Responses of forest carbon and water coupling to thinning treatments across multiple spatial scales

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Abstract

Background

Water-use efficiency (WUE) represents the coupling of forest carbon and water. Little is known about the responses of WUE to thinning at multiple spatial scales. The objective of this research was to use field measurements to understand short-term effects of two thinning treatments (T1: 4,500 stems ha$^{-1}$; and T2: 1,100 stems ha$^{-1}$) and the control (C: 27,000 stems ha$^{-1}$) on WUE (the ratio of leaf photosynthesis to leaf transpiration, or tree growth to tree transpiration, or net stand above-ground biomass (AGB) accumulation to stand transpiration) and the intrinsic WUE (WUEi, the ratio of leaf photosynthesis to stomatal conductance or the net stand AGB accumulation to canopy conductance) in a 16-year old natural lodgepole pine forest. Leaf-level measurements were conducted in 2017, while tree- and stand-level measurements were conducted in both 2016 (the normal year) and 2017 (the drought year).

Results

There was no significant effect of thinning on the tree- and stand-level WUE in 2016, while in 2017, only T2 exhibited significantly higher tree-level WUE (0.63 mm$^2$ kg$^{-1}$) than the C (0.06 mm$^2$ kg$^{-1}$), and the stand-level WUE values were significantly higher in the thinned stands, with the means of 0.34, 0.61 and 0.7 kg m$^{-3}$ for the control, T1 and T2, respectively. Stand-level WUEi was, however, significantly higher in the unthinned stands than in the thinned stands. In contrast, the leaf-level WUE values were not significantly different from each other, while the leaf-level WUEi was statistically higher in C than in the thinned stands. Thinning changed the WUE-VPD relationships at tree and stand levels, but not at the leaf level.
Conclusions

The effects of thinning on forest carbon and water coupling differed with the spatial scales and the metrics (WUE or WUEi) of water use efficiency. Lacking consistent responses of WUE metrics to thinning treatments across the spatial scales suggests that caution must be exercised when transferring and modeling WUE from one spatial scale to others. Both tree-level and stand-level WUE values in the more heavily thinning stands were significantly promoted under the drought condition, demonstrating that thinning can improve WUE and consequently support forests to cope with the drought effects.

**Keywords**: Water-use efficiency (WUE), thinning, *Pinus contorta*, gas exchange, upscaling, drought.
Background

Water-use efficiency (WUE), representing the coupling between carbon assimilation and water consumption of vegetation, is an important parameter in modelling responses of terrestrial carbon and water cycles to climate and land cover changes (Gentine et al., 2019; Liu et al., 2015; Tang et al., 2014; Zhang et al., 2018; Zhen et al., 2017). At the leaf level, water-use efficiency (leaf-level WUE) is calculated as the ratio of net photosynthetic assimilation to leaf transpiration. As exchanges of CO$_2$ and water vapor share the same diffusion pathway via stomata (Perez-Harguindeguy et al., 2013), intrinsic water-use efficiency (leaf-level WUEi, the ratio of net photosynthesis to stomatal conductance) is an alternative index for the leaf-level carbon and water coupling, which excludes influences from evaporative demand on leaf transpiration (Wieser et al., 2018). At the individual tree level, WUE (tree-level WUE) is expressed as the ratio of tree growth (e.g., basal area increments (BAI)) to the whole tree transpiration (Wullschleger et al., 1998). And at the ecosystem level, WUE can be quantified as the ratio of gross primary production to evapotranspiration or the ratio of net primary production to transpiration (e.g., Petritsch et al. (2007)). The responses of WUE at finner spatial levels (e.g., leaf and individual tree) provide valuable information for understanding and predicting behaviors of carbon and water processes of forest ecosystems at coarser spatial scales.

Holistic view on forest WUE across spatial scales is limited. Leaf-level studies are generally concentrated on the leaf-level WUEi that is detected from isotopic signatures of tree tissues, because when comparing with leaf-level WUE, the isotopic leaf-level WUEi only accounts for the ratio of intercellular and ambient CO$_2$ concentration, and can cover periods of low light, low temperature and dry conditions, while leaf-level WUE that is usually conducted by the gas exchange method, reflects optimal conditions of trees (near light saturation and optimal ranges of
temperature) due to limitations in conducting measurements in the field (e.g., during night) (Wieser et al., 2018). The leaf-level WUEi can also be measured from the gas exchange technique, and the discrepancy between WUEi of two different methods has been ascribed to the differences in the time scale (i.e., long term and short term) (Wieser et al., 2018) because the ratio of photosynthesis to stomatal conductance is positively linearly with the ratio of the leaf intercellular CO₂ concentration (Ci) to the atmospheric CO₂ concentration (Ca), and the Ci/Ca is also assumed to be linearly related to the carbon isotope discrimination by approximating the intercellular chloroplast concentration to Ci (Farquhar et al., 1989; Seibt et al., 2008). Some research have discovered that leaf-level WUEi tended to be a homeostatic trait (Comstock and Ehleringer, 1992; Fernández-de-Uña et al., 2016), while others found that leaf-level WUEi responded to changes in plant morphology (Brienen et al., 2017) and climatic controls (Adams et al., 2019). A review by Cernusak et al. (2013) suggested that environmental factors modified leaf-level WUEi, and internal physiology which varied with species, dampening its responses. However it remains unresolved the mechanism underlying the interactions between environmental and physiological determinants on leaf-level WUEi. On the other hand, leaf-level WUE is closer to the concept of net carbon and water exchange with atmosphere, especially in the context of climate change (Seibt et al., 2008). Besides, when scaling up from leaf to ecosystem levels there are more complications involved, including physical and physiological processes within the ecosystem, errors and uncertainties associated with measuring approaches and differences in the temporal and spatial resolutions, which lead to a lack of correspondence of WUE between different spatial levels (Guerrieri et al., 2016; Medrano et al., 2015). For instance, Guerrieri et al. (2016) found opposing patterns of WUE at leaf and ecosystem scales across a broader range of American forests. Ponton et al. (2006), however, showed a good agreement
between ecosystem WUE and WUEi by studying Douglas fir forest, aspen forest and grassland in Canada, and the differences in WUE among the three sites were most determined by variations in VPD. Contrast evidences imply cautions are needed in choosing the most representative indicators of carbon and water coupling and in the applicability of scaling from leaf to ecosystem levels.

Upscaling WUE from leaf to canopy levels requires knowledge of relationships between WUE and micrometeorological forcing. Ecosystems whose WUE exhibited good agreement across spatial scales, their WUE were fairly stable and mostly determined by VPD (Baldocchi et al., 1987; Lindroth and Cienciala, 1996; Morén et al., 2001; Ponton et al., 2006; Tang et al., 2006), which served as the basis for upscaling WUE from leaf to canopy levels. The relationship between WUE and VPD is also dependent on light intensity, because the stomata can react to decreases in photosynthesis under lower light intensity by closure, and thus lowering the transpiration processes (Bierhuizen and Slatyer, 1965; Linderson et al., 2012). Besides, water availability (e.g., soil water contents) can potentially influence the sensitivity of WUE to climatic variables through its effects on stomatal behaviors (Gimeno et al., 2016). Because of these WUE-micrometeorology relationships, it is generally expected that forest thinning treatments would affect the responses of WUE, because they affect the microclimate resulting in higher direct incident radiation reaching the ground, higher soil temperature, air temperature and wind speed, and lower air humidity within treated stands (Anderson et al., 2007; Bartos and Amman, 1989; Chen et al., 1999; Cutini and Mascia, 1996; Weng et al., 2007). To authors’ knowledge, no previous studies have examined the effects of thinning on microclimate sensitivity of WUE and WUEi except for the one reported that the isotopic WUEi became sensitive to annual precipitation after thinning (Fernandes et al., 2016). However, a limited number of studies
examined the effects of thinning on growth-climate and transpiration-climate relationships. For instance, Magruder et al. (2013) found that overstory thinning in red pine (*Pinus resinosa* Ait.) stands in Michigan at either the high or moderate thinning intensity, had the highest number of significant correlation coefficients between the radial growth and precipitation and temperature, whereas the unthinned had the lowest number of significant correlation coefficients. And enhanced sensitivities of tree transpiration to microclimatic controls under thinning treatments have also been observed in the beech (*Nothofagus antarctica*) forests in Argentina, maritime pine (*Pinus pinaster* Ait.) stands in Spain and cypress (*Chamaecyparis obtusa* and *Cryptomeria japonica*) stands in Japan (Gyenge et al., 2011; Jimenez et al., 2008; Tateishi et al., 2015). To date, few studies have been carried out on the effects of thinning on WUE at multiple spatial levels and under ambient conditions (Linderson et al., 2012; Tsamir et al., 2019). Modelling the forest carbon dynamics by using WUE relies on the established functions between WUE and VPD (Zhou et al., 2014), and thus, it is important to know if thinning changes the relationship between WUE and microclimate.

In the separate prior study in the young lodgepole pine forests in the interior of British Columbia, Canada, we examined the effects of juvenile thinning on tree-level radial growth, sap flow velocity and stand transpiration under drought and non-drought years, and found that significant differences of tree growth and sap flow velocity between light and heavy thinning treatments only occurred in the drought year, with the heavier thinning having a higher sensitivity of sap flow velocity to VPD (Wang et al., 2019). Building on this prior study, we further assess in this work the effects of juvenile thinning on WUE across the multiple spatial scales during the growing seasons of 2016 and 2017. The drought occurred in 2017 provided an excellent opportunity of evaluating the responses of WUE to thinning treatments under the drought
condition. In this study, we hypothesized: (1) the juvenile thinning would significantly affect leaf-level WUE and WUEi, tree-level WUE and stand-level WUE and WUEi; (2) there were consistent responses of WUE and WUEi to thinning treatments across various spatial scales; and (3) the thinning would alter the sensitivity of WUE to microclimatic variables at all three spatial scales.

Methods

Study area and experiment design

The study was conducted in an even-aged 16 years old lodgepole pine (*Pinus contorta* Dougl.) forest in the Upper Penticton Watershed (UPW) in the southern interior of British Columbia, Canada (49°39′34″N, 119°24′34″W). The site is around 1675 m a.s.l, generally south aspect, and have sparse understory vegetation. Soils were derived from granite parent rock, coarse sandy-loam in texture, with low water holding capacities, and were classified as Luvisol. Snow cover lasts from early November through middle of June. In June 2016, two thinning treatments (Treatment 1 (T1): 4,500 stems per ha; Treatment 2 (T2): 1,100 stems per ha) and one unthinned control (C: 27,000 stems per ha) were randomly assigned to three plots (20 m × 20 m each) of each block. There are 3 blocks, B1, B2 and B3 (25 m × 75 m each) and each block has 3 plots.

The leaf-level measurements were carried out in the growing season of 2017 (June to October) while the tree-level measurements were recorded since June 2016. The year of 2017 has been classified as a drought year based on the calculation of Standardized Precipitation Index (SPI). The mean daily temperature during the growing season of 2017 is 12.1°C, and the total growing
season precipitation is 37.4 mm. Detailed information on the study site and the experimental
design can be found from Wang et al. (2019).

Leaf-level measurements

Leaf-level WUE (μmol mmol⁻¹) was calculated as the ratio of leaf photosynthesis rate (μmol CO₂
m⁻² s⁻¹) to leaf transpiration rate (mmol H₂O m⁻² s⁻¹). Leaf-level WUEi (μmol mol⁻¹) was
calculated as the ratio of leaf photosynthesis rate (μmol CO₂ m⁻² s⁻¹) to stomatal conductance
(mol H₂O m⁻² s⁻¹). Leaf-level WUEi approximately equals to the product of leaf-level WUE and
the leaf to air vapour pressure deficit (i.e., leaf-level WUEi = leaf-level WUE × VPD, or
alternatively, leaf-level WUE = leaf-level WUEi/VPD), because the ratio of leaf transpiration to
VPD corresponds approximately to the conductance of water vapor via stomata (i.e., stomatal
conductance (gₛ)) (Gorthi et al., 2019; Linderson et al., 2012; Seibt et al., 2008; Wieser et al.,
2018).

Leaf-level WUE = \frac{leaf\ photosynthesis}{leaf\ transpiration} \tag{1}

Leaf-level WUEi = \frac{leaf\ photosynthesis}{stomatal\ conductance} \tag{2}

All the variables were measured by the instantaneous photosynthesis measurement system
(Model LI-6400XT, Licor, Lincoln, NE, USA) with an opaque conifer chamber (Model 6400-22)
and an external RGB light source (6400-18A). The measurements took place between 10:00 am
and 14:00 pm from June, 23rd, 2017, and were conducted approximately once per week in the
three blocks until the end of the growing season (October 2017). When the measurement was
conducted each time, five trees in each plot were selected, and for each tree, four bunches of needles orienting north, south, east and west at the similar location of tree canopy (around 1.5-1.7 m height) were measured for three times. In order to mimic the natural surrounding environment of measured leaves when conducting the chamber measurement, the temperature was set as the ambient temperature, the light conditions were set to reflect the ambient light levels, and the leaf chamber was sealed with gum in case of gas leaking. Flow rate was set at 500 μmol/s to minimize the modifications on the environmental variables by the equipment (Medrano et al., 2010).

Tree-level measurements

Tree-level WUE (mm² kg⁻¹) is calculated as the ratio of basal area increment (BAI, mm² d⁻¹) to tree transpiration (T, kg d⁻¹).

\[
\text{Tree-level WUE} = \frac{BAI}{T}
\]

(3)

Tree transpiration was calculated based on the sap flow of 5 trees per plot in B1 (namely sap flow trees). Sap flow was continuously measured by the Granier-type sap flow probes (Model TDP-30, Dynamax, Inc., Texas, USA) at the breast height (15 trees in total). The detailed descriptions on DBH and sap flow measurement and their related quality control can be found in Wang et al. (2019). BAI were calculated based on the diameter at the breast height (DBH) measured on the five sap flow trees per plot in B1 by an electronic caliper (Model:500-196-30, Mitutoyo Corporation, Japan) at the beginning and the end of each growing season of 2016 and 2017. Besides, DBH of 45 trees per plot across the three blocks were measured monthly by the
same electronic caliper in the two growing season.

Stand-level estimations

Stand-level WUE (kg m\(^{-3}\)) is calculated as the ratio of the stand net primary production (NPP, kg m\(^{-2}\) d\(^{-1}\)) to stand transpiration (T\(_{\text{stand}}\), mm d\(^{-1}\)). And stand-level intrinsic WUE (stand-level WUE\(_i\), kg m\(^{-3}\)) is calculated as the ratio of NPP to the canopy conductance (Gs, mm d\(^{-1}\)).

\[
\text{Stand-level WUE} = \frac{\text{NPP}}{\text{T}_{\text{stand}}} \quad (4)
\]

\[
\text{Stand-level WUE}_i = \frac{\text{NPP}}{\text{Gs}} \quad (5)
\]

Given that the studied stand is even-aged and mono-species forest with sparse understory, NPP is estimated by changes in the stand above-ground biomass (AGB\(_{\text{stand}}\), g) in each growing season, and T\(_{\text{stand}}\) (mm d\(^{-1}\)) is estimated from the tree transpiration, stand density and DBH distribution, which has been reported in our previous study (Wang et al., 2019). Tree AGB (AGB\(_{\text{tree}}\)) is estimated by the tree allometric equations based on 24-year-old lodgepole pine trees from a range of stand densities across the Yellowstone subalpine plateaus (Copenhaver and Tinker, 2014).

\[
\text{AGB}_{\text{tree}} (g) = 98.85 \times \text{basal diameter (cm)}^{1.99} \quad (6)
\]

\[
\text{AGB}_{\text{stand}} = \text{mean AGB}_{\text{tree}} \times \text{stand density} \quad (7)
\]

\[
\text{NPP} = \Delta \text{AGB}_{\text{stand}} \quad (8)
\]

Paired measurements of tree DBH and basal diameter from 180 trees across all three blocks were used to build a linear relationship between basal diameter and tree DBH, which has been also reported in our previous study (Wang et al., 2019).
Gs was used as a proxy to indicate the stomatal responses at the canopy level. It was calculated by the simplified inversion of Penman-Monteith equation by assuming that the VPD is close to the leaf to air vapor pressure deficit with no vertical gradient through canopy, and negligible water storage above the measurement point where sap flow probes were inserted (Ewers and Oren, 2000). This method have been applied in lodgepole pine forests (Reid et al., 2006). The conditions of our young lodgepole pine forests that have low canopy height (< 2.5m) and relatively open canopy (canopy closure <55%) generally satisfy the assumptions of the equation.

\[
Gs = \frac{\gamma E_{LA}}{\rho_a c_a VPD} \tag{9}
\]

Where, \(\gamma\) is the psychrometric constant (0.067 kPa K\(^{-1}\)); \(\lambda\) is the latent heat of vaporization calculated by Harrison’s equation, \(\lambda = 2.501 - 2.361 \times 10^{-3} T_a\) (Harrison, 1963); \(E_{LA}\) is the transpiration per leaf area, \(E/\text{LA (mms}^{-1}\)); \(\rho_a\) is air density (1.225 kg m\(^{-3}\)); \(c_a\) is the specific heat of air (1.0 \times 10^{-3} \text{MJ kg}^{-1} \text{K}^{-1}); \) VPD is vapor pressure deficit (kPa). All VPD data used in the equation are greater than 0.6 kPa to minimize the relative errors (<10%) (Ewers and Oren, 2000).

Monthly leaf area (LA, m\(^2\)) was also estimated from the tree allometric equations from Copenhaver and Tinker (2014).

\[
\text{LA (in m}^2\text{)} = 0.02 \times \text{basal diameter(in cm)}^{2.34} \tag{10}
\]

Leaf area index (LAI) was estimated by dividing LA by the projection coefficient (2.5) (Litton et al., 2004). The estimated mean monthly LAI in 2016 for C (0.99) matches relatively well with the field measurement (0.97).

Collection of climate data
Climatic variables including solar radiation (Rn, W m$^{-2}$), air relative humidity (RH, %), temperature (T, °C), precipitation (P, mm) and wind velocity (Wv, m s$^{-1}$) were continuously measured in each treatment in B1 by climate station (HOBO weather station, Onset Computer, Bourne MA, USA). The sensors were placed at the canopy level (approximately 2.5 m). VPD is calculated based on Goff–Gratch equation (Goff, 1946). Microclimate variables including leaf temperature, leaf VPD and incoming photosynthetically active radiation (PAR, µmol (photons) m$^{-2}$ s$^{-1}$), at the height of approximately 1.5 - 1.7 m, across three blocks (9 plots) at the mid-day on a weekly basis during the growing season, were recorded by the instantaneous photosynthesis measurement system (Model LI-6400XT, Licor, Lincoln, NE, USA). Soil volumetric water content (VWC) at two depth (20 and 40 cm) in three randomly selected locations per plot in B1 was measured by EC-5 sensors (Decagon, Pullman, WA, USA) at the interval of 20 minutes. Soil VWC at the 20 cm depth was also manually measured in the three blocks weekly by GS-1 portable measuring system (Decagon, Pullman, WA, USA).

Statistical analysis

Leaf-level WUE and WUEi were analysed by multi-factor AVOVA to investigate the effects of branch aspect, thinning, date and their interactions. Tree-level WUE was analyzed by ANCOVA with the initial DBH of the trees as covariate, and the thinning treatment and year (drought) as factors. Stand-level WUE was log-transformed before the two-way ANOVA analysis (Table S4), and stand-level WUEi was only analyzed in 2017 due to the requirement of the canopy conductance model on VPD (which should be greater than 0.6kPa). Model residuals were checked to meet the requirements of normality and homoscedasticity of variance (Figure S1-S2, Table S2-S4). Besides, monthly tree-level WUE, stand-level WUE and WUEi were also
calculated in order to examine their responses to microclimatic factors. However, the DBH
measurements in the September of 2016 and the August of 2017 were systematically greater than
the following months due to the misoperation, were thus excluded from analyses. Besides,
monthly stand-level WUEi were only available in the August of 2016 and the July and the
August of 2017 because their VPD conditions were allowed to apply the canopy conductance
model. Comparisons between two groups were performed with independent-t test, if data met the
requirements of homogeneity of variance and normality, otherwise, were tested by the Mann-
Whitney U test. Correlation was tested by Spearman test. A significance level of p ≤ 0.05 was
used for all analyses. All data were processed by R (R Core Team (2014) and SPSS for Windows
(SPSS, Inc., USA).

Results

Effects of thinning on leaf-level WUE and WUEi

Thinning did not significantly affect leaf-level WUE (p=0.47, Table S1), with mean leaf-level
WUE values for C, T1 and T2 being 5.50±3.04, 4.99±3.40 and 5.31±2.96 μmol mmol⁻¹,
respectively. However, thinning did significantly influence leaf-level WUEi (p < 0.001, Table
S1), with the averaged leaf-level WUEi for C, T1 and T2 being 93.14±55.55, 57.24±47.23 and
62.03±45.61 μmol mol⁻¹, respectively. Leaf-level WUEi in C was statistically higher than in T1
and T2 (both p < 0.001), while T1 and T2 did not significantly differ from each other (p=0.66).
(Figure 1, Table S1).
Figure 1. Leaf-level WUE (in left) and WUEi (in right) during the growing season of 2017.

Effects of thinning on tree-level WUE

The ANCOVA test showed that thinning significantly affected tree-level WUE (p = 0.033, Table S2). The mean tree-level WUE for C, T1 and T2 in 2016 were 0.14±1.01, 1.07±1.85 and 1.27±1.42 mm² kg⁻¹, respectively, and they were not significantly different from each other (p =0.45). The values were reduced to 0.06±0.30, 0.38±0.16 and 0.63±0.35 mm² kg⁻¹ in 2017, respectively, and only tree-level WUE between C and T2 was statistically different (p=0.009). Thus, the heavier thinning significantly improved tree-level WUE in the drought year.

However, the ANCOVA test showed that the drought did not significantly affect tree-level WUE (p=0.4, Table S2). And for each of the three groups, their tree-level WUE also did not statistically differ between years (all p>0.1). These were probably due to the large variances in the tree-level WUE of T1 and T2 in the non-drought year (Figure 2). In addition, there was no significant interaction effect between year (drought) and thinning (p=0.84, Table S2).
Figure 2. Tree-level WUE during the growing season of 2016 (in white) and 2017 (in gray).

Effects of thinning on the stand-level WUE and WUEi

The ANOVA test showed that the thinning did not have significant impacts on the stand-level WUE (p = 0.27, Table S4). In 2016, stand-level WUE was 1.57±0.347, 1.73±0.245, and 1.39±0.245 kg m\(^{-3}\) for C, T1 and T2, respectively, and there was not any significant differences among them (p = 0.89). In 2017, stand-level WUE was reduced to 0.339±0.20, 0.61±0.20, and 0.75±0.20 kg m\(^{-3}\) for C, T1, and T2, respectively, and C was significantly lower than T1 (p = 0.03) and than T2 (p = 0.005), suggesting the positive thinning effects only occurred in the drought year. However, there was no statistical difference between T1 and T2 (p = 0.18).

The drought significantly reduced stand-level WUE (p < 0.001, Table S4), while the interaction between thinning and drought did not play a significant role (both p > 0.1) (Table S4, Figure 3).

Stand-level WUEi in 2017 was 0.36±0.06, 0.13±0.02, and 0.12±0.04 kg m\(^{-3}\) for C, T1 and T2 respectively. C was statistically higher than T1 and T2 (both p =0.001), and there was no
significant difference between T1 and T2 ($p = 0.77$) (Figure 3).

![Stand-level WUE and WUEi in 2016 and 2017](image)

Figure 3. Stand-level WUE in 2016 and 2017, and stand-level WUEi in 2017.

(The upper panel showed stand-level WUE of 2016 in white, and that of 2017 in gray. The bottom panel showed the stand-level WUEi in 2017.)

Responses of leaf level WUE and WUEi to VPD and PAR

Leaf-level WUE did not significantly correlate with VPD ($p = 0.49$), while leaf-level WUEi
exhibited strongly positive relationship with VPD (spearman rho= 0.629, p<0.001). The responses of leaf-level WUEi to VPD did not apparently differ among C, T1 and T2, and were fit best with exponential equation (R$^2$ = 0.45) (Figure 4). However, both the leaf-level WUE and WUEi were significantly correlated with PAR, with correlation coefficients of 0.47 and 0.23, respectively (both p< 0.001). But there were no any significant differences in the responses of leaf-level WUE and WUEi to PAR among C, T1, and T2 (Figure S3-S4). The responses of leaf-level WUEi to VPD were lower when PAR was less than 500 μmol/m$^2$s (Figure 4). But when PAR was greater than 500 μmol/m$^2$s, increasing PAR did not obviously enhance the responses of leaf-WUEi to VPD (Figure 4).
Figure 4. Leaf-level WUEi versus VPD for C, T1 and T2, and for the four PAR groups.

(The upper panel showed the leaf-level WUEi versus VPD for C, T1, and T2 with a fitted exponential equation (leaf-level WUEi = 47.894×VPD^{1.2356} (R^2=0.45). The bottom panel showed the leaf-level WUEi versus VPD for the four PAR groups (unit: μmol/m²s) with fitted exponential equations (when PAR < 500: leaf-level WUEi = 6.1266e^{1.2208×VPD} (R^2=0.43) and when PAR >= 500: leaf-level WUEi = 17.825e^{1.0788×VPD} (R^2=0.47)).

Responses of tree-level WUE to VPD and transmitted solar radiation

Tree-level WUE was significantly correlated with VPD (rho=-0.63, p=0.02). The response of tree-level WUE to VPD in C was slightly lower than those of T1 and T2, and there was no apparent difference between T1 and T2 (Figure 5). At each treatment group, however, there were no significant correlations with VPD in C (rho = -0.9, p = 0.083), T1 (rho = -0.8, p = 0.13), and T2 (rho = -0.5, p = 1), probably due to the limited sampling data. There were also no any significant correlations between tree-level WUE and transmitted solar radiation either when
pooling the three groups together (p=0.86) or analysing the group individually (all p>0.1), but transmitted solar radiation influenced the responses of tree-level WUE to VPD (Figure 5).

![Graph showing tree-level WUE versus VPD for C, T1 and T2, and for the two transmitted solar radiation groups.](image)

Figure 5. Tree-level WUE versus VPD for C, T1 and T2, and for the two transmitted solar radiation groups.

(The upper panel showed the tree-level WUE versus VPD with fitted exponential equations for C (tree-level WUE = 7.3514e^{-4.992xVPD} (R^2=0.79)), and for T1 and T2 (tree-level WUE = 0.143e^{-3.336xVPD} (R^2 =
The bottom panel showed the tree-level WUE versus VPD for the two transmitted solar radiation groups (unit: W/m²) with fitted exponential equations (when the transmitted solar radiation < 90: tree-level WUE = $6.3938e^{-4.818eV_{PD}} (R^2=0.81)$ and when the transmitted solar radiation >= 90: tree-level WUE = $3.3011e^{-1.405eV_{PD}} (R^2=0.34)$).

Responses of the stand-level WUE and WUEi to VPD and transmitted solar radiation

The stand-level WUE was statistically correlated with VPD (rho=-0.51, p = 0.03, Figure 9) and transmitted solar radiation (rho=-0.72, p = 0.002) when C, T1 and T2 were analyzed together, but when the three groups were analyzed individually, only C exhibited a significant correlation between the stand-level WUE and VPD (rho=-0.94, p = 0.02), and no significant correlations with transmitted solar radiation were found in any of the three groups. Due to data deficiency, stand-level WUEi was only analyzed by pulling the three groups together, and it was only significantly correlated with transmitted solar radiation (rho=-0.95, p = 0.001, Figure 6).

![Graph showing stand-level WUE vs VPD]
Figure 6. Stand-level WUE versus VPD, and stand-level WUEi versus transmitted solar radiation for C, T1 and T2.

(The upper panel showed the stand-level WUE versus VPD with a fitted exponential equation for C (stand-level WUE = 3.0195e^{-1.368xVPD} (R^2=0.61). The bottom panel showed the stand-level WUEi versus transmitted solar radiation with a fitted exponential equation (stand-level WUEi = 8.5971e^{-0.025xVPD} (R^2=0.86).)

Discussion

The effects of thinning on WUE across the spatial scales

Contrast to our hypothesis, the leaf-level WUE values were not significantly different among C, T1 and T2, while the leaf-level WUEi was statistically higher in C than in the thinned stands, and there were no significant differences between the two thinning intensities. Our result on the leaf-level WUEi is in accordance with some studies showing that thinning had no effects on WUEi
based on the isotope method for Scots pine (*Pinus Sylvestris*) and maritime pine (*Pinus pinaster*) (Navarro-Cerrillo et al., 2016), Aleppo pine (*Pinus halepensis* Mill.) (Fernandes et al., 2016), and black pine (*Pinus nigra* Arn.) (Martin-Benito et al., 2010) stands in Mediterranean forests. But our result is in contrast to the studies which were conducted by the leaf gas-exchange measurements in young paper birch (*Betula papyrifera* Marsh.) stands (Wang et al., 1995) and saplings of oak (*Quercus cerris* L.) and ash (*Fraxinus ornus* L.) forests (D’Alessandro et al., 2006). They attributed the increased leaf-level WUEi to increased PAR induced by their thinning treatments (D’Alessandro et al., 2006; Wang et al., 1995).

In our study, VPD, instead of PAR, might explain the discrepancy between the responses of leaf-level WUE and WUEi, and the significant higher leaf-level WUEi in C. It is because that leaf-level WUEi approximates the product of leaf-level WUE and VPD (Linderson et al., 2012; Wieser et al., 2018), and VPD during the leaf measurement periods was statistically higher in C than in T1 and T2 (both p <0.001) with no significant differences between T1 and T2 (p=0.29, Figure S5). Besides, in comparison with temperature and PAR, VPD is the only microclimate variable that exhibited significant differences between the control and the thinned stands during the leaf measurement periods (Figure S5). As VPD is determined by temperature and RH, and RH is influenced by soil evaporation and plant transpiration (Arx et al., 2013; Danehy and Kirpes, 2000; Fischer et al., 2007), higher VPD in C can be attributed to a lower soil water content and a higher stand transpiration in C, both of which were shown in our previous study at the same site (Wang et al., 2019). Besides, VPD indicates the atmospheric drought (Burkhardt and Pariyar, 2016), and increased WUEi were commonly observed under drought conditions (Andrés et al., 2018; Fernandes et al., 2016; Linares and Camarero, 2012). Therefore, higher leaf-level WUEi in C suggests that the unthinned stand experienced severer water stress than the
thinned stands.

However, VPD could either increase (Arx et al., 2013; Guenther et al., 2012; Weng et al., 2007), or remain unchanged (Chan et al., 2004; Meyer et al., 2001; Monteith et al., 1991; Porté et al., 2004) with increasing thinning intensity. Therefore, though microclimates play important roles in determining leaf-level WUEi, their effects may be site specific. We also acknowledge that the field measurements in our study were conducted from 10 am to 14 pm, and consequently, the results may not fully represent the effects of thinning on mean daily leaf-level WUE and WUEi at the 24-hour basis. Such a shortcoming could be addressed by the isotopic method, which will be considered in our future study.

At the tree level, we found significant and positive impacts of thinning on tree-level WUE (p =0.03). Our result agrees with the studies showing that thinning increased tree-level WUE in Aleppo pine (Pinus halepensis Mill.), Silvertop (Eucalyptus nitens) (Deane and Maiden) and Norway spruce (Picea abies [L.] Karst.) forests (Fernandes et al., 2016; Forrester et al., 2012; Gebhardt et al., 2014), by observing the enhanced tree transpiration accompanied with a higher increase of tree growth. Besides, Park et al. (2018) found that the significant difference of tree-level WUE between the heavy thinning and the control stands occurred only in high growth years in a 50-year-old Korean pine (Pinus koraiensis) stands. The enhancement of tree-level WUE was mainly attributed to a lower water stress under a more intense thinning treatment (Cabon et al., 2018; del Campo et al., 2014; Gebhardt et al., 2014; Park et al., 2018). Therefore, the effects of thinning on tree-level WUE depend on how thinning releases tree competition for resources (e.g., water, light and nutrients) as indicated by Fernández-de-Uña et al. (2016), and thus they can be more pronounced in the drought year, as observed by Park et al. (2018). This has been confirmed by our study that increasing tree-level WUE accompanied with thinning intensity was more
obvious in the drought year (2017) than in the normal year (2016), and the magnitude of tree-level WUE was generally higher in the normal year (Figure 2).

The discrepancy of WUE between the tree and leaf levels in 2017 may be attributed to the respiratory processes (Medrano et al., 2015), the tree carbon allocation pattern under droughts (Pretzsch et al., 2012), and the night transpiration (Escalona et al., 2013; Medrano et al., 2015). Stem respiration, however, was greater in the thinned stands (Lavigne, 1988), and thus is unlikely to contribute to possible explanation of our observation. Under water stress, lodgepole pine tends to allocate more biomass to their root system to improve water acquisition (Pretzsch et al., 2012), which may serve as a potential explanation. Besides, in order to infer the effect of the night transpiration, we calculated the percentage of the tree daily nocturnal sap flow (from 1 h after sunset to 1 h before dawn according to the local time (Medrano et al., 2015)) to the tree daily total sap flow, for the night sap flow is usually partitioned into the night transpiration and the stem refilling (Forster, 2014). We found that tree daily night sap flow accounted for 29.7±26.1%, 11.9±14.1% and 6.5±11.9% of the total tree daily sap flow in 2017 for C, T1 and T2, respectively, and they were significantly different from each other (all p <0.001). If the stomata was open during night for tree transpiration in our study site during the experiment period, it was possible that the trees in C had the highest daily non-productive water consumption, while those in T2 had the lowest, contributing to a significantly low tree-level WUE in C but high tree-level WUE in T2. The fact that nocturnal transpiration reduced whole-plant WUE and consequently caused lack of correspondences of WUE between leaf and whole-plant scales have been well documented (Escalona et al., 2013; Medrano et al., 2015), in which the higher tree night transpiration corresponded to higher atmospheric water deficits and soil water contents mainly
through their effects on stomata (Dawson et al., 2007; Kavanagh et al., 2007). But in our study, the night-time VPD was generally highest in T2, followed by C, and then T1 (Figure S5), and soil water contents were sustainably higher in the thinned stands (Wang et al., 2019), so that VPD and soil water contents alone cannot explain the patterns of nocturnal tree sap flow found in our study. However, under drought condition, stomata conductance can be unrelated to VPD and soil water contents, which leads to a great amount of unavoidable water loss through epidermis (e.g., 6-8% of daily transpiration under well-watered condition in comparison with 19-20% of daily transpiration under droughts) (Cavender-Bares et al., 2007). This may be the explanation for our study. Nevertheless, our further study will examine the mechanisms behind the phenomenon in more details.

The responses of the stand-level WUE to the thinning treatments were slightly different from the tree-level WUE in this study. The lack of significance of the overall thinning effects on the stand-level WUE was probably due to the large variations of the stand-level WUE in 2016. Except for that, the stand-level WUE exhibited good correspondences with the tree-level WUE. As expected, the unthinned stand had the least tree growth but the highest stand transpiration during the two growing seasons (Wang et al., 2019), and the difference between the unthinned and thinend stands was more pronounced in the drought year. Besides, our result on the stand-level WUE is within the range of the AGB-based WUE (Forrester et al., 2012), and agrees well with the studies reporting that thinning increased stand-level WUE (Forrester et al., 2012) and droughts decreased WUE (ecosystem WUE or GPP/ET) (Gao et al., 2017). In fact, stand-level or ecosystem WUE can either increase (Sinacore et al., 2019), remain unchanged (Sinacore et al., 2019) or even decrease (Gao et al., 2017) under drought conditions, which is subject to various factors including forest characteristics (e.g., mixed or monospecific), tree species and
environmental conditions. The reduced stand-level WUE in the drought year in our study is attributed to the fact that the decrease in stand transpiration accompanied with greater reduction in net accumulation of the stand aboveground biomass under the drought.

Surprisingly, the stand-level WUEi was significantly higher in C than in the thinned stands with no significant difference between T1 and T2. The net accumulation of the stand above-ground biomass was significantly higher in C than T1 (p = 0.004) and T2 (p = 0.001), and there was no significant difference between T1 and T2 (p = 0.11). Canopy conductances, however, were not significantly different among the three groups (all p > 0.1, except for the comparison between T1 and T2 (p = 0.053)). Therefore, the pattern of the stand-level WUEi in our study was driven by the net stand above-ground biomass accumulation during the growing season. This indicates that the higher stand density in C compensated for the decreased individual tree growth, leading to a higher net stand above-ground biomass accumulation in the unthinned stands than the thinned ones. Although T2 had the highest individual tree growth, the low density in T2 counterbalanced the improved individual tree growth under the thinning treatment.

Responses of WUE and WUEi to microclimate under the thinning treatments and the implications for upscaling and modelling

Thinning changed the responses of WUE to microclimate at the tree and stand levels, but to our surprise, thinning did not change the sensitivity of the leaf-level WUE to PAR, and leaf-level WUEi to VPD and PAR. And the relationship between the leaf-level WUE with VPD fits the exponential increasing function (Figure 4), which is different from the exponential decay function established by Linderson et al. (2012) for beech (Fagus sylvatica L.) forests. The differences may be attributed to the differences between the studied conifer and deciduous species,
because we found that the exponential decay relations between WUE and VPD were mostly reported from the studies on deciduous species e.g., oak-hickory *(Quercus and Carya* spp.) (Baldocchi et al., 1987), sugar maple *(Acer saccharum)*, hemlock *(Tsuga canadensis)*, yellow birch *(Betula alleghaniensis)*, basswood *(Tilia americana)*, and American hophornbeam *(Ostrya virginiana)* (Tang et al., 2006), and basket willow *(Salix viminalis* L.) (Lindroth and Cienciala, 1996), except for the Norway spruce *(Picea abies* L. Karst) (Morén et al., 2001). It may also be due to the impacts of the drought in 2017: under the water stress, increasing VPD may result in greater reduction in stomatal conductance than photosynthesis, leading to a higher WUEi (Linares and Camarero, 2012).

The discrepancy between the leaf-level WUE and WUEi to microclimatic factors in this study was due to the distinct sensitivity of leaf transpiration and stomatal conductance, as leaf transpiration is regulated corroborately by stomatal conductance and boundary layer conductance to water vapor (Defraeye et al., 2014), the former of which depends on the density, size and degree of opening of stomata, while the latter is determined by the air movement and leaf morphology (Martin et al., 1999). The different responses of the leaf-level WUE and WUEi to VPD suggest that caution must be taken in selecting a proper WUE metric for upscaling from leaf to stand levels.

Similar with the leaf-level WUEi, the tree-level WUE was significantly correlated with VPD, and their relationship was modulated by the transmitted solar radiation (Figures 6 and 7). Such relationship between the tree-level WUE and VPD fits the exponential decay function, which is in agreement with the previous study (Lindroth and Cienciala, 1996). Although there was no significant correlation between tree-level WUE and leaf-level WUE and WUEi (both p >0.1) probably due to limited sampling data, our study agrees with the research reporting that tree-
level WUE was primarily a function of VPD (Table 1) (Bierhuizen and Slatyer, 1965; Lindroth and Cienciala, 1996; Park et al., 2018).

Thinning changed the responses of tree-level WUE to VPD, resulting in the apparent lower tree-level WUE in C than in T1 and T2 at each VPD level (Figure 5). It is apparent in the Figure 5 that the lower responses of the tree-level WUE to VPD in C was because C generally had a lower transmitted solar radiation, and thus lower responses of the tree-level WUE to VPD.

At the stand level, the response of the stand-level WUE to VPD was similar to the tree-level response, exhibiting the exponential decay relationship in this study. This has also been described by Lindroth and Cienciala (1996) and Kuglitsch et al. (2008). However, thinning seemed to affect the response of the stand-level WUE to VPD, although the correlation was only significant in C in our study. Besides, both the stand-level WUE and WUEi were significantly correlated with transmitted solar radiation, which was probably because the stand-level WUE and WUEi were primarily driven by the net above-ground biomass accumulation as discussed in the previous section, and the net above-ground biomass accumulation is dependent on the light availability (Jarčuška and Barna, 2011). Besides, we did not find any significant relationship between the stand-level WUE and WUEi and the tree-level WUE (both p>0.2). Therefore, whether the stand-level WUE can be predicted from the tree-level WUE requires further study.

Table 1. Correlation coefficient between WUE with VPD, light intensity and soil water content.

| WUE across spatial scales | VPD | Light intensity (PAR or Transmitted solar radiation) | Soil water content at 20 cm | Soil water content at 40 cm |
|---------------------------|-----|-----------------------------------------------------|-----------------------------|-----------------------------|
| Leaf-level WUE           | -0.03 | 0.47***                                               | -0.25                       | -0.37**                     |
|                  | Leaf-level WUEi | 0.63*** | 0.23*** | -0.47*** | -0.55*** |
|------------------|-----------------|---------|---------|----------|----------|
| Tree-level       |                 |         |         |          |          |
| Tree-level WUE   | -0.63**         | 0.05    | 0.11    | 0        |
| Stand-level      |                 |         |         |          |          |
| Stand-level WUE  | -0.51**         | -0.72***| -0.16   | -0.27    |
| Stand-level WUEi | -0.37           | -0.95***| -0.24   | -0.48    |

Light intensity includes PAR at the leaf level and transmitted solar radiation at tree and stand levels. Star indicates the significant level at 0.01***, 0.05**, and 0.1*;

Last but not least, although thinning did not affect the sensitivity of the leaf-level WUE and WUEi, the changes in microclimate resulting from thinning can still lead to the differences in WUE between the control and thinned stands (e.g., leaf-level WUEi and tree-level WUE). Clearly, microclimate is critical in evaluating the effects of thinning from the perspective of carbon and water coupling. It also plays an important role in other ecological functions of forests, such as seed germination (Dunlap and Helms, 1983; van Mantgem et al., 2006; Zald et al., 2008), species diversity (Dodson et al., 2008; Godefroid et al., 2006), soil nutrient cycling (Concilio et al., 2005; Gray et al., 2002; Moghaddas et al., 2008; Zhu et al., 2007), microhabitats for insects and animal (Chan et al., 2004; Chen et al., 1999; Meyer et al., 2001; Rambo and North, 2009) as well as reduction of wildfire (Bigelow and North, 2004; Whitehead et al., 2006) and mountain pine beetle attacks (Amman and Logan, 1998; Bartos and Amman, 1989). Previous research suggested that the changes of microclimate under forest management are predictable, as microclimate is closely related to vegetation structure, elevation and microtopography (Arx et al., 2013; Frey et al., 2016; Hardwick et al., 2015; Ma et al., 2010). It is very important for forest
silvicultural practices to create suitable microclimate conditions to improve forest ecological services. Our study provides the evidence of the short-term effects of thinning on WUE from leaf to stand levels. As juvenile thinning generates progressive crown and rooting system development and fast growing in understory vegetation (Aussenac, 2000; Bigelow and North, 2004; Chan et al., 2004; Elkin et al., 2015; Sohn et al., 2013; Trentini et al., 2017), their effects are likely dynamic, and their long-term implications requires continuous investigation and monitoring.

Conclusions

Our study provided direct field evidences on the responses of WUE and WUEi to juvenile thinning treatments between the non-drought and drought conditions at the multiple spatial scales. We found that: (1) WUE and WUEi responded differently to the thinning treatments at the same spatial level as well as across the different spatial scales; (2) thinning affected the sensitivites of WUE to VPD at the tree and stand levels, but not the at the leaf level; (3) only under the drought condition, thinning significantly improved the tree- and stand-level WUE, demonstrating that thinning can promote forest resilience to the drought effects; and (4) microclimate played an important role in forest carbon and water coupling.

Declarations

Availability of data and materials
The data supporting this research are included within the article and its additional files. Additional data are available upon request to corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Y. Wang, X. Wei and A. del Campo designed the study; Y. Wang and X. Wei led the field data collection. Y. Wang did the data analyses; Y. Wang, X. Wei and A. del Campo led the writing of the manuscript. All authors contributed to the drafts and gave final approval for publication.

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