SCIENTIFIC REP🅞RTS

OPEN

Dynamic spatial patterns of leaf traits affect total respiration on the crown scale

Xiaolin Wang, Hongxuan Zhou, Fengsen Han, Yuanzheng Li & Dan Hu

Temporal and spatial variations of leaf traits caused conflicting conclusions and great estimating errors of total carbon budget on crown scales. However, there is no effective method to quantitatively describe and study heterogeneous patterns of crowns yet. In this study, dynamic spatial patterns of typical ecological factors on crown scales were investigated during two sky conditions, and CEZs (crown ecological zones) method was developed for spatial crown zoning, within which leaf traits were statistically unchanged. The influencing factors on hourly and spatial variations of leaf dark respiration (R_d) were analysed, and total crown respiration (R_t) was estimated based on patterns of CEZs. The results showed that dynamic spatial patterns of air temperature and light intensity changed significantly by CEZs in special periods and positions, but not continuously. The contributions of influencing factors on variations of R_d changed with crown depth and sky conditions, and total contributions of leaf structural and chemical traits were higher during sunny days than ecological factors, but lower during cloudy days. The estimated errors of R_t may be obviously reduced with CEZs. These results provided some references for scaling from leaves to crown, and technical foundations for expanding lab-control experiments to open field ones.

The carbon budget of plants is crucial to the ecosystem carbon balance[1–3], and crown accounts for most plant carbon budget[4,5]. Typically, the crown carbon budget was estimated by the summary of individual leaf gas-exchange with the assumption that the crown was uniform[6–9]. However, recent studies show that noteworthy crown heterogeneities appeared in several crowns resulted that leaf structural and functional traits greatly varies with crown positions[3,7–10].

Heterogeneities of some ecological factors on a crown scale had been reported, especially in high trees. Light intensity (photosynthetic photon flux density, I) and air temperature (T) decline markedly with crown depth[3,11], and T generally declines with crown depth due to within-crown shading[12,13]. However, there is currently little research on the spatial heterogeneity of relative air humidity (RH) and CO_2 concentration (C_{air}), which are also related to leaf traits. Leaf dry mass per area (LMA), leaf nitrogen and carbon content per area (N_a and C_a) have been found to respond independently to crown position, which are essential for the analysis of heterogeneous crowns[6,14–17]. LMA and N_a decrease with crown depth mostly, which are considered to be related to the vertical light gradients in crowns, but the significant decline did not always occur[3,6,8,16,18]. C_a is due to the comprehensive effects of various crown factors, but very few studies have described its spatial heterogeneity[16]. Until now, the spatial heterogeneity of ecological factors, leaf structural and chemical traits still require further study.

Leaf dark respiration (R_d) is a large fraction of the total carbon budget in plant from day to night, which is affected by environmental factors and leaf structural and chemical traits[14–16,19–21]. To our knowledge, the variation of R_d across the whole crown and contributions of influencing factors in diurnal course and crown positions still remain inconclusive largely due to a lack of quantitative and accurate research[5,16,21]. Total crown respiration (R_t) account for up to two-thirds of total tree respiration[7,10], the estimation of R_t on crown scale using gas-exchange technology fails to taking the heterogeneity of crown into consideration[22,23]. It is not convenient to estimate R_t and study the response of leaf traits to various environments on crown scales.

To solve the problems, it is crucial to describe and quantify heterogeneities of crown traits. Because of the various temporal and spatial patterns of crown traits, the spatial zoning of a crown is considered to be one better way to describe quantitatively spatial heterogeneities of crown traits. However, it is quite difficult to investigate lots of

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, State Key Laboratory of Urban and Regional Ecology (SKLURE), Beijing 100085, PR China. Correspondence and requests for materials should be addressed to D.H. (email: hudan@rcees.ac.cn)
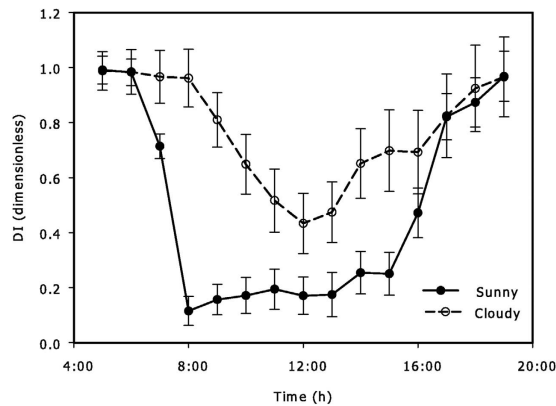
Figure 1. Diurnal changes in the incident diffuse index (*DI*) during sunny (●) and cloudy (○) days in summer. All values are means ± SE; $n = 20$.

leaf gas-exchange traits automatically and continuously on crown scales in open field observations. Fortunately, many reports have suggested that leaf structural and functional traits were directly affected by ecological factors[3,5,24–26]. Temporal and spatial variations of ecological factors on crown scales can be obtained quantitatively. In view of this fact, variations of ecological factors were a solid foundation for spatial crown zoning. Through observations in open field, the diurnal dynamic patterns of typical ecological factors on a crown scale were surveyed. Reliable statistical techniques were used to quantitatively analyse the spatial significant differences of ecological factors. If there was no significant difference for any ecological factor between two adjacent crown areas, we considered the two areas as one "crown ecological zone (CEZ)". Therefore, a CEZ was defined as a crown profile where ecological factors and leaf traits were unchanged statistically, and all CEZs were combined to be a complete coverage of a whole crown. Many researches indicated that ecological factors in different sky conditions (sunny or cloudy) were quite different[10,11,27], thus, we assumed two different types of CEZs gradient patterns under two different sky conditions.

The heterogeneity of canopy traits does not always occur in all the crowns and all the time, in this study we took the crown of *Prunus lannesiana Wils.* as study target which is a common type of widely planted deciduous trees. Leaf structural and chemical traits and the diurnal dynamics of R_d across the crown during two sky conditions were analysed based on CEZs. We further explored the impact of a heterogeneous crown by calculating and comparing the R_t both in a heterogeneous crown and a "uniform crown". We hypothesized that: (1) the crown could be zoned by CEZs, based on the dynamic patterns of T and I on crown scales, and the CEZs method is a sufficient and replicated method for spatial crown zoning to deal with heterogeneous crowns; (2) R_t is affected by dynamic patterns of leaf traits on crown scales, and the CEZs method of estimating R_t is reliable and practical compared to traditional ways; (3) the dominant influencing factors on variation of R_d are largely different between each pair of CEZs, during two sky conditions. In this paper, the traditional gas-exchange technology and spatial crown zoning method are combined to deal with the problem of scale transformation from leaves up to crown, and we emphasize the spatial variations of leaf traits on a crown scale. Additionally, we expect to achieve a better understanding of the impacts of typical ecological factors and leaf structural traits on R_d on a heterogeneous crown scale. This study will serve as a methodological reference for studying the response of the spatial heterogeneity of crown leaf respiration to local micro-climate changes.

Results

Temporal and spatial heterogeneity of ecological factors on a crown scale. The diffuse index (diffuse fraction of total radiation, *DI*) was higher at dawn (5:00–7:00) and dusk (17:00–19:00) than that during the rest of a day under two sky conditions (Fig. 1). *DI* during cloudy conditions was significantly higher than that during sunny conditions from 8:00 to 16:00 (Fig. 1) ($p < 0.001$). The vertical variation of I on a crown scale could be described with unimodal curves during two sky conditions, and the maximum I appeared at 13:00 (Fig. 2a,b). The fluctuating range of I during sunny days was 8.14–1430.40 μmol m^{-2}·s^{-1}, with an average of 301.39 μmol m^{-2}·s^{-1}, while during cloudy days, it was 5.48–821.98 μmol m^{-2}·s^{-1}, with an average of 192.69 μmol m^{-2}·s^{-1} (Fig. 2a,b). The significance of I appeared between each pair of C1, C2, and C3 during sunny days (Fig. 2a), while it appeared between each pair of C1, C2, C3, and C4 during cloudy days ($p = 0.005$) (Fig. 2b). Hence, the spatial heterogeneity of the vertical variation I showed a 3-CEZs and 4-CEZs gradient pattern respectively under sunny and cloudy conditions (Supplementary Fig. S4).

The hourly T showed a periodic change during sunny conditions, peaked at 31.4 °C in the meridian hour (13:00), and dropped down to 19.6 °C at 5:00 with an average of 24.1 °C (Fig. 3a). During cloudy conditions, a similar periodic change occurred in which the fluctuating range was 15.0–23.2 °C, peaked from 15:00 to 16:00 and dropped to the minimum at 5:00 (Fig. 3b). There were significant differences in T between each pair of the areas of C1, C2, and C3 from 8:00 to 15:00 ($p = 0.004$), while there was no significant difference between each pair of areas from C4 to C10 during sunny conditions. No significant difference was observed during the rest of a sunny day and all the cloudy day. From 12:00 to 15:00 during sunny conditions, there were significant differences in T between the sub-areas s1 and s2 ($p = 0.005$) but no significant difference between each pair of the sub-areas s2, s3, and s4 (Fig. 3a). Hence, the spatial heterogeneity of T showed a 4-CEZ gradient pattern during sunny conditions (Supplementary Fig. S5).
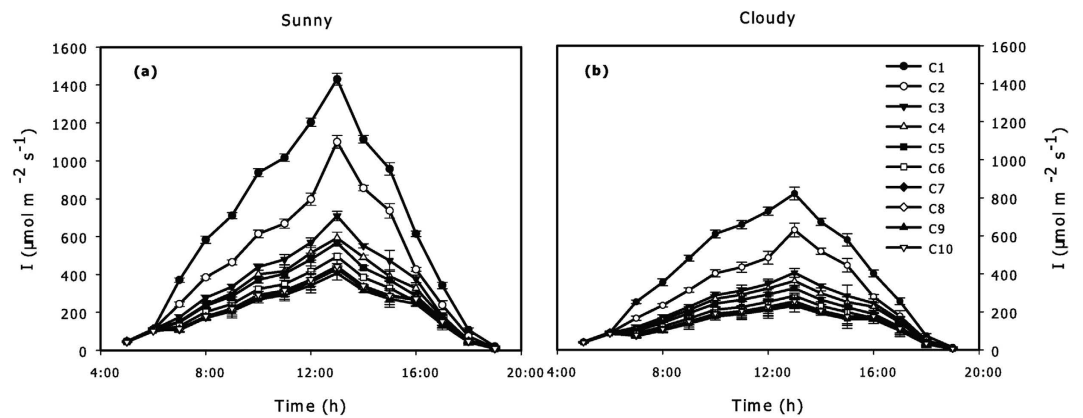
Figure 2. Diurnal changes in the incident photosynthetic photon flux density (*I*) in ten observed areas during sunny (**a**) and cloudy (**b**) conditions in summer. Different symbols are used to indicate different areas: ● = C1, ○ = C2, ▼ = C3, △ = C4, ■ = C5, □ = C6, ◆ = C7, ◇ = C8, ▲ = C9, ▽ = C10. All values are means ± SE; *n* = 120.
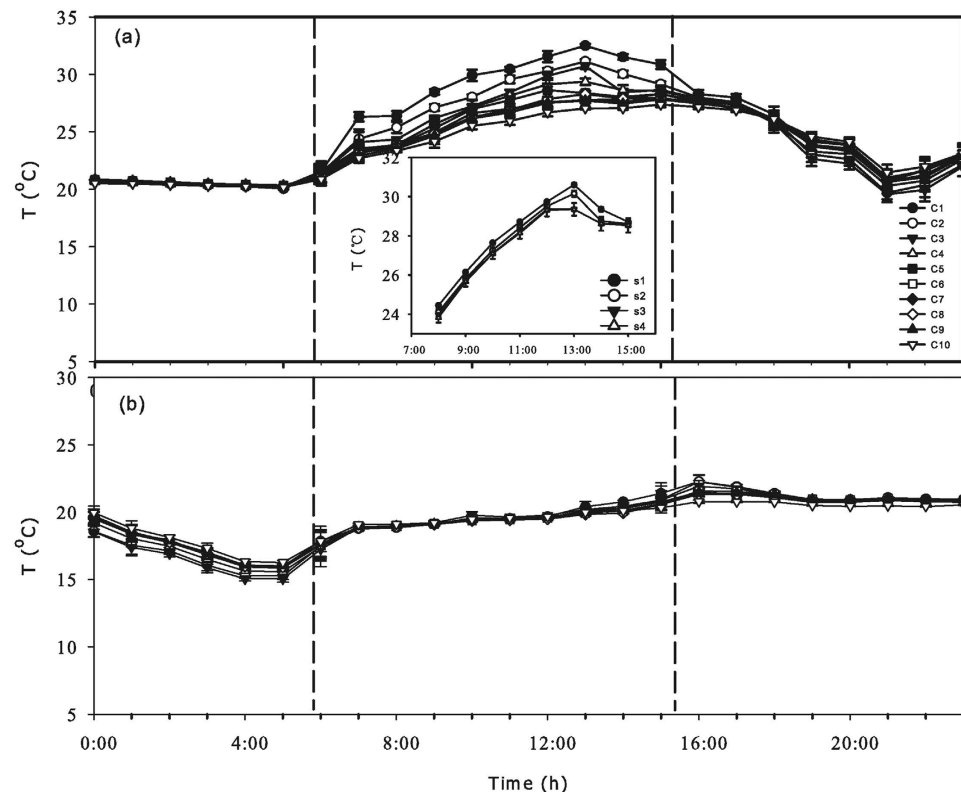

Figure 3. The hourly average air temperature (*T*) of ten observed areas (C1-C10) during sunny (**a**) and cloudy (**b**) conditions in summer. Different symbols are used to indicate different areas: ● = C1, ○ = C2, ▼ = C3, △ = C4, ■ = C5, □ = C6, ◆ = C7, ◇ = C8, ▲ = C9, ▽ = C10. The inserted figure is hourly average T of sub-areas (s1, s2, s3, s4) at 8:00 to 16:00 during sunny condition. Different symbols are used in inserted figure to indicate different sub-areas: ● = s1, ○ = s2, ▼ = s3, △ = s4. All values are means ± SE; *n* = 36.

It can be seen that both the hourly *RH* and C_{air} showed periodic changes during the two sky conditions, but there were no significant differences in *RH* (Fig. 1a,b) and C_{air} (Fig. 1c,d) among the ten crown areas under the two sky conditions at our current temporal and spatial resolutions of observation. During sunny conditions, the fluctuating range of *RH* was 25.1–74.8% across the measurement period and peaked at 23:00, which lasted until 5:00 the next day; the minimum of *RH* appeared at 14:00 (Fig. 4a). During cloudy conditions, the fluctuating range of *RH* was 35.8–99.5%, the maximum *RH* appeared at 16:00 and 23:00, and the minimum *RH* also appeared at 14:00 (Fig. 4a). The C_{air} peaked at 5:00 and then dropped to the minimum at 17:00 under the two sky
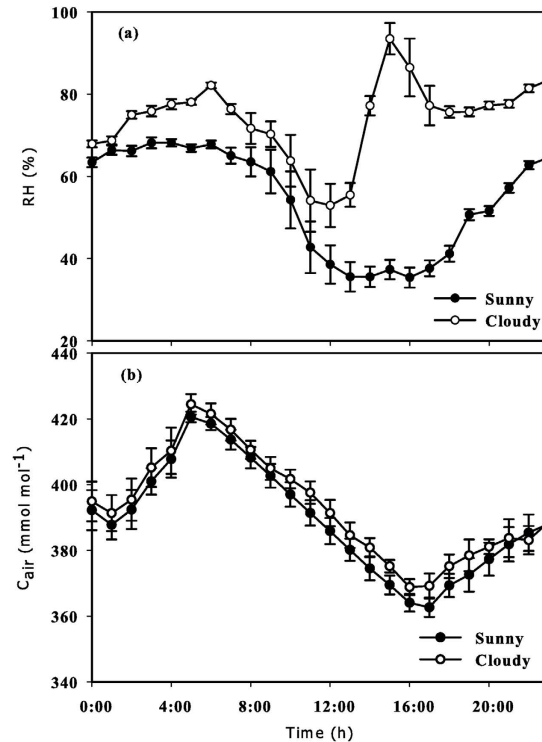
Figure 4. The hourly average air relative humidity (*RH*, **a**) and CO$_2$ concentrations (*C$_{air}$*, **b**) of the crowns under sunny (●) and cloudy (○) conditions in summer. All values are means ± SE; $n = 36$ (**a**) and 120 (**b**).

conditions. The *C$_{air}$* of the crown areas fluctuated between 358.2 and 423.0 μmol·mol^{-1} during sunny conditions and fluctuated between 363.5 and 428 μmol·mol^{-1} during cloudy conditions (Fig. 4b).

Therefore, it came up with a spatial crown pattern of ecological factors in a vertical direction based on the spatial heterogeneity of *T* and *I* under sunny and cloudy conditions. The spatially heterogeneous patterns of ecological factors divided the whole space of the crown into four different CEZs (D1, D2, D3, D4) during sunny conditions and into another four CEZs (E1, E2, E3, E4) during cloudy conditions (Supplementary Fig. S7).

Variation of *LMA*, *C$_a$*, *N$_a$*, and *R$_d$* between CEZs.

LMA on a crown scale decreased with crown depth, and there were significant differences between each pair of the four CEZs (p = 0.005) in the sunny pattern except for D3 and D4 (Fig. 5a); there were significant differences between each pair of the four CEZs in the cloudy pattern (p = 0.006) (Fig. 5b). There were significant differences in *C$_a$* and *N$_a$* between each pair of the CEZs during two sky conditions (p = 0.004) (Fig. 5c,d,e,f).

R$_d$ changed rapidly in the sunny days with a fluctuating range of 0.499–2.243 μmol CO$_2$ m^{-2}·s^{-1} and became gentle in the cloudy days with a fluctuating range of 0.483–1.321 μmol CO$_2$ m^{-2}·s^{-1} (Fig. 6a,b). In sunny days, *R$_d$* peaked at 12:00 to 13:00 in the CEZs of D2, D3, and D4, while it peaked at 11:00 in the CEZs of D1 and then dropped to a minimum at 3:00 to 5:00 (Fig. 6a); there were significant differences between each pair of the four CEZs (D1, D2, D3, D4) in every hour from 8:00 to 19:00 (p = 0.004), except the CEZ of D1 from 12:00 to 14:00, but there was no significant difference during the rest of the day. In cloudy days, the *R$_d$* peaked at 14:00 to 15:00 and then dropped to a minimum at 3:00 to 5:00; the significant differences between each pair of four CEZs (E1, E2, E3, E4) only occurred from 10:00 to 18:00 (Fig. 6b) (p = 0.01). The stomatal conductance (*G$_s$*) appeared similar changes between CEZs under two sky conditions (Fig. 6c,d). *G$_s$* in the daytime considerably decreased with the depth of CEZs, while there was no obvious difference between each pair of CEZs in the nighttime.

Contributions of ecological factors and leaf traits to the hourly and spatial variations of *R$_d$*.

In sunny days, the independent effects of the explanatory variables *T*, *I* and *RH* were significant for the change of hourly *R$_d$* in each CEZ (Fig. 7a,c,e,g), while *T* had the highest independent and total (independent + joint) contributions among ecological factors (Fig. 7a,c,e,g); all the explanatory variables had higher joint contributions than independent contributions during sunny conditions (Fig. 7a,c,e,g). In cloudy days, the independent effect of *I* was significant for hourly variation of *R$_d$* in each CEZ, while other variables were not significant (Fig. 7b,d,f,h); *I* had a higher independent contribution than the joint contribution in each CEZ (Fig. 7b,d,f,h). The independent contributions of *T* decreased with the depth of CEZs, while the independent contributions of *I* increased with the depth of CEZs under both sky conditions (Fig. 7). *LMA*, *N$_a$*, and *C$_a$* had higher contributions on spatial variations of *R$_d$* than ecological factors (*T* and *I*) under sunny conditions, and *C$_a$* had the highest total contribution on spatial variations of *R$_d$* among all variables (Fig. 8a). In contrast, *I* had higher contributions than leaf structural and
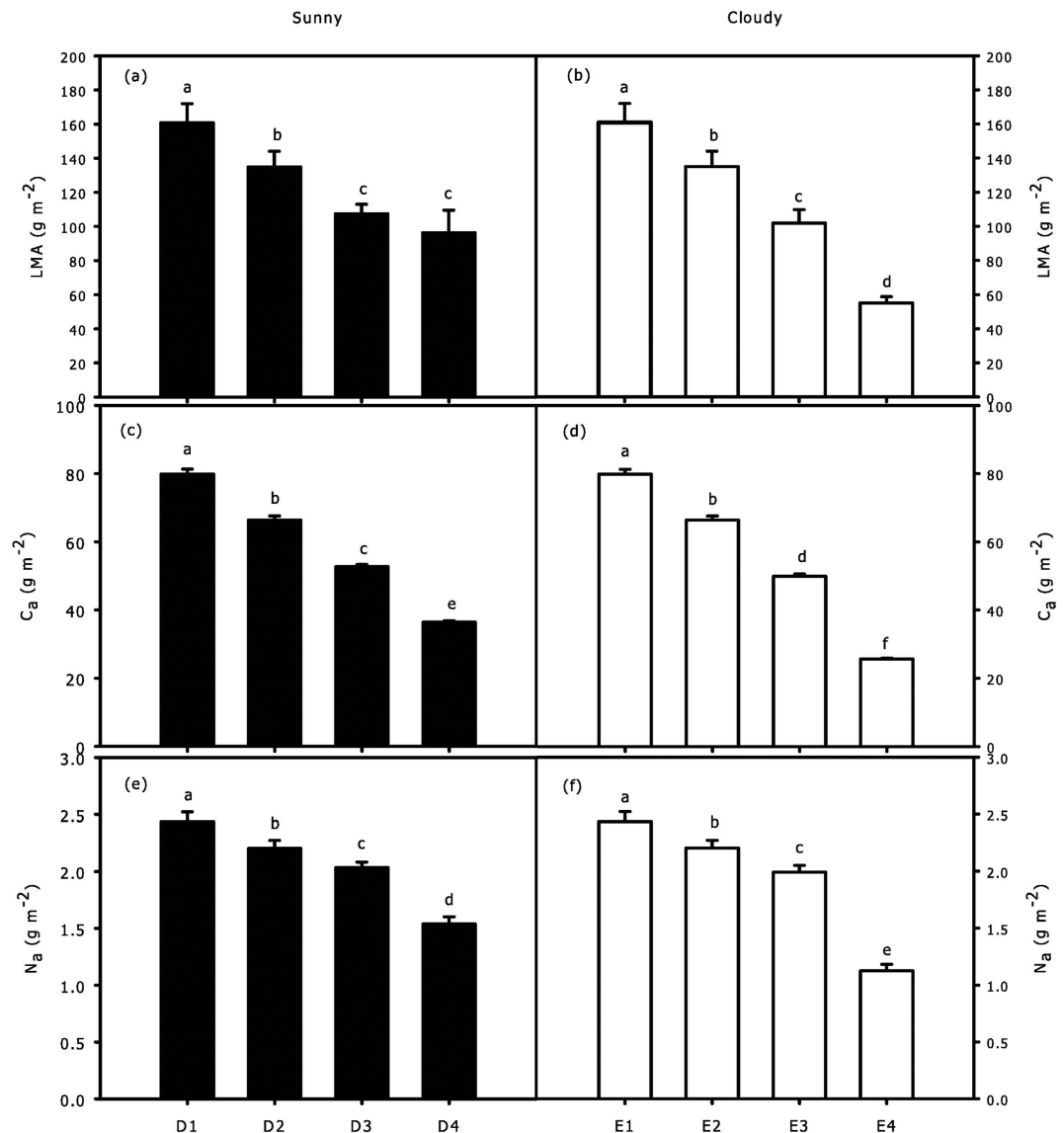
Sunny   Cloudy

Figure 5. Variation of LMA (**a,b**), C_a (**c,d**) and N_a (**e,f**) of leaves between each pair of CEZs (D1, D2, D3, D4 under sunny condition and E1, E2, E3, E4 under cloudy condition) on a crown scale. Identical letters indicate homogeneous groups with statistically insignificant differences (p > 0.05). All values are means ± SE; $n = 30$.

chemical traits (LMA, N_a, and C_a) under cloudy conditions (Fig. 8b). Thus, the relative importance of variables for the spatial variation of R_d was different between two sky conditions.

Estimation of crown respiration in CEZ patterns and a uniform crown. The cumulative respiration rates (R_{day}) of CEZs were higher in sunny conditions than cloudy conditions (Fig. 9a), and R_{day} were significantly different between each pair of CEZs under both sky conditions (p < 0.001), except between D3 and D4. The CEZ-based R_t was lower than D1-based (when considering the crown as uniform for D1) and D2-based R_t, while it was higher than D3-based and D4-based R_t during sunny conditions (Fig. 9b). The CEZ-based R_t was lower than E1-based, E2-based, and E3-based R_t, while it was higher than E4-based R_t during cloudy conditions (Fig. 9b).

Discussion

The aim of the study presented here was to explore a technological method to quantitatively describe the heterogeneity of a crown, and apply the method to study crown-dependent variation of leaf traits and estimate R_t more accurately. The aim was reached in defining CEZs across the crown based on the dynamic diurnal variations of ecological factors. All the measured ecological factors in a CEZs were statistically unchanged in any time of the day, and leaf morphologies (Supplementary Fig. S11) and functional traits were considered to be stable in the same CEZ, assuming that they were hardly affected by growth-regulating substances.
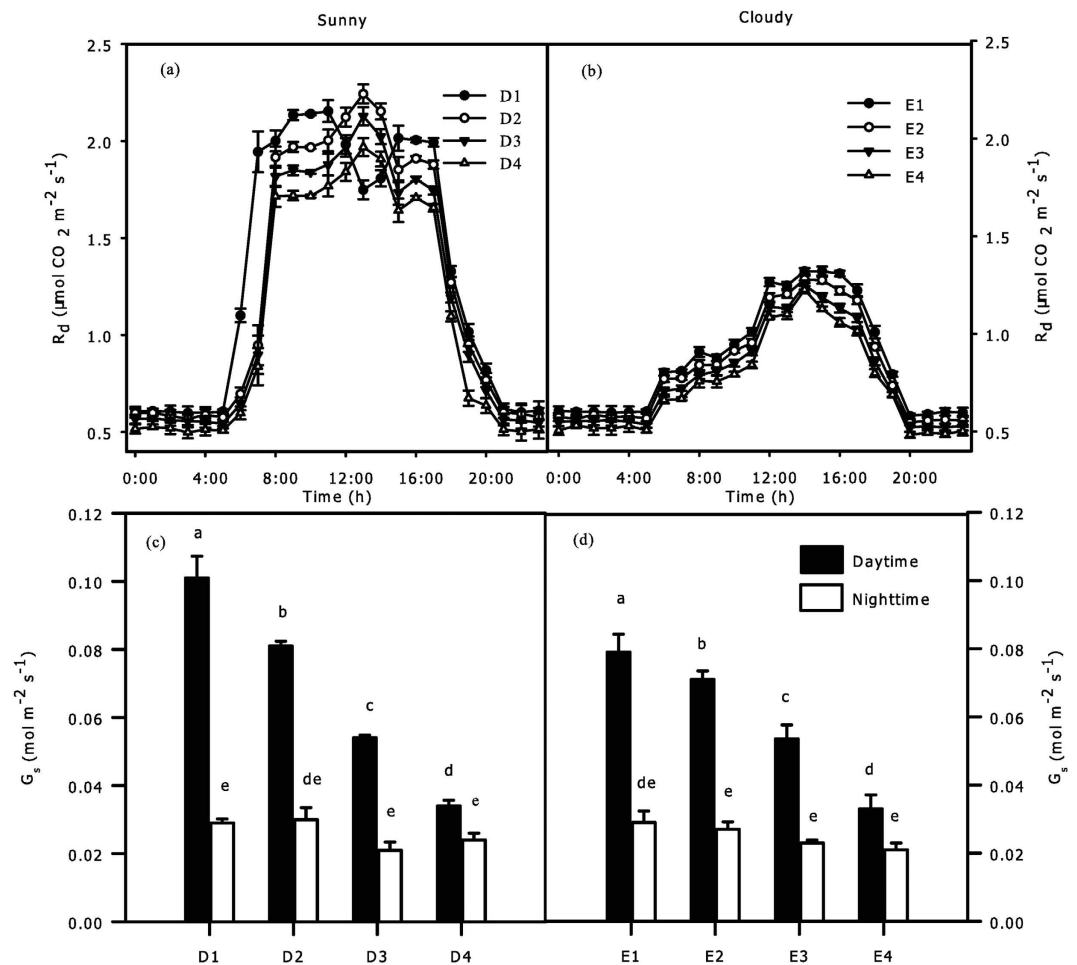
Figure 6. Spatial variations of leaf respiration rate (R_d, **a,b**) stomatal conductance (G_s, **c,d**) on a crown scale during sunny (**a,c**) and cloudy (**b,d**) conditions in summer. The hourly R_d was shown at every hour (**a,b**), ● = D1 and E1, ○ = D2 and E2, ▼ = D3 and E3, △ = D4 and E4. G_s of daytime (closed bar) was the average stomatal conductance in days, and G_s of night-time (open bar) was the average in nights. All values are means ± SE; $n = 18$.

The CEZs based on temporal and spatial heterogeneity of *T* and *I*. According to our results, there were no significant differences in the hourly *RH* and C_{air} during the two sky conditions (Fig. 4). The spatial variations of *T* and *I* appeared in special periods of a day and between some special areas (Figs 2 and 3). The *T*, *RH*, C_{air} and *I* in a horizontal orientation measured in each area had no significant difference regardless of areas and sky conditions (Supplementary Fig. S7). Therefore, *T* and *I* were the main reference indicators for spatial crown ecological zoning.

The spatial patterns of *T* and *I* was different under the two sky conditions (Figs S5 and S6) based on the statistical significances among areas and sub-areas; the property of incident light was a major factor underlying this phenomenon. During sunny conditions, leaves were illuminated from a single direction, which tended to be divided into brightly illuminated and heavily shaded areas[10,11,28]. Under diffuse conditions, irradiance illuminating into a crown comes from a larger fraction of the sky hemisphere, so shading is minimized. Thus, crowns are more evenly illuminated under diffuse irradiance than direct irradiance[10,11]. In our results, the *DI* during cloudy conditions was significantly higher than that during sunny conditions for most of the day (Fig. 1).

Leaf temperature changes with air temperature simultaneously, and the correlation between them is extremely notable; thus, *T* is a good indicator of leaf temperature[29]. The diurnal period with significant differences for *T* (9:00–15:00) was consistent with the period with a higher value of *I* in sunny days. Consequently, the hourly *T* changed almost simultaneously with *I*. However, *T* was also affected by physiological processes and airflow within a crown[30–32]; thus, the vertical variations of *T* might be more sensitive. As such, it appeared two significant parts in sub-areas due to the statistical significances of *T* (Fig. 3a). During cloudy conditions, weak heating rate of low irradiance and heat loss caused by airflow within a crown might result in non-significant variation of *T*, the spatial difference in *T* on a crown scale was not obvious (Fig. 3b). Thus, the spatial pattern of *T* was similar to that of *I*, and both were not changed continuously, but discretely by CEZs in term of statistics.
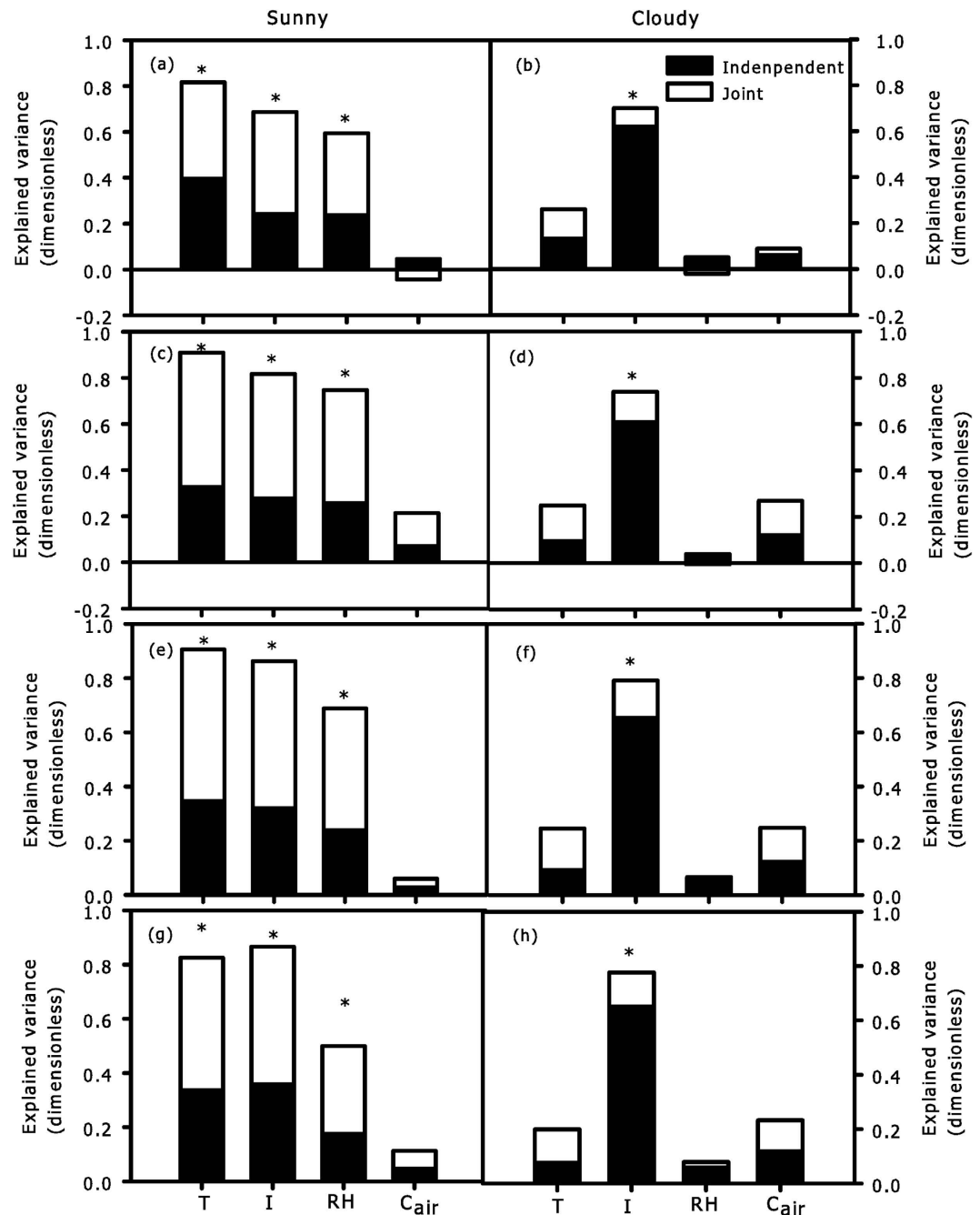
Figure 7. The independent (closed bar) and joint (open bar) explanatory variance of ecological factors for hourly R_d in each CEZ during sunny (**a,c,e,g**) and cloudy (**b,d,f,h**) conditions as estimated by hierarchical partitioning, in which '*' denotes that the independent effect due to this variable was significant at p < 0.05.

Variations of R_d between CEZs on a crown scale. There was no actual consensus about how R_d varies with crown depth in spite of many reports on heterogeneous crowns[6,20]. Our results showed that the significant differences of R_d in different CEZs only appeared in special periods of the day, which were also different between the two sky conditions (Fig. 6a,b). Hence, we considered that time and position of sampling caused the inconsistent conclusions of current studies, excluding variations in species. Despite the fact that T directly affects R_d, our investigation showed that R_d at predawn was very low but sharply rose after day break (Fig. 6a,b), while T did not dramatically change in this period (Fig. 3). Leaf stomata are the channels for CO_2 and O_2, and their behaviours are greatly affected by light[33]; thus, the spatial variation of leaf stomatal conductance (G_s) might be the reason for the conflict. Figure 6b showed that G_s was much lower in the night-time than daytime (p < 0.01), and there were nearly no significant differences between each pair of CEZs at all night-time periods in this crown. Tarvainen *et al.* showed that G_s was the main reason for differences in gas exchange between daytime and night-time, and the low value of G_s resulted in no difference in gas exchange in crown positions[34]. Hence, the limited G_s was the
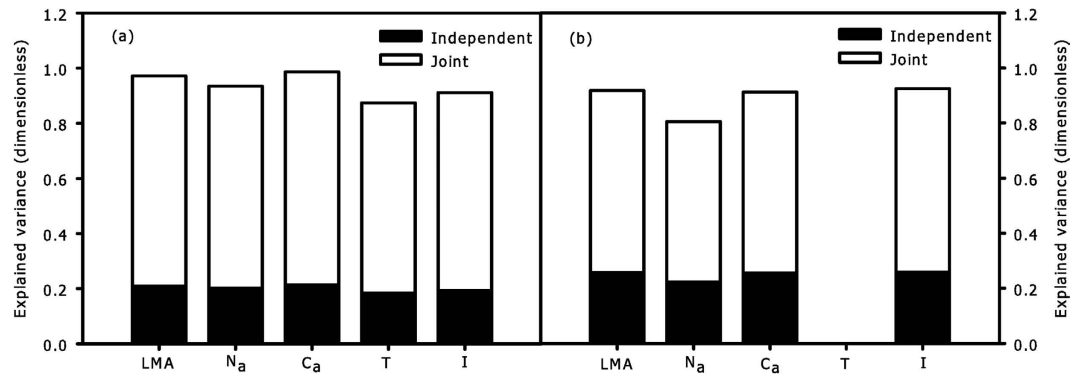
Figure 8. The independent (closed bar) and joint (open bar) explanatory variance of the factors for spatial R_d during sunny (**a**) and cloudy (**b**) conditions, as estimated by hierarchical partitioning, in which '*' denotes that the independent effect due to this variable was significant at p < 0.05.
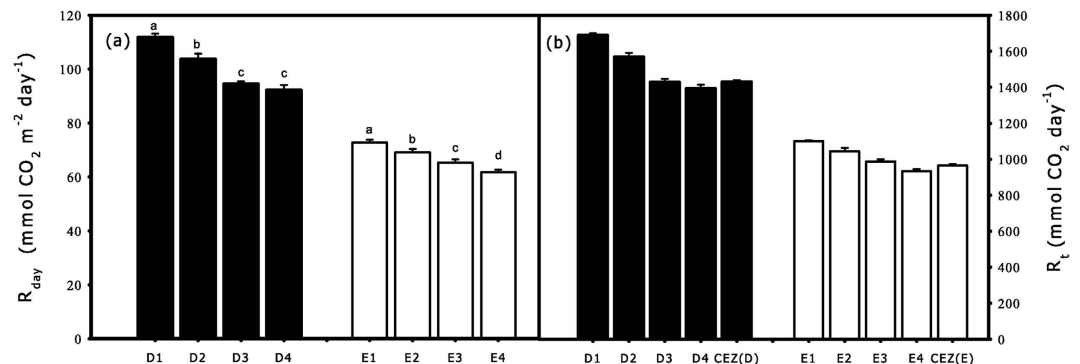

Figure 9. The cumulative respiration rates (R_{day}) of CEZs (D1, D2, D3, D4, and E1, E2, E3, E4) and total crown respiration (R_t) estimated by a uniform R_d and CEZs method on crown scales. R_{day} and R_t were shown under sunny (closed bar) and cloudy (open bar) conditions, CEZ(D) and CEZ(E) were R_t estimated by using CEZs method respectively under sunny and cloudy conditions. Identical letters indicate homogeneous groups with statistically insignificant differences (p > 0.05). $n = 6$.

reasons for the lower R_d and the non-significant differences among CEZs in night-time. Furthermore, the temporary decline of R_d from 11:00 to 13:00 in sunny days might be due to stomata closure at noon.

The study of influencing factors of hourly and spatial variations of R_d using CEZs.
To clarify the influencing factors on spatial variation of R_d on the crown scale, the vertical variation of R_d and R_d-related factors should be described firstly. Leaves from one CEZ were substantially different from other CEZs in R_d and some related factors. Although leaves from plenty of sampling positions might greatly covered difference, lots of repetitions are needed to obtain obvious gradients of leaf traits across the crown. Moreover, the investigations of hourly variation of R_d need to be carried out in a constant R_d. The hourly variation of R_d can be investigated in one CEZ, and the influencing factors of that can be compared between the CEZs. In this study, the CEZs method took full use of the advantage of dynamic pattern of canopy traits to study the contributions of influencing factors on temporal and spatial variation of R_d on the crown scale.

Tissue constructions need a period for accumulation, so we assumed there were no significant differences in LMA, N_a and C_a between every hour, the ecological factors were the only direct factors influencing the change of hourly R_d. However, a long period of variations in T and I could cause differences in leaf structural traits of different CEZs (Fig. 5); thus, the spatial heterogeneity of R_d between CEZs was affected by ecological factors and leaf structural traits. In this case, the results of hierarchical partitioning showed that T, I and RH had significant independent contributions on the hourly variations of R_d during sunny conditions (Fig. 7a,c,e,g), but only I during cloudy conditions (Fig. 7b,d,f,h). Light intensity decreases severely with depth of crown, and light is considered to be the key factor of leaf traits in lower crowns[3,35]. Due to thermo-regulation of leaves, lower crowns with higher leaf density could be less sensitive than upper crowns. As a consequence, the independent contributions of T tended to be higher in upper crown than that in lower crown, while the independent contributions of I tended to be higher in lower crown during two sky conditions (Fig. 7). None of explained variance had significant independent contribution to spatial variation of R_d, and a far greater portion of the explained variance was related to the joint contributions (Fig. 8). As such, the interaction of multiple factors played a more important role in influencing the spatial variation of R_d. In addition, C_a more comprehensively reflected the response of leaves to

ecological factors[16], C_a had the highest total contribution to spatial variations of R_d of all variables (Fig. 8), which further confirms that variation of R_d was affected more by the joint rather than the independent effects of the factors.

Variation of R_t on the crown scale with the help of CEZs.

Many studies attempted to extrapolate the gas-exchange parameters to crown level, and the accuracy of a method depended on the sampling positions of the crown[36,37]. In the previous reports, sampling positions were selected by the uniform method, such as the "big-leaf model"[38]. The positions of the crown affect R_d and its response to ecological factors on the crown scale[3,7]. Our results demonstrated that crown position, sampling time and sky conditions could have significant effects on R_d (Fig. 6a,b). The multilayer model has taken leaf variations into account, it fails to utilize these variations. Furthermore, the sampling positions are selected by using the normalized Gaussian distances, although the mathematical accuracy could be admitted, it fails to select the representative positions[39]. The CEZs in this study provided better reference systems of notable significance in leaf traits, and the positions from all the CEZs were considered good representations of the crown. Compared to the CEZs method, the R_t were overestimated when selecting positions in upper and middle crown (CEZs of D1, D2, E1, and E2), and underestimated when selecting positions in lower crown (CEZs of D3, D4, E3, and E4) (Fig. 9b). Furthermore, simplifying assumptions regardless of sky conditions tended to result in an overestimation or underestimation of the actual R_t (Fig. 9b). Thus, the CEZs method which had taken sky conditions and the spatial patterns of the crown into consideration can reduce the estimation errors of R_t.

Feasibility and simplicity of the CEZs method.

Two 4-CEZ gradient patterns reasonably reflected the spatial patterns of ecological factors and leaf traits and reduced the errors in estimating R_t to the greatest possible extent. However, ecological factors and leaf traits on a crown scale are affected by climate, seasons, species, and crown types. By the same technology, we investigated the CEZs gradient patterns of the selected trees in spring and autumn (Supplementary Fig. S9), and other trees of different species (Supplementary Fig. S10) to indicate the applicability of the method. The CEZs were similar in all the investigated trees, although the patterns of CEZs were not always the same. Due to the same CEZ gradient patterns for a crown of *Prunus lannesiana Wils.*, the estimation on R_t in autumn was consistent with that in summer (Supplementary Fig. S12). And the contributions of ecological factors and leaf traits on the hourly and spatial variation of R_d in autumn were nearly the same with that in summer because of the constant leaf structural and chemical traits in full-developed crowns (Supplementary Figs S13 and S14).

It was worth noting that the height of D3 was only 2.5% of the whole crown, and the R_{day} of D3 was non-significant with D4. In addition, D3 occurred from 12:00 to 15:00 in a day, while other CEZs occurred from 8:00 to 15:00. For most of the day, ecological factors of D3 showed the same variation with D4 except for 12:00–15:00. Furthermore, the sub-dividing areas by 0.1m is beyond the level that most models would require, and undetectable in field collections. Thus, in field applications, we suggest combining D3 and D4 under sunny conditions. Leaf traits of the lower percentage of 80% (sunny days) and 70% (cloudy days) were statistically unchanged in our studies, which was consistent with non-significance of T, I, LMA, and N_a between lower crown areas reported in numerous studies[10,21,40]. Meanwhile, more accurate scales of observation might generate more convincing results and help to identify much more defined boundaries of CEZs.

Conclusions

With this study, the temporal and spatial heterogeneities of ecological factors (T, I, RH, C_{air}) in *Prunus lannesiana Wils.* were clarified on the fully-developed crown scale in north temperate regions (Beijing): the vertical variations of T and I occurred in special periods of a day, and were different between two sky conditions. T and I were not continuously decreased with depth of crown, but decreased discretely by CEZs, even if the observational accuracy of the crown decreased to 0.1m. With the fact of temporal-spatial features of T and I in the crown scale, it came up with CEZs gradient patterns which were used to indicate the spatial patterns of a crown. The CEZs method provides a system of crown with obvious inter-zones differences and intra-zone homogeneity. With the help of CEZs, the contributions of influencing factors on hourly variations of R_d of *Prunus lannesiana Wils.* and their relationship with depth of crown were analysed for the first time, and the contributions of fully-developed leaf morphologies on spatial variations of R_d were compared with ecological factors. The estimated values of R_t in north temperate regions (Beijing) were affected by dynamic spatial patterns of leaf traits (LMA, N_a, C_a, R_d) on crown scales, which could be displayed clearly using CEZs method, thus, estimation of R_t with CEZs method was proved to be less errors than previous methods.

Our results may help to establish effective technical methods to estimate total crown respiration and better understand spatial response of ecological factors and leaf traits of a crown to environmental conditions in north temperate regions (Beijing).

Materials and Methods

Field sites and plant materials.

This experiment was conducted in an urban green space in Haidian District, Beijing City of China during July-September 2014. Beijing is located in a northern temperate zone from 115.7°E-117.4°E, 39.4°N-41.6°N. The experimental site was a monsoon-influenced humid continental climate with a hot and humid summer with the mean air temperature of 24.4 °C and precipitation of 503.1 mm. The sunshine duration during July-September was 230–245 hours per month. The site was a typical plant community primarily composed of trees, shrubs and herbaceous plants. The study site was not blocked by high buildings or large trees. The selected trees of *Prunus lannesiana Wils.* were described in Supplementary Table S1. The sky conditions were determined with the following method: 1) the sunny and cloudy days were selected according to weather reports by China Meteorological Administration. 2) visual observation during experimental period

was used to exclude the diurnal temporary changes of sky condition. The cloudy sky condition was defined as the solar disc is invisible[41], and there is no clear bonder of shade in the crown. 3) besides, in many ecological studies, the diffuse index (diffuse fraction of total radiation, *DI*) was used to separate days into sunny and cloudy, *DI* mostly remained less than 0.27 during sunny days while more than 0.65 during the sunny and cloudy days, respectively[10,41–43]. Microclimatic characteristics during sunny and cloudy conditions were measured and are shown in Supplementary Table S2.

Ecological factors on a crown scale. We took a range of 4.0 m heights of the crown as survey areas, covering almost all the areas of a crown based on the tree's structural parameters while taking into account the precision and resolution of the detectors. The survey areas were artificially divided into ten sequential areas (labelled as C1, C2, C3, C4, C5, C6, C7, C8, C9, C10) in a vertical direction where C1 was the 90–100% relative height of the whole crown, and each area contained 10% range of crown and sufficient leaves to measure leaf traits. The details of this technique are described in Supplementary Information (Supplementary Fig. S1).

The spectral composition of light transmitted into the crown and the hourly *I* (over the wavelength of 400–800 nm) in the crown areas was observed by using a fibre optic spectrometer equipped with a cosine corrector (AvaSpec-ULS 2048XL, Avantes, Netherland) in each area (C1-C10), from 5:00 to 19:00. In each area, we randomly performed sampling of 20 crown points and calculated the average of the observational data of six days during the two sky conditions.

The *T* and *RH* of each area were observed with sensors of air temperature and relative humidity, and were recorded with data loggers (Onset HOBO Data Loggers, Pocasset, Massachusetts, USA). The logging interval was 1 min, and the obtained data were averaged every 10 min. Thus, 6 numerical values for each sensor were acquired in one hour. According to the statistical results, much smaller intervals of sampling needed to be performed to distinguish the variation of *T* in the C3 area. Thus, we divided the area of C3 equally into four sub-areas (s1, s2, s3, s4); each sub-area was 0.1 m height (Supplementary Fig. S2). We also took an average of the six-day observational data of the variables *T* and *RH* during the two sky conditions.

The C_{air} was measured by a portable open-flow gas-exchange system (Li-6400, Li-Cor Inc., Lincoln NE, USA) that logged every hour in each area. We randomly performed sampling of 20 crown points and took an average of the observational data of six days during the two sky conditions.

The values of the variables *I*, *T*, *RH* and C_{air} in a crown horizontal orientation were also measured in each vertical area and sub-area, and the statistical significance was analysed using the same method as for the vertical orientation (Supplementary Fig. S4).

Leaf traits on a crown scale. *LMA* was measured on leaves randomly sampled in each CEZ before dawn (4:00). Leaves were scanned using a leaf area meter (Li-3100, LI-COR, Inc.) to measure surface areas, then placed into a drying oven. After 15 min at 105 °C, leaves were dried at 85 °C until the weight no longer changed. LMA was calculated as a unit area of dry weight. We collected 50 samples in each CEZ. C_a and N_a were acquired with an elemental analyser (Vario EL III, Elementar, Germany) using powdery leaves; nine samples were collected in each CEZ.

Measurements of R_d were made with a portable open-flow gas-exchange system (Li-6400, Li-Cor Inc), and a workbench was used in high positions. Environmental conditions within the cuvette were controlled to match the measured air conditions. Leave of each CEZs were dark acclimated for a minimum of 20 min before measurements began, and the measurement of respiration was recorded only when gas exchange had equilibrated (taken to be when the rate of CO_2 efflux was stable and the coefficient of variation for CO_2 partial pressure differential between the sample and reference was <1%). The R_d of each CEZ was measured at 1-hour interval, and all the collected leaves were fully self-expanded and of a similar age.

Estimation of crown respiration. The R_t of each zone was calculated from equation (1), where R_{day} was the cumulative respiration rates of each CEZ throughout the day, and calculated by trapezoidal integration over R_d in 24 hours by equation (2); S_n was the estimated area of a single leaf, which is the average of leaf areas in a CEZ. Number of leaves in unit volume was calculated, *N* was the number of all leaves in a CEZ, and was estimated with the total volume of a CEZ, assuming that the CEZ was a round estrade.

$$R_t = R_{day} \times S_n \times N \tag{1}$$

$$R_{day} = \int_0^{24} R_d \tag{2}$$

Therefore, the R_t based on CEZs was the summary of the R_t of CEZs, and the R_t based on a 'uniform crown' was calculated by using the constant R_d of a certain CEZ.

Statistical analysis. The observed values of ecological factors at every hour were analysed statistically with one-way ANOVA in R-software (R 3.0.3: A programming environment for data analysis and graphics © 2014) to calculate the statistical significance of the difference among areas (confidence band of 95%). The significance of the differences in leaf traits between CEZs was analysed statistically via one-way ANOVA in R-software. Correlation analysis were made with the related modules in R-software.

The hierarchical partitioning method in R-software was used to identify the individual variables that significantly affected R_d in a crown. This method could provide a calculation of the independent and joint contribution with all other variables. This analysis was completed using the 'hier.part package' version 5.1[44] that was a part of the R statistical package. Hierarchical partitioning depends on monotonic relationships between the response and

predictor variables[45,46]. Hence, the data were log transformed to improve the linearity of relationships between R_d and other variables. Statistical significance was accepted at the upper 95% of confidence limit[44]. The graphics software SigmaPlot (Version 10.0) was used to create artwork.

References

1. Ayub, G., Smith, R. A., Tissue, D. T. & Atkin, O. K. Impacts of drought on leaf respiration in darkness and light in Eucalyptus saligna exposed to industrial-age atmospheric CO_2 and growth temperature. *New Phytol.* **190,** 1003–1018, doi: 10.1111/j.1469-8137.2011.03673.x (2011).
2. Huntingford, C. *et al.* Simulated resilience of tropical rainforests to CO_2-induced climate change. *Nature Geosci* **6,** 268–273, doi: 10.1038/ngeo1741 (2013).
3. Weerasinghe, L. K. *et al.* Canopy position affects the relationships between leaf respiration and associated traits in a tropical rainforest in Far North Queensland. *Tree Physiol* **34,** 564–584, doi: 10.1093/treephys/tpu016 (2014).
4. Ryan, M. G., Lavigne, M. B. & Gower, S. T. Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. *J. Geophys. Res.* (*Atmospheres*) **102,** 28871–28883, doi: 10.1029/97JD01236 (1997).
5. Xu, C. Y. & Griffin, K. L. Seasonal variation in the temperature response of leaf respiration in Quercus rubra: foliage respiration and leaf properties. *Funct. Ecol.* **20,** 778–789, doi: 10.1111/j.1365-2435.2006.01161.x (2006).
6. Ellsworth, D. S. & Reich, P. B. Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. *Oecologia* **96,** 169–178, doi: 10.1007/bf00317729 (1993).
7. Griffin, K. L., Turnbull, M. & Murthy, R. Canopy position affects the temperature response of leaf respiration in Populus deltoides. *New Phytol.* **154,** 609–619, doi: 10.1046/j.1469-8137.2002.00410.x (2002).
8. van de Weg, M. J., Meir, P., Grace, J. & Ramos, G. D. Photosynthetic parameters, dark respiration and leaf traits in the canopy of a Peruvian tropical montane cloud forest. *Oecologia* **168,** 23–34, doi: 10.1007/s00442-011-2068-z (2012).
9. Post, H., Hendricks Franssen, H. J., Graf, A., Schmidt, M. & Vereecken, H. Uncertainty analysis of eddy covariance CO_2 flux measurements for different EC tower distances using an extended two-tower approach. *Biogeosciences* **12,** 1205–1221, doi: 10.5194/bg-12-1205-2015 (2015).
10. Urban, O. *et al.* Impact of clear and cloudy sky conditions on the vertical distribution of photosynthetic CO_2 uptake within a spruce canopy. *Funct. Ecol.* **26,** 46–55, doi: 10.1111/j.1365-2435.2011.01934.x (2012).
11. Williams, M., Rastetter, E. B., Van der Pol, L. & Shaver, G. R. Arctic canopy photosynthetic efficiency enhanced under diffuse light, linked to a reduction in the fraction of the canopy in deep shade. *New Phytol.* **202,** 1267–1276, doi: 10.1111/nph.12750 (2014).
12. Baldocchi, D. D., Wilson, K. B. & Gu, L. How the environment, canopy structure and canopy physiological functioning influence carbon, water and energy fluxes of a temperate broad-leaved deciduous forest—an assessment with the biophysical model CANOAK. *Tree Physiol* **22,** 1065–1077, doi: 10.1093/treephys/22.15-16.1065 (2002).
13. Zweifel, R., Böhm, J. P. & Häsler, R. Midday stomatal closure in Norway spruce—reactions in the upper and lower crown. *Tree Physiol* **22,** 1125–1136, doi: 10.1093/treephys/22.15-16.1125 (2002).
14. Wright, I. J. *et al.* The worldwide leaf economics spectrum. *Nature* **428,** 821–827, doi: 10.1038/nature02403 (2004).
15. Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J. & Villar, R. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol.* **182,** 565–588, doi: 10.1111/j.1469-8137.2009.02830.x (2009).
16. Coble, A. P. & Cavaleri, M. A. Light acclimation optimizes leaf functional traits despite height-related constraints in a canopy shading experiment. *Oecologia* **177,** 1131–1143, doi: 10.1007/s00442-015-3219-4 (2015).
17. Niinemets, Ü., Keenan, T. F. & Hallik, L. A worldwide analysis of within-canopy variations in leaf structural, chemical and physiological traits across plant functional types. *New Phytol.* **205,** 973–993, doi: 10.1111/nph.13096 (2015).
18. Dewar, R. C., Tarvainen, L., Parker, K., Wallin, G. & McMurtrie, R. E. Why does leaf nitrogen decline within tree canopies less rapidly than light? An explanation from optimization subject to a lower bound on leaf mass per area. *Tree Physiol* **32,** 520–534, doi: 10.1093/treephys/tps044 (2012).
19. Atkin, O. K. *et al.* Global variability in leaf respiration in relation to climate, plant functional types and leaf traits. *New Phytol.* **206,** 614–636, doi: 10.1111/nph.13253 (2015).
20. Villar, R., Ruiz-Robleto, J., Ubera, J. L. & Poorter, H. Exploring variation in leaf mass per area (LMA) from leaf to cell: An anatomical analysis of 26 woody species. *Am. J. Bot.* **100,** 1969–1980, doi: 10.3732/ajb.1200562 (2013).
21. Coble, A. P. & Cavaleri, M. A. Light drives vertical gradients of leaf morphology in a sugar maple (Acer saccharum) forest. *Tree Physiol* **34,** 146–158, doi: 10.1093/treephys/tpt126 (2014).
22. Buckley, T. N., Cescatti, A. & Farquhar, G. D. What does optimization theory actually predict about crown profiles of photosynthetic capacity when models incorporate greater realism? *Plant, Cell Environ.* **36,** 1547–1563, doi: 10.1111/pce.12091 (2013).
23. Weng, E. S. *et al.* Scaling from individual trees to forests in an Earth system modeling framework using a mathematically tractable model of height-structured competition. *Biogeosciences* **12,** 2655–2694, doi: 10.5194/bg-12-2655-2015 (2015).
24. Wright, I. J. *et al.* Irradiance, temperature and rainfall influence leaf dark respiration in woody plants: evidence from comparisons across 20 sites. *New Phytol.* **169,** 309–319, doi: 10.1111/j.1469-8137.2005.01590.x (2006).
25. Ow, L. F., Griffin, K. L., Whitehead, D., Walcroft, A. S. & Turnbull, M. H. Thermal acclimation of leaf respiration but not photosynthesis in Populus deltoides×nigra. *New Phytol.* **178,** 123–134, doi: 10.1111/j.1469-8137.2007.02357.x (2008).
26. Heskel, M. A., Bitterman, D., Atkin, O. K., Turnbull, M. H. & Griffin, K. L. Seasonality of foliar respiration in two dominant plant species from the Arctic tundra: response to long-term warming and short-term temperature variability. *Funct. Plant Biol.* **41,** 287–300, doi: 10.1071/FP13137 (2014).
27. Kanniah, K. D., Beringer, J. & Hutley, L. Exploring the link between clouds, radiation, and canopy productivity of tropical savannas. *Agr Forest Meteorol* **182–183,** 304–313, doi: 10.1016/j.agrformet.2013.06.010 (2013).
28. Mercado, L. M. *et al.* Impact of changes in diffuse radiation on the global land carbon sink. *Nature* **458,** 1014–1017, doi: 10.1038/nature07949 (2009).
29. Leuzinger, S. & Körner, C. Tree species diversity affects canopy leaf temperatures in a mature temperate forest. *Agr Forest Meteorol* **146,** 29–37, doi: 10.1016/j.agrformet.2007.05.007 (2007).
30. Bauerle, W. L., Bowden, J. D., Wang, G. G. & Shahba, M. A. Exploring the importance of within-canopy spatial temperature variation on transpiration predictions. *J. Exp. Bot.* **60,** 3665–3676, doi: 10.1093/jxb/erp206 (2009).
31. Leuzinger, S., Vogt, R. & Körner, C. Tree surface temperature in an urban environment. *Agr Forest Meteorol* **150,** 56–62, doi: doi: 10.1016/j.agrformet.2009.08.006 (2010).
32. Schurgers, G., Lagergren, F., Mölder, M. & Lindroth, A. The importance of micrometeorological variations for photosynthesis and transpiration in a boreal coniferous forest. *Biogeosciences* **12,** 237–256, doi: 10.5194/bg-12-237-2015 (2015).
33. Lawson, T. Guard cell photosynthesis and stomatal function. *New Phytol.* **181,** 13–34, doi: 10.1111/j.1469-8137.2008.02685.x (2009).
34. Tarvainen, L., Wallin, G., Rantfors, M. & Uddling, J. Weak vertical canopy gradients of photosynthetic capacities and stomatal responses in a fertile Norway spruce stand. *Oecologia* **173,** 1179–1189, doi: 10.1007/s00442-013-2703-y (2013).
35. Niinemets, Ü., Cescatti, A., Rodeghiero, M. & Tosens, T. Complex adjustments of photosynthetic potentials and internal diffusion conductance to current and previous light availabilities and leaf age in Mediterranean evergreen species Quercus ilex. *Plant, Cell Environ.* **29,** 1159–1178, doi: 10.1111/j.1365-3040.2006.01499.x (2006).

36. Prieto, J. A. *et al.* A leaf gas exchange model that accounts for intra-canopy variability by considering leaf nitrogen content and local acclimation to radiation in grapevine (Vitis vinifera L.). *Plant, Cell Environ.* **35,** 1313–1328, doi: 10.1111/j.1365-3040.2012.02491.x (2012).

37. Medlyn, B. E., Pepper, D. A., O'Grady, A. P. & Keith, H. Linking leaf and tree water use with an individual-tree model. *Tree Physiol* **27,** 1687–1699, doi: 10.1093/treephys/27.12.1687 (2007).

38. De Pury, D. G. G. & Farquhar, G. D. Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. *Plant, Cell Environ.* **20,** 537–557, doi: 10.1111/j.1365-3040.1997.00094.x (1997).

39. Leuning, R., Kelliher, F. M., De Pury, D. G. G. & Schulze, E. D. Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies. *Plant, Cell Environ.* **18,** 1183–1200, doi: 10.1111/j.1365-3040.1995.tb00628.x (1995).

40. Legner, N., Fleck, S. & Leuschner, C. Within-canopy variation in photosynthetic capacity, SLA and foliar N in temperate broad-leaved trees with contrasting shade tolerance. *Trees-Struct Funct* **28,** 263–280, doi: 10.1007/s00468-013-0947-0 (2014).

41. Li, D. H. W., Lau, C. C. S. & Lam, J. C. Overcast sky conditions and luminance distribution in Hong Kong. *Build. Environ.* **39,** 101–108, doi: 10.1016/j.buildenv.2003.06.001 (2004).

42. Roderick, M. L., Farquhar, G. D., Berry, S. L. & Noble, I. R. On the direct effect of clouds and atmospheric particles on the productivity and structure of vegetation. *Oecologia* **129,** 21–30, doi: 10.1007/s004420100760 (2001).

43. Urban, O. *et al.* Ecophysiological controls over the net ecosystem exchange of mountain spruce stand. Comparison of the response in direct vs. diffuse solar radiation. *Glob. Chang. Biol.* **13,** 157–168, doi: 10.1111/j.1365-2486.2006.01265.x (2007).

44. R Core Team. R: A programming environment for data analysis and graphics. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/packag=hier.part (2013).

45. Heikkinen, R. K., Luoto, M., Virkkala, R. & Rainio, K. Effects of habitat cover, landscape structure and spatial variables on the abundance of birds in an agricultural–forest mosaic. *J. Appl. Ecol.* **41,** 824–835, doi: 10.1111/j.0021-8901.2004.00938.x (2004).

46. Wang, H. *et al.* Water, heat, and airborne pollutants effects on transpiration of urban trees. *Environ. Pollut.* **159,** 2127–2137, doi: 10.1016/j.envpol.2011.02.031 (2011).

Acknowledgements

Author Contributions

Original ideas and research topic was put forward and designed by X.W. and D.H.; X.W., H.Z., F.H., Y.L. and D.H. undertook all sampling and analysis; all authors contributed to the interpretation of the research results, writing and revisions of manuscript.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Wang, X. *et al.* Dynamic spatial patterns of leaf traits affect total respiration on the crown scale. *Sci. Rep.* **6**, 26675; doi: 10.1038/srep26675 (2016).