars (5 cm in height, 20 cm in diameter) were randomly placed and inserted at 3 cm depth in each plot, and the measured soil respiration was used to indicate the total soil respiration ($R_s$). In each plot, a trenched subplot (1 m in length, 1 m in width, and 0.5 m in depth) was outfitted and surrounded by PVC sheets to prevent lateral root entry and to minimize nutrient and water exchange. Trenching was conducted in August 2011, about 3 months before treatments, allowing the decomposition of the roots inside the trenched plots after trenching. All vegetation was removed from the trenched subplot during the experiment. A soil collar inserted to 3 cm depth was placed in each subplot and the measured soil respiration was used to indicate soil heterotrophic respiration ($R_h$) due to the absence of plant biomass. Autotrophic respiration ($R_a$) was calculated as the difference between $R_s$ and $R_h$ (Dacal et al., 2020). All soil collars were inserted before any treatments were applied, and remained in place throughout the study. Soil respiration was measured twice a month from December 2011 to November 2012, and each measurement took place between 9 a.m. and 12 p.m. on a sunny day. Proportions of lime application induced soil respiration ($R_{LI}$) to total soil respiration were calculated as either $R_{LI} = (R_{LA}-R_{CK})/R_{CK} \times 100\%$ or $R_{LI} = (R_{L+UR}-R_{UR})/R_{UR} \times 100\%$, where $R_{CK}$, $R_{LA}$, $R_{L+UR}$, $R_{UR}$ is soil respiration in CK, LA, L+ UR, and UR treatment, respectively. Proportions of understory respiration ($R_U$) to total soil respiration was calculated as either $R_U = (R_{UR}-R_{CK})/R_{CK} \times 100\%$ or $R_U = (R_{L+UR}-R_{LA})/R_{LA} \times 100\%$, where $R_{CK}$, $R_{LA}$, $R_{L+UR}$, $R_{UR}$ is soil respiration in CK, LA, L+UR, and UR treatment, respectively. Annual soil average respiration rate was calculated by the sum of the soil respiration rate from each month divided by sampling time (12 months). Both soil moisture (volumetric moisture content) and temperature near the soil collar were measured at a depth of 0–5 cm using a probe attached to the Li-8100.

## 2.3. Soil sampling and soil bio-physical-chemical properties measurement

Soil samples were collected in December 2011 and Feb, May, Sep, December 2012, respectively. The surface litter was carefully removed prior to samplings. In each plot within each plantation, five sites were randomly selected and soil cores were collected from 0 to 10 cm depth. The soil cores from each plot were pooled. All soil samples were clear of visible stones and roots, then sieved (2 mm), and portioned into two sub-samples. Of these, one sub-sample destined for physio-chemical analysis was stored at 4°C and the other one destined for phospholipid fatty acid analysis (PLFAs) was stored at −20°C. Soil microbial community composition and abundance were estimated using PLFAs analysis (Bossio and Scow, 1998). When applying PLFAs analysis, soil bacterial abundance was estimated using PLFA markers cy19:0, cy17:0, a17:0, 17:0, i17:0, i16:0, 16:1ω7, i15:0, a15:0, and 14:0, while soil fungal abundance was determined using 18:2ω6,9c. The soil microbial community composition was estimated using the ratio of total fungal to bacterial abundance (F:B ratio) (Xiao et al., 2022). Soil pH was measured using a 1:2.5 soil: water mixture. Soil dissolved organic carbon (DOC) in fresh soil sample was extracted with 0.5 M $K_2SO_4$ and then measured with a TOC analyzer (TOC-VCPH Shimadzu Corp., Shinagawa, Japan). Soil $NH_4^+$-N and $NO_3^-$-N in fresh soil samples were extracted with 2 M KCL and then measured with a flow injection auto-analyzer (FIA, Lachat Instruments, USA).

## 2.4. Measurement of fine root biomass of *Eucalyptus*

Fine root biomass of *Eucalyptus* was determined in August 2012, when the trees grew most vigorously. Nine samplings were randomly sampled within each plot at a depth of 40 cm with an 8 cm diameter soil corer. Each soil core was divided into four sections based on horizon (0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm), and each section of soil was soaked and carefully sieved through a 0.5 mm mesh sieve to isolate roots, which were sorted by size (less than 5 mm or 5 mm in diameter) and vitality (living or dead according to color, and elasticity) In plantations, the understory vegetation is dominated by the fern *Dicranopteris dichotoma*, whose roots are rhizome and adventitious, and the fine roots can be hardly distinguished. In addition, the roots of *Dicranopteris dichotoma* were harder than those of *Eucalyptus* due to their higher lignin content (Yang et al., 2021). The roots were then dried in an oven (65°C) until they reached a constant mass and weighed.

## 2.5. Statistical analyses

Statistical analyses were performed in SPSS 18.0 (SPSS, Chicago, IL, USA). All data met assumptions of normality and homogeneity of variance in the present study. A two-way repeated-measure analysis of variance (ANOVAs) was conducted to determine the effect of LA, UR, and their interactions on soil temperature, moisture, pH, DOC, $NH_4^+$-N, $NO_3^-$-N, soil respiration rate ($R_s$, $R_h$, and $R_a$), and soil microbial communities. A one-way ANOVA and least significant difference tests (LSD) were then employed to test the significant difference among treatments on soil annual average respiration rate and *Eucalyptus* fine root biomass. In all cases, the statistical significance was set at $P \leq 0.05$. The relationships between soil respiration ($R_s$, $R_h$, and $R_a$) and soil temperature or soil moisture were tested by exponential regression and linear models, respectively. The temperature sensitivity ($Q_{10}$) of soil respiration ($R_s$, $R_h$, and $R_a$) was established using the following equations (Dacal et al., 2020):

$R = ae^{bT}$
$Q_{10} = e^{10b}$
where $R$ is $R_s$, $R_h$, or $R_a$, T is the temperature of the soil at a depth of 0–5 cm, and a and b are the regression coefficients.

## 3. Results

### 3.1. Effect of lime application and understory clearance on soil respiration and soil physical-chemical properties

Soil respiration ($R_s$, $R_h$, and $R_a$) exhibited clear temporal patterns, with the lowest respiration rate occurring in December and the highest in September (Figures 1A, B). Overall, the soil respiration rate ranged from 0.67 to 4.21 $\mu$mol $m^{-2}$ $s^{-1}$ (Figure 1). Lime application reduced $R_s$ and $R_a$, as well as their annual average rates (Table 1 and Figures 1A, C), while it increased $R_h$ and the annual average rate irrespective of understory removal (Table 1 and Figure 1B). Understory clearance reduced both $R_s$ and $R_h$ and their annual average rates (Table 1 and Figures 1A, B). It also inhibited $R_a$ and the annual average rate (Table 1 and Figure 1C). Moreover, the LA treatment decreased $R_s$ by 16.48% compared to the CK treatment, and the L+UR treatment decreased $R_s$ by 4.49% relative to the UR treatment (Figure 2A). The UR treatment decreased $R_s$ by 21.43% compared to the CK treatment, while the L+UR treatment decreased $R_s$ by 10.24% relative to the LA treatment (Figure 2B). Both understory clearance and lime application increased the proportion of heterotrophic respiration to total soil respiration (Supplementary Figure 1A), while decreased the proportion of autotrophic respiration to total soil respiration (Supplementary Figure 1B).

Lime application significantly increased soil pH, and soil $NO_3^-$-N content, but decreased soil moisture, with no significant impact on soil temperature and soil $NH_4^+$-N content (Table 1, Figure 3, and Supplementary Table 1). Understory clearance elevated soil temperature by 0.74°C, but decreased soil moisture content and soil $NO_3^-$-N content (Table 1, Figure 3, and Supplementary Table 1). In addition, understory clearance caused a trend of decline in DOC ($P = 0.06$). No significant interactions of lime application and understory clearance were observed on soil moisture, soil temperature, and soil physical-chemical properties during the experimental period (Table 1 and Supplementary Table 1).

### 3.2. Effect of lime application and understory clearance on soil microbial communities and fine root biomass

The soil microbial biomass ranged from 453 to 2,701 ng $g^{-1}$ for each treatment during the experiment. Sampling time caused a significant effect on soil microbial biomass and communities. Lime application significantly decreased both soil fungal and bacterial PLFAs, and the soil total PLFAs, despite that the F:B ratio remained unchanged (Table 2). Understory clearance significantly decreased the fungal and total PLFAs without affecting bacterial PLFAs, overall leading to a reduced F:B ratio. No interactive effects of lime application and understory clearance were found on the composition of soil microbial communities.

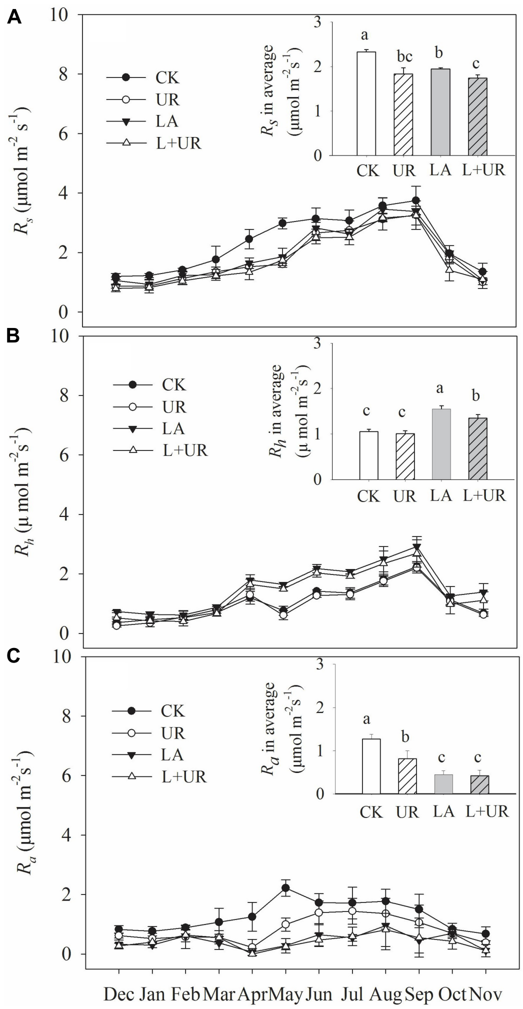


FIGURE 1
Variations of $R_s$ (A), $R_h$ (B), and $R_a$ (C) under different treatments from December 2011 to November 2012. The inserted figure indicate the annual average of soil respiration ($R_s$, $R_h$, and $R_a$) under different treatments. Different lowercase letters among treatments indicate significant difference ($P \leq 0.05$). Values are means with standard errors, $n$ = 3. Control (CK), understory removal (UR), lime application (LA), and lime application with understory removal (L+UR).

Eight months after treatment, the fine root biomass, was 35.8 ± 3.0, 31.2 ± 2.7, 38.5 ± 3.9, and 39.1 ± 3.7 g $m^{-2}$ for CK, UR, LA, and L+UR, respectively. Neither lime application nor understory clearance had a significant impact on *Eucalyptus* fine root biomass (Table 2 and Figure 4).

### 3.3. Relationships between soil respiration and soil temperature or soil moisture

Soil temperature was found to be significantly positively correlated with $R_s$, $R_h$, and $R_a$, with the exception of $R_a$ under

TABLE 1 Effects of lime application (LA), understory removal (UR), and their interactions (LA × UR) on soil respiration ($R_s$, $R_h$, and $R_a$), soil temperature (ST), and soil moisture (SM) in a southern Chinese *Eucalyptus* plantation.

| Treatments | $R_s$ | $R_h$ | $R_a$ | $ST_{UT}$ | $ST_T$ | $SM_{UT}$ | $SM_T$ |
|---|---|---|---|---|---|---|---|
| | μ mol $m^{-2}$ $s^{-1}$ | μ mol $m^{-2}$ $s^{-1}$ | μ mol $m^{-2}$ $s^{-1}$ | °C | °C | % | % |
| Control | 2.3 (0.0)a | 1.1 (0.0)c | 1.3 (0.1)a | 21.0 (1.4) | 21.8 (1.5) | 20.6 (2.1) | 20.3 (1.9) |
| UR | 1.8 (0.1)bc | 1.0 (0.0)c | 0.8 (0.1)b | 22.1 (1.5) | 22.4 (1.5) | 19.3 (2.2) | 17.7 (2.0) |
| LA | 1.9 (0.0)b | 1.6 (0.0)a | 0.5 (0.1)c | 21.3 (1.3) | 21.9 (1.5) | 18.6 (1.7) | 19.9 (2.2) |
| L+UR | 1.7 (0.0)c | 1.4 (0.0)b | 0.4 (0.1)c | 22.1 (1.5) | 22.5 (1.5) | 18.4 (2.0) | 17.2 (2.3) |
| The effects of LA, UR, and their interaction by a two-way repeated-measure ANOVAs | | | | | | | |
| UR | ** | ** | * | * | * | * | * |
| LA | ** | ** | ** | ns | ns | ** | ** |
| LA × UR | * | ns | * | ns | ns | ns | ns |

Different lowercase letters in the same column indicate significant difference among treatments ($P < 0.05$). All values are means with standard errors in the parentheses ($n = 3$). $ST_{UT}$, soil respiration in untrenched plot; $ST_T$, soil respiration in trenched plot; $SM_{UT}$, soil moisture in untrenched plot; $SM_T$, soil moisture in trenched plot. ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$.

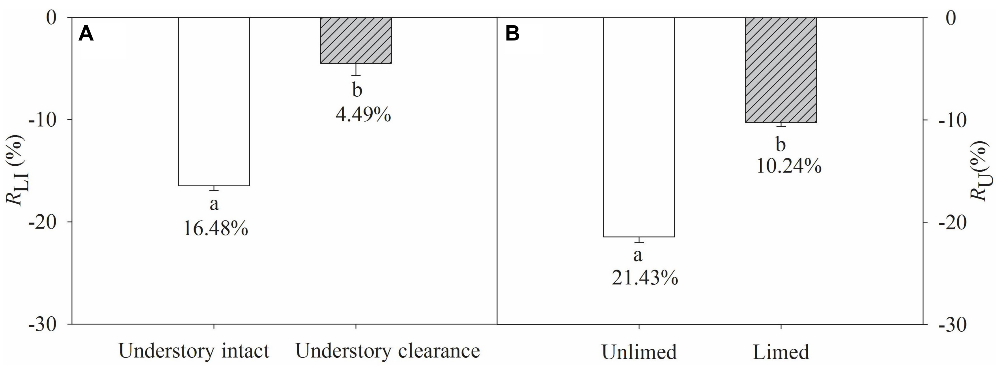


FIGURE 2
**(A)** Proportions of lime application induced soil respiration to total soil respiration ($R_{LI}$) in the presence and absence of understory vegetation; **(B)** proportions of understory removal caused changes in soil respiration to total soil respiration ($R_U$) in unlimed and limed soil. Values are means with standard errors, $n$ = 3. Different lowercase letters indicate significant difference ($P \leq 0.05$).

lime application (Figures 5A–C). Based on exponential regression models, regardless of the treatment applied, soil temperature explained 56–82% of the variation in $R_s$, 33–59% of variation in $R_h$, and 58–92% of variation in $R_a$. The temperature sensitivity ($Q_{10}$) varied from 2.08 to 2.21 in $R_s$, from 1.83 to 2.37 in $R_h$, and from 2.3 to 2.35 in $R_a$, regardless of treatment (Figures 5A–C). Neither lime application nor understory removal had a significant effect on $Q_{10}$ of soil respiration and its components. Linear regression modeling showed that the effect of soil moisture on $R_s$, $R_h$, and $R_a$ was negligible (Figures 6A–C).

## 4. Discussion

Studies have shown that lime application can increase $R_s$ by increasing plant carbon input, improving soil properties and structure, and activating the activities of soil microorganisms (Ingvar Nilsson et al., 2001; Dada and Ewulo, 2011; Jiang et al., 2018; Abalos et al., 2020). However, in the present study, we did not find such a positive relationship between lime application and $R_s$, and lime application significantly reduced soil respiration rate irrespective of understory removal, which is consistent with the first hypothesis. Generally, soil respiration mainly includes soil heterotrophic respiration and autotrophic respiration, therefore, the effect of lime application on soil respiration depends on the balance of these two components. Although we found that lime application tended to increase $R_h$, this treatment also led to the consistent inhibition of $R_s$, primarily due to the inhibition of $R_a$ (Figures 1A–C), with the extent of inhibition varying according to the occurrence of understory vegetation. Fine root respiration is the primary contributor to total root respiration (Chen et al., 2011). Nevertheless, lime application had no effect on *Eucalyptus* fine root biomass (Figure 4), thus the reduction in understory plant root respiration is likely responsible for the decreased $R_a$ under lime application. This conclusion is further supported by the finding that lime application caused a greater reduction in $R_s$ when the understory vegetation was intact (Figure 2A). Indeed, in the *Eucalyptus* plantation we studied, the understory vegetation was almost dominated by *Dicranopteris dichotoma*, which is considered as an indicator plant for acidic soils in tropical and subtropical regions. Its roots are densely distributed close to the soil surface, therefore, the root growth and associated root respiration are thus negatively affected by higher soil pH under lime application (Yang et al., 2021; Zhang et al., 2021). Børja and Nilsen (2009) and Lin et al. (2015) also explored that lime application can inhibit plant root growth and decomposition. Notably, lime application

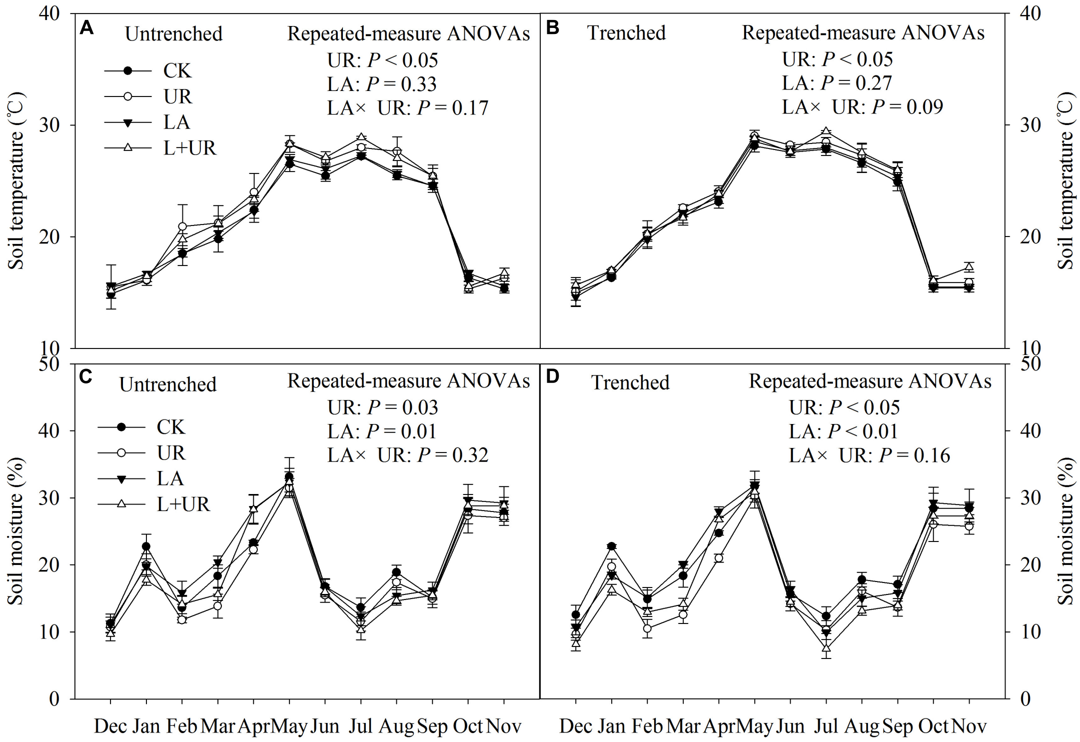


FIGURE 3
**(A,B)** Are variations of soil temperature under different treatments in untrenched and trenched plot, respectively; **(C,D)** are variations of soil moisture under different treatments in untrenched and trenched plot, respectively, from December 2011 to November 2012. The inserted statistical results are from a two-way repeated-measure ANOVAs. Values are means with standard errors, $n$ = 3.

**TABLE 2 Effects of lime application (LA), understory removal (UR), and their interactions (LA × UR) on soil microbial PLFAs, and *Eucalyptus* fine root biomass in a southern Chinese *Eucalyptus* plantation.**

| Treatments | Fungal PLFAs | Bacterial PLFAs | F:B | Total PLFAs | Fine root biomass |
|---|---|---|---|---|---|
| | ng g$^{-1}$ | ng g$^{-1}$ | | ng g$^{-1}$ | g m$^{-2}$ |
| Control | 185 (6)a | 1,441 (9)a | 0.13 (0.00)a | 1,626 (4)a | 35.8 (3.1) |
| UR | 134 (6)b | 1,369 (30)a | 0.10 (0.01)b | 1,505 (27)b | 31.2 (2.7) |
| LA | 142 (8)b | 1,206 (18)b | 0.12 (0.01)b | 1,348 (24)c | 38.5 (3.9) |
| L+UR | 110 (8)c | 1,064 (56)c | 0.11 (0.00)b | 1,174 (63)d | 39.1 (3.7) |
| The effects of UR, LA, and their interaction by a two-way repeated-measure ANOVAs | | | | | |
| UR | ** | ns | * | * | |
| LA | ** | ** | ns | ** | |
| LA × UR | ns | ns | ns | ns | |

Different lowercase letters in the same column indicate significant difference among treatments ($P < 0.05$). All values are means with standard errors in the parentheses ($n$ = 3). ns, $P > 0.05$; *$P < 0.05$; **$P < 0.01$.

increased $R_h$ (**Table 1** and **Figure 1B**), but decreased both fungal and bacterial PLFAs (**Table 2**), implying that some microbial populations were inhibited at higher soil pH but others with low carbon use efficiency were promoted in growth and activity (Xiao et al., 2022). Consequently, lime application stimulated soil microbial activities and associated $R_h$ following the findings of other researchers (Ingvar Nilsson et al., 2001; Dada and Ewulo, 2011). Given that the inhibitory effect of lime application on soil respiration was significantly greater when the understory was intact compared with when the understory was removed (**Figure 2A**), our results suggest that understory vegetation of *Eucalyptus* plantation can play a crucial role in regulating the effects of lime application on soil respiration. It should be noted that the effect of lime application on $R_s$ has been found to vary with forest, lime formulation, treatment time, and lime application rate (Klaus et al., 2001; Børja and Nilsen, 2009).

Consistent with the second hypothesis, understory clearance caused a significant reduction in $R_s$ (**Figure 1**), which is in accordance with the majority of previous work in subtropical plantations (Wan et al., 2015; Zhao et al., 2022). Studies on natural tropical forests, subtropical plantations, and boreal forests show that understory vegetation can affect $R_s$ by modulating $R_h$ and $R_a$

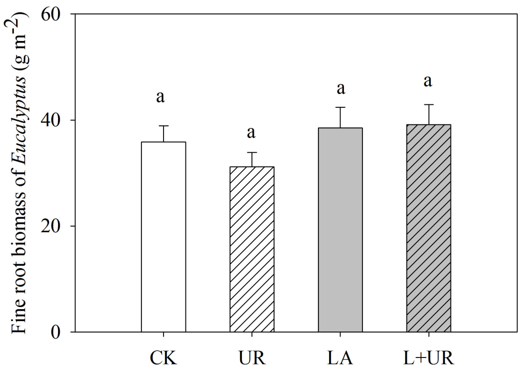


FIGURE 4
*Eucalyptus* fine root biomass after treatments were applied 8 month. Values are means with standard errors, $n$ = 3. Different lowercase letters indicate significant difference ($P$ < 0.05).

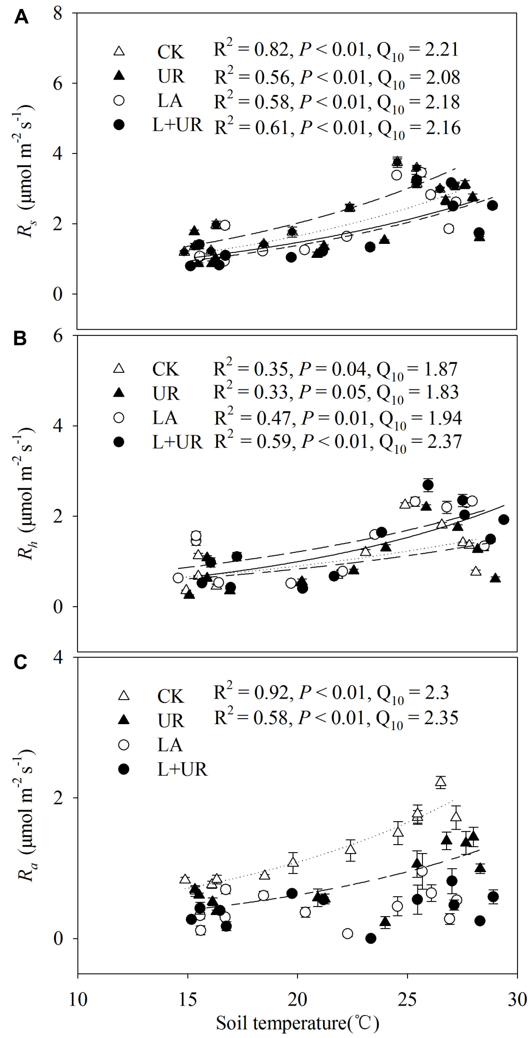


FIGURE 5
**(A–C)** Are $R_s$, $R_h$, and $R_a$ in relation to soil temperature at 5 cm depth across the four treatments, respectively. CK, dotted line; UR, short-long line; LA, long-dash line; L+UR, solid line.

(Fukuzawa et al., 2015; Subedi et al., 2019; Dacal et al., 2020). In the present study, understory clearance allowed a greater amount of solar radiation to reach the soil surface, and increased soil temperature by 0.74°C, which may stimulate $R_h$, as soil temperature was significantly positively correlated with $R_h$ (**Figure 5**). Overall, however, we found that understory clearance had a negative effect on $R_h$. This result may be due to the reduction of DOC and soil $NO_3^-$-N content caused by the clearance of understory vegetation (**Supplementary Table 1**), because the growth of soil microbiota is often limited by the accessibility of soil nutrients and carbon substrates (Subedi et al., 2019). The decreased soil total PLFAs after understory clearance can further support this finding (**Table 2**). Furthermore, despite the effects of understory clearance on *Eucalyptus* root respiration and *Eucalyptus* fine root biomass were negligible (**Figure 4**), it decreased $R_a$, suggesting that the inhibition of $R_a$ was mainly due to the reduction in understory root respiration. Overall, understory clearance reduced $R_s$ by decreasing both $R_h$ and $R_a$. These observations highlight that understory vegetation can play a crucial role in modulating soil microorganisms and carbon cycling in subtropical plantations. In addition, our previous studies have found that understory vegetation had a positive effect on *Eucalyptus* fine root biomass and productivity in 4-year-old plantations (Wan et al., 2014, 2015), while this was not the case in the present study with plantations in 6-year-old, implying that the positive effect of understory vegetation on the growth of *Eucalyptus* is age-dependent.

Following with the third hypothesis, $R_s$ was decreased when lime application was combined with understory clearance (**Table 1** and **Figure 1**). In the present study, the annual average of $R_h$ under the L+UR treatment were significantly higher than those under the CK treatment (**Figure 2B**), indicating that the increase in $R_h$ due to lime application was greater than that the decrease in $R_h$ after understory clearance. Therefore, when lime application and understory clearance was combined, the increased $R_h$ by lime application can be partially offset by the reduction in $R_h$ caused by understory clearance, and the other by the reduction in $R_a$ caused by understory clearance. Given that both lime application and understory clearance had negligible effects on *Eucalyptus* root respiration, we believe that the combined lime application and understory clearance can inhibit soil respiration, mainly by reducing root respiration associated with understory vegetation. In the context of the prevalence of lime application and understory clearance in subtropical plantations (Moore and Ouimet, 2021; Wan et al., 2021), our results suggest that the combined effect of lime application and understory clearance should be considered to understand and predict carbon cycling in the plantation ecosystems.

Overall, lime application and understory removal, and their combined application reduced soil respiration, suggesting that both forest management practices have the potential to increase soil carbon stocks of subtropical *Eucalyptus* plantations. However, whether lime application or understory removal contributes to carbon sequestration in subtropical *Eucalyptus* plantation requires further study. Soil microbial abundance and activity generally have positive effects on plant growth, however in this study, lime application and understory significantly reduced soil microbial biomass. Therefore, we advise that plant productivity and soil carbon stocks should be considered when taking lime

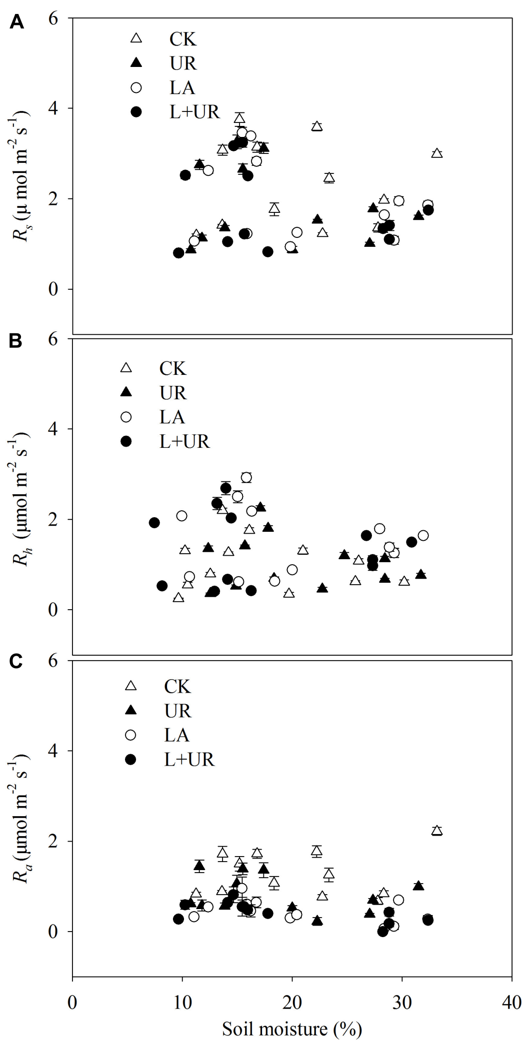


FIGURE 6
**(A–C)** Are Soil respiration ($R_s$, $R_h$, and $R_a$) in relation to soil moisture at 5 cm depth across the four treatments, respectively.

application and understory removal measures in subtropical *Eucalyptus* plantations.

## 5. Conclusion

In conclusion, lime application inhibited soil respiration by decreasing the root respiration of understory vegetation. Understory clearance reduced soil respiration by inhibiting both soil heterotrophic respiration and understory root respiration. The interaction of lime application and understory clearance resulted in a reduction in soil respiration, which was mainly due to the decrease in understory root respiration. These results demonstrate that the effects of lime application on soil respiration are highly modulated by understory vegetation, highlighting the important role of understory vegetation in regulating soil carbon balance in subtropical *Eucalyptus* plantations. Given the critical role of soil respiration in regulating soil carbon cycling, our findings will contribute to understanding and predicting the effect of forest management practices on soil carbon storage and global carbon cycling in a changing climate.

## Data availability statement

The original contributions presented in this study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

JL performed the study data collection, analyzed the results, wrote the original draft, and reviewed the manuscript. RL performed the formal analysis and visualization. JX analyzed the results and reviewed the manuscript. SF designed the experiment, reviewed the manuscript, and wrote the final manuscript. SW designed the experiment, writing—review and editing, and acquired funding. All authors contributed to the article and approved the submitted version.


## Funding

This study was financed by the National Natural Science Foundation of China (Nos. 41867007 and 32060267) and the Double Thousand Plan of Jiangxi Province (jxsq2018102058).

## Acknowledgments

We thank the editor and the reviewers for their constructive comments on this manuscript.


## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2023.1136474/full#supplementary-material

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