Stand age rather than soil moisture gradient dominantly regulates the compromise between plant growth and water use of *Eucalyptus urophylla* in hilly South China

Running title：Tree water use in relation to stand age and soil moisture

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Large-scale cultivation and short-term rotation of *Eucalyptus* trees for economic reasons have led to excessive consumption of soil water, raising broader ecological and environmental concerns. Therefore, exploring the balance between water use and plant growth of *Eucalyptus* trees has become increasingly important but remains understudied. Here we hypothesized that stand age and soil moisture gradient can both regulate such balance, and examined this hypothesis by collecting a field dataset of *Eucalyptus urophylla* plantations that respectively capture three age categories (i.e. young age of 3-4 years, mature age of 6-7 years, and old age of >25 years) and span three soil moisture gradients along a hilly slope in South China. The datasets collected in Jan/2018-Dec/2019 included 1) continuous measurements of tree sap flow and soil moisture and 2) periodic measurements of leaf water potential (Ψ_{leaf}), tree biometric parameters, and stand leaf area index (*LAI*). With the data, we derived the monthly tree transpiration (E_{L}), annual growth rate, and tree water use efficiency (*WUE*). Our results showed that stand age importantly regulated plant growth and water use, as old trees transpired more water than young and mature trees, while the young trees have significantly higher *WUE* due to their relatively higher growth rate and lower water consumption. In contrast, we didn't observe significant differences in tree transpiration along with soil moisture gradients at each age level, suggesting stand age rather than soil moisture gradient dominantly regulates the growth vs. water-use compromise. Our results also showed that the old trees can maintain a more stable water consumption, suggesting that they are less sensitive to environmental seasonality and thus more stable. Collectively, our study provides important insights into the management and ecosystem stability of *Eucalyptus* plantation in South China.

Keywords: *Eucalyptus*, stand age, soil moisture gradients, tree water use, tree growth rate, water use efficiency.

1 Introduction

Attributable to high productivity and important economic values, *Eucalyptus* has been widely planted in tropical and subtropical areas (Whitehead and Beadle, 2004). By now there are more than 4 million hectares of *Eucalyptus* plantations in South China, and the planting area is still increasing (data from State Forestry Bureau of China). Given the high water consumption and the relentless utilization of soil water resulted from the consecutive short-term rotations (6-7 years in South China) of *Eucalyptus* plantations, it has become imperative to evaluate the relationship between tree growth and transpiration under current *Eucalyptus* silvicultural systems with critical insights into the optimal afforestation practice that can best balance the timber production and water demand.

Numerous studies have claimed that tree growth and transpiration are age- or size- dependent, namely trees grow rapidly at their early age, peak at the middle age, and decrease in their growth while maintaining relatively stable transpiration thereafter (Ryan et al., 1997; Taylor and MacLean, 2005; Xu et al., 2012). The age- and size-induced reduction in tree growth and water use in older and taller trees was traditionally attributed to a less efficient hydraulic transport, or a reduced biomass allocation to stem, or a limitation of nutrient uptake (Ryan and Yoder 1997; Mencuccini 2002). These possible mechanisms have been experimentally and separately supported, but a consensus has not yet been reached (Wu et al., 2018; Baret et al., 2018), as several studies have reported a continuously increase of tree growth and transpiration with tree age or size (Stephenson et al., 2014; Sillett et al., 2015; Tfwala et al., 2019). For instance, Stephenson et al. (2014) conducted a global analysis of 403 tropical and temperate tree species and showed that the biomass growth rate increases continuously with tree size for most species. Sillett et al. (2015) claimed little evidence for negative effects of age on tree-level productivity for studied tree species, but reported an increasing trend of annual biomass increments with stand age. These inconsistent experimental results highlight that a site-specific or multi-faceted biological processes rather than a universal mechanism can explain the changes in forest productivity and water use with stand age (Baret et al., 2018).

Age effect on tree growth and transpiration of *Eucalyptus* has been previously investigated with the main focus on those plantations in Australia. Almeida et al. (2007) reported a reduction in tree transpiration, leaf area index, and annual increment when the stand age exceeded 3~4 years in fast-growing *Eucalyptus* plantations. Buckley et al. (2012) and Macfarlane et al. (2010) compared the transpiration of regrowth and mature *Eucalyptus* trees in South Australia and found the transpiration rate of regrowth trees was twice more than that of the mature trees. Similarly, the results of Forrester et al. (2010) also showed that tree transpiration, stand leaf area index, and periodic annual increments of aboveground biomass peaked at about age of 4-6 years, then declined when stand age reached 8 years. These previous studies highlighted the effects of structural adjustment with stand age on tree transpiration, but lacked the possible changes in tree hydraulic properties with the increasing age. Generally, tree hydraulic properties that show strong dependency on stand age critically regulate tree water transport efficiency, and thus drive the dynamic of tree individual level transpiration rate (Zhu et al., 2015; Sperry and Love, 2015). Moreover, the variability in soil moisture availability has been claimed to exert important controls on tree water use through adjusting the hydraulic properties in response to different soil moisture conditions (Renninger et al., 2014; Grossiord et al., 2018; Gao et al., 2020), but haven't yet been fully tested, especially under the real-world practice that *Eucalyptus* plantation is often mixed with the variability in both stand age and soil moisture availability.

Tree water use efficiency (*WUE*), defined as the amount of transpired water relative to the increment of produced biomass (or carbon), is an integrated indicator that accounts for all events occurring during biomass accumulation (Hubbard et al., 2010). This index directly relates tree water use to productivity and potentially varies with stand age and environmental factors (Forrester et al., 2010; Battie-Laclau et al., 2016). Water availability is generally regarded as an essential factor that strongly affects growth, plant transpiration, resource use, and biomass partitioning in planted forests (White et al., 2014). Under soil water deficit conditions, trees are expected to reduce their transpiration through stomatal control, leading to a decrease in transpiration but an increase in *WUE*. For instance, the manipulated water inputs experiment demonstrated that tree transpiration decreased with the excluded precipitation treatment and suggested that the studied trees deeply rely on precipitation water sources during the peak of growing season (Besson et al., 2014; Grossiord et al., 2018; Ouyang et al., 2020). Kwon et al. (2018), who intended to explore the *WUE* of forests with different ages and precipitation regimes in the Pacific Northwest, showed that the summer maximum of *WUE* was 2.5 times higher in semi-arid climate than that in mesic condition. Moreover, they claimed that the effect of drought stress on *WUE* was much more pronounced in young pine than in mature pine, confirming the age effect on plants' *WUE*.

In South China, the rotation lengths of *Eucalyptus* plantations for timber production are usually 6-7 years with canopy closure generally occurring within 5-6 years. The harvest usually comes at the peak of *Eucalyptus* growth, making it difficult to explore the age effect on tree transpiration and growth and thus causing controversies (Shi et al., 2012; Ouyang et al., 2018). In the present study, we used the sap flow technique to accurately and continuously monitor long-term water transpiration of *E. urophylla* and evaluated the combined impact of stand age and soil moisture gradients on tree growth and *WUE*. Three *E. urophylla* plantations aged at 3-4, 6-7, and more than 25 years were chosen to typically reflect the current *Eucalyptus* silvicultural practice mode in hilly South China. We hypothesized that tree transpiration, growth rates, and *WUE* would decline with stand age, and compared with the sufficient soil moisture conditions, the lower soil moisture content could suppress transpiration but increase *WUE*, especially for the young trees. Our main objectives include (1) to determine the effect of stand age on transpiration of *Eucalyptus* under different soil moisture gradients, and to understand the potential mechanism for the varied tree transpiration by examining the changes of physiological and hydraulic traits, such as leaf water potential and hydraulic conductance; (2) to explicitly assess the age-dependent tree growth under different soil moisture contents; and (3) to analyze the age-dependent variation in *WUE* derived from the increment of tree biomass and the integrated measured transpiration under different soil moisture gradients at the individual tree level.

2 Materials and Methods

2.1 Study area and experimental design

The experiment was carried out during the period from January 2018 to December 2019 in Heshan National Field Research Station of Forest Ecosystem, located in Heshan County, Guangdong Province, China (22° 41' N, 112°54' E) (Figure S1). Basic climate condition in this area has been described in Ouyang et al. (2020). Three *E. urophylla* plantations, aging at 3-4 years (young trees), 6-7 years (mature trees), and more than 25 years (old trees) referring to sites A, B, C, respectively, were selected for this study. The average plant spacing was 3 m × 2.5 m, and no fertilization was conducted across all the growth stages. In general, the young trees (3-4 years) are in the stage of rapid growth with no heartwood formed. The mature trees have reached their growth peak, at which the rotation is performed (6-7 years). The growth of old trees is assumed to be stable. The three plantations grew on 3 different hillsides with similar inclinations of ~20° and roughly faced southeast. The elevation of the chosen hillsides is approximately 80 m and the distance between every two hillsides is within 1 km. Stand density was 2400 (site A), 2267 (site B), and 1867 (site C) trees ha^{-1}, respectively. Along the slopes from the top to the bottom, we randomly set up three plots with a size of 20 m × 15 m to represent different soil moisture gradients. For simplification, we denominated the plots from hilltop to bottom as Slope Top, Mid-Slope, and Slope Bottom with a total of 9 plots for the experiment. Along with the slope, we hypothesized that the soil water contents gradually increased from the Slope-Top to Slope Bottom along with the slope. Additionally, for each plot, we dug soil profiles to determine the soil depths. The results show that the soil depth decreases significantly with the slope (Table 1), which can represent a good soil water gradient, despite the soil water content may be similar across different slope positions. Soil texture of the chosen stands was clay loam, with the pH of the topsoil (0-30 cm) ranging from 3.98 to 4.41. Similar contents of soil organic matter, total nitrogen, and total phosphorous were observed among the three stands, and the values were 22.05-37.40 g kg^{-1}, 1.08-1.60 g kg^{-1}, and 0.22-0.26 g kg^{-1}, respectively (Table 2).

2.2 Meteorological parameters and soil water content

A 1.5 m high mast with meteorological sensors was installed within the meteorological observation field of Heshan Station. The sensors included a temperature (*T*) and relative humidity (*RH*) probe (HC2-S3, Rotronic Inc., Switzerland), and a photosynthetically active radiation (*PAR*) sensor (SQ-110, Apogee Instruments, Inc., USA). Vapour pressure deficit (*VPD*) was calculated using the relative humidity and air temperature data proposed by Campbell and Norman (1998) as follows:

$$VPD = a \times \exp\left(b \times T/(T+c)\right) \times (1-RH) \quad (1)$$

where *T* is the air temperature (°C), *RH* is the air relative humidity of (%), and a, b and c are constants with values of 0.611, 17.502, and 240.97, respectively. Three SM150 sensors (Delta-T Devices, Ltd., Cambridge, UK) were used to continuously monitor the soil water contents (*SWC*, at a depth of 30 - 50 cm) in each plot. All the meteorological and soil water data were recorded every 10 minutes during the experimental period using the data loggers (DL2e, Delta-T Devices, Ltd., Cambridge, UK). The precipitation was recorded by a tipping-bucket rain gauge in the same meteorological observation field.

2.3 Sap flow and tree transpiration

Seven *E. urophylla* trees per plot and a total of 21 trees per stand age across all the three plots were selected for sap flow monitoring, for which the lab-made Granier's thermal dissipation probes (TDB) were applied. Detailed information for the sap flow measurement was described in Ouyang et al. (2020). The sap flux density (J_s, g H_2O m^{-2} s^{-1}) was derived from the temperature difference between the upper heated and lower reference probes according to the following equation (Granier, 1987):

$$J_s = 119\left(\frac{\Delta T_m - \Delta T}{\Delta T}\right)^{1.231} \quad (2)$$

where ΔT_m is the maximum temperature difference under zero-flux conditions, and ΔT is the instantaneous temperature difference. Sap flow readings were collected also with 10-min resolution by the Delta-T data loggers. According to our prior analysis of wood anatomy, the *E. urophylla* is a diffuse-porous species, with no heartwood formed of young *E. urophylla*, while the sapwood widths of mature and old trees are slightly thicker than the TDP probe's length. Thus, the J_s at the outermost 20 mm can represent the average value. The actual whole tree transpiration (E) was obtained by multiplying the sap flux density with the sapwood area (A_s). To remove the effect of tree size on tree water consumption, we used normalized tree transpiration (E_L, kg m^{-1}) expressed as E/DBH by following the proposal of Besson et al. (2014), in such way we could minimize the size-induced individual differences and ensure reasonable comparison of the quantity of individual tree transpiration among different stand ages.

2.4 Leaf water potential and whole-tree hydraulic conductance

Measurements of leaf water potential were performed in August and December of 2018 and 2019, representing the wet and dry seasons, respectively. At each site, the predawn (Ψ_{pd}) and midday water potentials (Ψ_{md}) were measured using 3~5 twigs with intact leaves from sampled trees for two consecutive sunny days. In other words, during each season, we chose six consecutive days (two days for each site) of fine weather to complete water potential measurements, by which we aimed to minimize potential effects of environmental fluctuations (particularly from weather conditions) on the measurement results. We cut off the twigs with leaves and immediately determined the leaf water potential with PMS-1000 pressure chambers (PMS Instrument, Corvallis, OR, USA). Three to five replicates were made for each treatment (age × soil moisture gradient). At the time shortly before dawn, a water equilibrium occurs between soil and canopy leaves when sap flux is zero, the measured Ψ_{pd} is considered to be equal to the soil water potential (Ψ_{soil}) within the root zone (Besson et al., 2014). We followed the Darcy's law that describes the water transport from soil to canopy leaves (Cochard et al., 1996) and determined the whole tree hydraulic conductance as:

$$K = \frac{E_L}{\Psi_{\text{soil}} - \Psi_{\text{md}}} \quad (3)$$

where K is the whole tree hydraulic conductance from the soil to the leaves (kg day^{-1} m^{-1} MPa^{-1}), E_L is the normalized whole tree transpiration during the measurement period.

2.5 Tree growth and stand leaf area indices

Tree diameter at breast height of 1.3 m above ground (*DBH*) and tree height (*H*) were semiannually measured using a *DBH* ruler and a Tandem-360R/PC altimeter (Suunto, Finland), respectively. The stem cross-sectional area is the sapwood area (A_s) for young *E. urophylla* since no heartwood formed at this age. We applied the equation to calculate the sapwood area of the mature and old trees by following the method of Zhu et al., (2015). For each site at the quarterly scale, we measured the stand leaf area indices (*LAI*) with a LI-2000 Plant Canopy Analyzer (Li-Cor, Inc., Lincoln, NE). The average *LAI* values were obtained from the image data of randomly captured 12-15 sample points.

2.6 Tree biomass and water use efficiency

Water use efficiency (*WUE*) was defined here as the ratio of the increment of whole tree biomass to integrated whole tree transpiration (ΔBiomass/*E*). It was not possible to harvest every tree, therefore, we applied the allometric equation to determine the whole tree biomass based on the data of *DBH* and *H* (Campoe et al., 2012). Here we used the allometric equation proposed by Xue et al. (2009). The equations describing the relationships between tree biomass of each sub-component and the associated *DBH* and *H* were established as follows:

$$\text{Biomass}_{stem} = 0.004861\times(DBH^2H)^{1.22}\ (R=0.99) \quad (4)$$

$$\text{Biomass}_{branch} = 0.002861\times(DBH^2H)^{1.04}\ (R=0.96) \quad (5)$$

$$\text{Biomass}_{leaf} = 0.406064\times(DBH^2H)^{0.31}\ (R=0.94) \quad (6)$$

$$\text{Biomass}_{bark} = 0.001866\times(DBH^2H)^{1.24}\ (R=0.99) \quad (7)$$

$$\text{Biomass}_{root} = 0.004264\times(DBH^2H)^{1.06}\ (R=0.99) \quad (8)$$

2.7 Statistical analysis and calculations

The two-way ANOVA (Tukey's HSD) was used to test the significance level of stand age and slope position on the biometric parameters, tree transpiration, leaf water potential, *SWC* and *WUE*. The difference of *LAI* values in different sites was tested by the one-way ANOVA (Tukey's HSD) (SPSS Inc. 2016). In this study, we denoted significant differences among the treatments when $p < 0.05$. To establish the correlations between the transpiration and *PAR* or *VPD*, the linear regression ($y = ax + b$) was performed in Origin 8.0, where a and b are fitting parameters.

Due to the power or probe failure, data missing is a common problem when conducting sap flow studies over a long period. To fill the data gaps, we firstly established linear regressions using the available data of sap flux density and *PAR* for every single tree in each month, and then calculated the missing data from the corresponding *PAR* values based on the equations. Finally, the actual whole tree transpiration (E, kg month^{-1}) for every sample tree was obtained by multiplying the sap flux density with the sapwood area, which was used for the calculation of *WUE*.

3 Results

3.1 Meteorological parameters

Monitored meteorological parameters varied over the 2-year study period (Figure 1). The highest and lowest values of mean monthly *PAR*, *T*, and *VPD* occurred in May and January of the Year 2018, and in September and March of the Year 2019, respectively. The two experimental years shared similar mean annual *T* and *RH*, with values of 24.0 and 24.6 ℃, and 68.0% and 66.3% in the Year 2018 and 2019, respectively. Mean monthly *PAR* and *VPD* (both were derived from daytime values) varied considerably with the values ranging from 412 to 882 μmol m^{-2} s^{-1} and 0.68 to 2.04 kPa, respectively. Similar temporal trends were observed between the two years for mean monthly meteorological variables, namely higher values often occurring during the wet seasons (from April to September), whereas lower values mainly occurring in the later dry seasons (from November to March of next year).

Total annual precipitation (*P*) at the research site was much higher in 2018 (1787.7 mm) than in 2019 (1309.6 mm), and unevenly distributed with 87.2% and 83.7% of the total falling from April to September in the two respective years (Figure 2). Notably, almost no rainfall events occurred in December 2018 and November 2019.

Corresponding with the seasonal variation in precipitation, soil water content (*SWC*) also varied with time and reached the seasonal maximum of ~ 40% during the wet months, whereas the minimum *SWC* (20.0%, 16.8%, and 20.7% in site A, B, and C, respectively) occurred during the dry months (Figure 3). Our statistical results showed that the SWC values of Slope Bottom were significantly higher than those of Slope Top and Mid-Slope Bottom. Variations in averaged SWC during the dry or wet season among the three sites were also observed, and site C usually possessed relatively higher SWC than site A (Figure 3, $p < 0.05$). As the soil depths of different slope positions were significantly different (Table 1), varied soil water storage among the different slope positions could be expected in this study, i.e. the Slope Bottom could have a larger soil water storage than the other two slope positions, and the soil water storage decreased with the elevating hill slope position, forming a gradient of water supply along the slope.

3.2 Seasonal and annual transpiration

Monthly changes of E_L and monthly averaged E_L for all plots were presented in Figure 4. The old trees transpired significantly more water than the young and mature trees, but the young and mature trees shared similar E_L during most of period ($p < 0.05$). Young trees growing at Slope Bottom generally possessed a significantly lower transpiration than those growing at Slope Top. Similar temporal changes in transpiration were presented for young and mature trees, with peak E_L values generally occurring in the wet and early dry seasons. Different from the young and mature trees, the old trees had relatively more stable E_L during the whole period, with values fluctuating around 2500 kg m^{-1}.

To examine the influence of environmental drivers and *SWC* on transpiration, we checked the linear relationships between E_L and *PAR*, *VPD* or *SWC* at a monthly scale. Except for the young trees growing at the slope bottom, significant linear relationships were established between E_L and *PAR/VPD* (Figure 5, R^2 values ranging from 0.21 to 0.62, $p < 0.05$). In addition, we separately analyzed the correlations between the transpiration and *PAR* or *VPD* in the dry and wet seasons. Fitted parameters and R^2 values were presented in the Table S1. The available data showed that the slopes of fitting equations in the dry season were significantly higher than those in the wet season. However, there was no significant linear relationship between the E_L and *SWC* ($p > 0.05$). These results might indicate less limitation of soil water on tree transpiration, even under the condition of dry season.

3.3 Leaf water potential and hydraulic conductance

Leaf water potential during wet and dry seasons varied among the three sites (Figure 6). Predawn leaf water potential (Ψ_{pd}) was relatively high (> -0.3 MPa) and constant among the three different age groups, ranging from -0.05 to -0.23 MPa, and -0.12 to -0.20 MPa in wet and dry seasons, respectively. Statistical analysis showed that soil moisture gradients had no significant effect on midday leaf potential (Ψ_{md}) and leaf-soil water potential difference ($\Delta\Psi$, Ψ_{pd}-Ψ_{md}) (Figure 6, p<0.05). The significant age-related differences of whole tree hydraulic conductance (*K*) were presented and the young trees usually possessed significantly lower *K* than those mature and old trees under wet season. Significant linear regressions between $\Delta\Psi$ and corresponding E_L were established, with an R^2 value of 0.34 (Figure S2, $p < 0.05$).

3.4 Tree growth and stand *LAI*

Values of *DBH* significantly increased with stand age, and tree height (*H*) values of mature and old trees were significantly higher than those of young trees (Figure S3, $p < 0.05$). The young trees had significantly higher annual *DBH* and *H* increments than mature and old trees. No significant effect of soil moisture gradient on the tree growth was observed during the whole experimental period (Figure 7c, d).

Significant differences in stand *LAI* among three sites were observed for all three stands (Figure 8, $p < 0.05$). The young trees site generally possessed significantly lower *LAI* values during the whole period, whereas the other two sites presented similar *LAI*, with average values being 0.65, 1.14, and 1.04 for sites A, B, and C, respectively. Higher *LAI* values usually occurred in the wet seasons. As the Super Typhoon Mangkhut hit on September 16, 2018, stand *LAI* decreased sharply and then recovered in the later period.

3.5 Water use efficiency

The annual *WUE* values for the different sites were listed in Table 3. Young and mature trees shared similar annual *WUE* that was significantly higher than that of old trees ($p < 0.05$). The soil moisture gradient did not pose a significant influence on tree annual *WUE* for all three sites ($p > 0.05$).

4 Discussion

4.1 The effects of stand age and soil moisture on transpiration and associated hydraulic mechanism

Previous studies investigating the age effect on tree transpiration of *Eucalyputs* were carried out mainly in Australia (Forrester et al., 2010; Macfarlane et al., 2010; Mendham et al, 2011; Buckley et al., 2012). For instance, Roberts et al. (2001) reported that the mean daily tree transpiration of *Eucalyptus sieberi* aged 14, 45, and 160 years, were 10.6, 21.8, and 49.4 L day^{-1}, respectively. Another study (Alcorn et al., 2013) showed that the mean daily transpiration of the individual *E. pilularis* and *E. cloeziana* trees (both tree species were 5-6 years old) ranged from 9 L day^{-1} to 16 L day^{-1}. In this study, the whole tree transpiration for young, mature, and old trees during the whole experimental period was in the range of 3~15, 8~20, and 12~32 L day^{-1}, respectively. The relatively lower whole tree transpiration at a similar stand age in Australia could be attributed to the less precipitation compared to the South China. Our study demonstrates that, with sufficiently high precipitation and solar radiation, the adequate supply of soil water allows vigorous water consumption of old and tall trees, especially during the high rainfall wet season.

We attributed the temporal variation of E_L to the changing climatic conditions, which was supported by the linear relationships between E_L and *PAR*/*VPD* (Figure 5). With higher *PAR* and *VPD*, trees undoubtedly transpired more water during the wet season and the early stage of the dry season. Compared with the large fluctuation in E_L of young *Eucalyptus* trees over the experimental period, the old trees maintained a relatively stable E_L. The differential response of E_L to the *PAR*/*VPD* in the dry and wet seasons further suggests the important role of *LAI* in affecting the relationships between transpiration and environmental factors. In the dry season, the decreased *LAI* means reduced leaf area for transpiration. Meanwhile, the higher *PAR* and *VPD* in the early time of dry season were beneficial to transpiration, consequently leading to higher slopes of fitting equations. Moreover, differing from previous studies reporting significantly higher transpiration in younger *Eucalyptus* trees than in older trees (Macfarlane et al., 2010; Hubbard et al., 2010), the highest E_L was observed in the old trees, while the young trees growing at the bottom of the slope experienced the lowest E_L in our case. Previous studies have reported that the ratio of sapwood area to leaf area declined with tree age and attributed the higher transpiration of regrowth and young eucalypt forest to the higher *LAI*, greater foliage cover, and smaller basal area (implying a higher ratio of sapwood area/basal area) (Buckley et al.,2012; Macfarlane et al., 2010). Nevertheless, the young trees in this study possessed relatively lower sapwood area and *LAI* values (namely a smaller transpiring leaf area) than those of mature and old trees during the whole period, which should potentially limit the water transport, consequently leading to the lower E_L. A previous study that analyzed tree transpiration from 94 published studies conducted across various sites and explored relationships between morphological traits and whole tree water use for 130 tree species suggested that tree water use increases with *DBH* (Tfwala et al., 2019), partly supporting one of our major findings that the old trees with larger *DBH* could transpire more water than young trees.

Other important factors affecting tree transpiration included soil water availability and the water transport capacity (Köhler et al., 2010; Besson et al., 2014). In this study, we found no significant correlations between E_L and *SWC* values for every treatment (p > 0.05), partly due to the narrow range of *SWC* in three sites, especially in site B and C for most periods of time. Although there are differences in soil water storage among the slope positions, the comparable E_L indicated that soil moisture was not the limiting factor. As suggested by Christina et al. (2017), roots of 2 years old *Eucalyptus* could reach the water table at a depth of 12 m, which enables the access to large quantities of water stored in deep soil layers at the early growth stage. According to the observation data from Heshan Station, the water table ranged from 0.47 m to 2.10 m during 2018, with the average depth of the groundwater level of 1.32 m, which could explain the less impact of soil moisture gradients on tree transpiration. Additionally, the measured Ψ_{md} ranged between -0.7 and -1.5 MPa that were higher than -3.0 MPa, a threshold at which a certain degree of loss in hydraulic conductivity occurs (Klein et al., 2014), implying that fine roots in the rhizosphere layer (about 30-40 cm) were less water-stressed. As the tree height increased with tree age and greater height could lead to more negative leaf water potentials (Liu et al., 2019), the old trees in this study usually possessed a relatively lower Ψ_{md} but higher $\Delta\Psi$ values than the young trees to help maintain higher tree transpiration.

4.2 The effects of stand age and soil moisture on tree growth and water use efficiency

We observed that the young trees experienced a higher growth rate than old trees, which is consistent with some earlier studies (Taylor and MacLean, 2005; Aakala et al., 2013; Tfwala et al., 2019), while the other works showed little evidence for negative effects of stand age on tree growth and productivity (Johnson and Abrams, 2009; Sillett et al., 2015; Molina et al., 2019). Numerous hypotheses have been developed to explain the age-related decline in growth, including the increased hydraulic resistance, nutrient limitation, reductions in photosynthesis, and reduced allocation to stem production (Xu et al., 2012; Martínez-Vilalta et al., 2007). For example, it was reported that trees are less efficient in transporting water or nutrients to their leaves and pay a higher carbon cost with increasing tree height (Mencuccini 2002), but the old trees in this study still possessed comparable $\Delta\Psi$ and K (Figure 6), and utilized relatively more water, suggesting less constrain in water transport. Therefore, we tend to think that the reduced growth rate of old trees was not associated with age-related hydraulic constraints.

We found the variations in annual *WUE* were tightly linked with stand age and climate factors. Compared with the old trees, the relatively lower E_L and comparable biomass increment resulted in the higher annual *WUE* for young and mature trees. Similar results were also reported in Forrester et al. (2010), in which the *WUE* of *E. globulus* peaked at around age 4-5 years and then declined with stand age. As proposed by Forrester et al. (2010), the decline of *WUE* with increasing age could be a result of exacerbated competition for resources as the stands develop. Consistent with the E_L, tree growth rates and *WUE* generally did not show a significant difference with the changes in soil moisture gradients. Similarly, Ngugi et al. (2003) showed that three different 7-month-old *E. clones* exhibited similar water use efficiency at high, medium, and low water availability. Also because the climate in our study area is warm and wet, and the soil water contents in the rooting zone remained sufficient along with the slopes, we further concluded that water availability is not a limiting factor for trees' growth and *WUE* in this study area. As suggested by Santini et al. (2016), trees with small conductive areas and hydraulic conductivity usually exhibited large *WUE* values. However, as stated, the *Eucalyptus* trees planted along with the hillside at the same site shared similar sapwood area. Also, the leaf water potential and the whole-tree hydraulic conductance were approximately constant regardless of the soil moisture gradients.

4.3 Implications

As pioneer tree species for plantation restoration in South China, the extensive and intensive planting of *Eucalyptus* and the short-term rotation have caused environmental effects and public concerns. In this study, we have provided evidence that the young *Eucalyptus* trees experienced a higher growth rate in *DBH* or *H* but relatively less water transpiration, leading to a higher water use efficiency. Differing from the young trees, the old trees maintained a relatively stable water consumption and water use efficiency under the seasonally changing environmental condition. This difference to some extent indicates the optimal utilization of current resources by young *Eucalyptus* and the good adaptation of old *Eucalyptus* to the fluctuating environment. Since the changes in the length and intensity of drought events (for instance, even more severe and prolonged drought) in the future could be more frequent and intensify the adverse environmental impact of the consecutive rotation of *Eucalyptus* plantation, our finding of relatively higher *WUE* values (less tree transpiration with high growth rates) in young and mature trees further suggests that the current planting practice of *Eucalyptus* in South China can best mitigate the threats to the replenishment of soil moisture.

5 Conclusion

We investigated the combined effect of stand age and soil moisture gradients on tree transpiration, growth rates, and *WUE* of *Eucalyptus* in South China. Young *Eucalyptus* trees possessed relatively less water transpiration but higher growth rates, consequently leading to a higher *WUE*. Attributed to the large sapwood area, water potential differences and hydraulic conductivity, the old trees utilized more water, while displayed lower growth rates and *WUE*. Along with the slopes, the young trees growing at the bottom of the slope possessed the lowest E_L, but no consistent effect of slope position on the trees' E_L was observed. We also did not find that the soil moisture gradients posed a significant influence on tree growth and *WUE*, and suggested that the warm and wet climate in the study area, as well as the sufficient soil water contents in the rooting zone, should be the key reasons for the similar tree growth and *WUE* along with the slopes. Moreover, the temporal changes of E_L among the three age series *Eucalyptus* trees suggested the young trees were more susceptible, while the old trees exhibited good adaptability to the environmental variability. To some extent, our results supported the rationality of *Eucalyptus* planting practices under current climate conditions in South China.

Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 Soil depths (cm, mean ± standard deviation) at different slope positions in the three experimental sites.

	Site A	Site B	Site C
Slope Top	86 ± 3.1	84 ± 3.6	119 ± 4.0
Mid-Slope	130 ± 2.5	119 ± 4.0	176 ± 3.6
Slope-Bottom	>250	>250	>250

site A: young trees; site B: mature trees; site C: old trees;

Slope Top: trees growing at the top of the sites, Mid-Slope: trees growing at the middle of the sites, and Slope Bottom: trees growing at the bottom of the sites.

Table 2 Soil physicochemical characteristics of three experimental sites.

Site	Soil texture	pH	SOM (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})
A	Clay loam	4.41 ± 0.10	22.05 ± 2.19	1.18 ± 0.13	0.22 ± 0.04
B	Clay loam	4.05 ± 0.13	37.40 ± 3.30	1.60 ± 0.07	0.26 ± 0.01
C	Clay loam	3.98 ± 0.08	31.95 ± 2.19	1.08 ± 0.18	0.26 ± 0.03

site A: young trees; site B: mature trees; site C: old trees; SOM: soil organic matter.

Table 3 Annual water use efficiency (*WUE*) of the sampled trees in different sites and slope positions.

	Site	Slope Position		
		Slope Top	Mid-Slope	Slope Bottom
Year 2018	A	10.2 ± 1.30[a]b[b]	9.37 ± 1.65b	8.63 ± 1.13a
	B	10.4 ± 0.77b	11.7 ± 1.37b	13.8 ± 3.63b
	C	6.29 ± 0.97a	5.46 ± 1.51a	7.90 ± 2.16a
Year 2019	A	10.6 ± 2.88b	10.9 ± 2.48b	12.1 ± 1.96b
	B	9.91 ± 1.81b	8.54 ± 1.07b	10.1 ± 1.79b
	C	8.13 ± 0.70a	6.84 ± 1.73a	6.71 ± 0.79a

Site A: young trees; Site B: mature trees; Site C: old trees; Slope Top: trees growing at the top of the sites, Mid-Slope: trees growing at the middle of the sites, and Slope Bottom: trees growing at the bottom of the sites;

[a] The values presented in the columns are mean ± standard deviation;

[b] Different small letters indicate differences among the three age sites (p <0.05);

Table 4 Two-way ANOVA (Tukey's HSD) on annual water use efficiency (*WUE*) and leaf area index (*LAI*) among different sites and years.

	Site		Year		Site × Year	
	F value	Sig.	F value	Sig.	F value	Sig.
Annual *WUE*	29.989	0.000	0.000	0.999	7.051	0.002
LAI	13.177	0.000	13.712	0.001	0.210	0.811

Table 5 Two-way ANOVA (Tukey's HSD) on predawn leaf water potential (Ψ_{pd}), midday leaf water potential (Ψ_{md}), leaf to soil water potential difference ($\Delta\Psi$, Ψ_{pd} - Ψ_{md}), and whole tree hydraulic conductance (K) among different sites and periods (including wet and dry season).

	Site		Season		Site × Season	
	F value	Sig.	F value	Sig.	F value	Sig.
Ψ_{pd}	2.218	0.115	38.980	0.000	15.491	0.000
Ψ_{md}	2.340	0.101	3.545	0.062	7.571	0.010
$\Delta\Psi$	0.040	0.961	7.457	0.007	2.917	0.047
K	16.042	0.000	3.342	0.071	5.105	0.008