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Research

Linking leaf dark respiration to leaf traits and reflectance spectroscopy across diverse forest types

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Summary

- Leaf dark respiration (R_{dark}), an important yet rarely quantified component of carbon cycling in forest ecosystems, is often simulated from leaf traits such as the maximum carboxylation capacity (V_{cmax}), leaf mass per area (LMA), nitrogen (N) and phosphorus (P) concentrations, in terrestrial biosphere models. However, the validity of these relationships across forest types remains to be thoroughly assessed.
- Here, we analyzed R_{dark} variability and its associations with V_{cmax} and other leaf traits across three temperate, subtropical and tropical forests in China, evaluating the effectiveness of leaf spectroscopy as a superior monitoring alternative.
- We found that leaf magnesium and calcium concentrations were more significant in explaining cross-site R_{dark} than commonly used traits like LMA, N and P concentrations, but univariate trait–R_{dark} relationships were always weak ($r^2 \leq 0.15$) and forest-specific. Although multivariate relationships of leaf traits improved the model performance, leaf spectroscopy outperformed trait–R_{dark} relationships, accurately predicted cross-site R_{dark} ($r^2 = 0.65$) and pinpointed the factors contributing to R_{dark} variability.
- Our findings reveal a few novel traits with greater cross-site scalability regarding R_{dark}, challenging the use of empirical trait–R_{dark} relationships in process models and emphasize the potential of leaf spectroscopy as a promising alternative for estimating R_{dark}, which could ultimately improve process modeling of terrestrial plant respiration.

Introduction

Land plant respiration, a major component of the global carbon (C) cycling and vegetation–climate interactions (H. Wang *et al.*, 2020), releases *c.* 60 Pg C yr^{-1} to the atmosphere, that is six times higher than human-induced CO_2 emissions (Ciais *et al.*, 2014). About half of C released by plant respiration is derived from leaf dark respiration (R_{dark} – i.e. nonphotorespiratory mitochondrial CO_2 release) that takes place during the day and night (Atkin *et al.*, 2007; Huntingford *et al.*, 2017), with the

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release of CO_2 being coupled to production of energy in the form of adenosine triphosphate (ATP) and reducing equivalents. Leaf R_{dark} plays an important role in nitrogen (N) assimilation, C skeleton synthesis and the regulation of redox balance (Atkin *et al.*, 2015; Garcia *et al.*, 2022). Because of the strong kinetic response of R_{dark} to temperature, R_{dark} is typically converted to a rate at a standardized temperature, such as 25°C (R_{dark25}), reflecting respiratory capacity (Atkin & Tjoelker, 2003; Atkin *et al.*, 2007; Davidson *et al.*, 2023b). As a result, R_{dark25} has long been identified as a crucial biochemical parameter in terrestrial biosphere models (TBMs) (Huntingford *et al.*, 2017), and accurate representation of R_{dark25} in TBMs greatly influences the precision of simulations of terrestrial plant productivity and C cycling (Schwalm *et al.*, 2010; Atkin *et al.*, 2015; Butler *et al.*, 2021). However, R_{dark25} is highly variable, in response to differences in energy demand among contrasting plant species, plant functional groups, ecosystem types, climate, soil conditions and acclimation (Lambers & Oliveira, 2008; Atkin, 2011; Atkin *et al.*, 2015; Reich *et al.*, 2016). Therefore, efficient and accurate characterization of leaf R_{dark25} across ecosystems – particularly among different forest types – will be crucial if we are to improve representation of plant respiration in TBMs. The ability to rapidly quantify variation in R_{dark25} is also essential if we are to develop a more thorough mechanistic understanding of the factors that drive R_{dark25} in nature.

R_{dark25} is closely intertwined to photosynthetic metabolism through reliance of respiration on substrates from photosynthesis and demands for respiratory products (e.g. ATP) to support maintenance and export processes that are closely linked to photosynthesis (Fernie *et al.*, 2004; Fan *et al.*, 2021). Because Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the largest and most energy demanding enzyme in the photosynthetic process, TBMs commonly assume R_{dark25} to be proportional to the maximum carboxylation capacity of the enzyme Rubisco standardized to a reference temperature of 25°C (V_{cmax25}) in TBMs (Schwalm *et al.*, 2010; Atkin *et al.*, 2015). An example is how R_{dark25} and V_{cmax25} are set by a commonly assumed coefficient (R_{dark25} : V_{cmax25} ratio = 0.015) in several TBMs (Clark *et al.*, 2011; Atkin *et al.*, 2017; Fan *et al.*, 2021). Alternatively, R_{dark25} can be extrapolated using its empirical relationships with other relatively easy-to-measure leaf traits, such as leaf mass per area (LMA), leaf N and phosphorus (P) concentrations, which have more extensive spatial coverage (Reich *et al.*, 1998b; Atkin *et al.*, 2015; Rowland *et al.*, 2016; Ren *et al.*, 2023). However, the relationships between R_{dark25} and leaf traits vary in nature, influenced by multiple biotic and abiotic variables (Wright *et al.*, 2006; Reich *et al.*, 2008; Atkin *et al.*, 2015; O'Leary *et al.*, 2017), leading to uncertainties that restrict the fine-scale R_{dark25} explanations and predictions within and across forest biomes.

Besides the widely studied leaf economics traits (i.e. V_{cmax25}, LMA, leaf N and P concentrations) that are related to R_{dark25}, other leaf elements might also crucially explain R_{dark25} variability (Atkin, 2011; O'Leary *et al.*, 2017; Tcherkez *et al.*, 2024). Magnesium (Mg), a core component of the energy transfer process in leaf respiratory metabolism, plays a crucial role in ATP synthesis and hydrolysis through the Mg-ATP/ADP complex, which can bind up to 50% of the total cellular Mg concentration (Maguire & Cowan, 2002; Cakmak & Yazici, 2010; Chen *et al.*, 2018). Manganese (Mn) shares similar ionic radii with Mg, and the two elements can substitute for each other in metal-binding sites, resulting in co-regulation of respiration rates in plants (Bloom & Kameritsch, 2017). Calcium (Ca) can affect leaf respiration processes as it acts as both the signal transduction ion that binds to a variety of respiratory-related carriers (e.g. mitochondrial aspartate/glutamate carrier and ATP-Mg/Pi carrier) (Bhosale *et al.*, 2015; Rueda *et al.*, 2016), and the activator of mitochondrial dehydrogenase (Satrústegui *et al.*, 2007). Potassium (K) can differentially regulate the activities of glycolysis and tricarboxylic acid cycle (TCA) involved in mitochondrial respiration processes (Okamoto, 1967, 1968; Cui *et al.*, 2019). Sulfur (S) in thioredoxin participates in the redox regulation that largely affects plant respiratory function, by binding to respiratory proteins (Balmer *et al.*, 2004; Gelhaye *et al.*, 2004). Despite their importance for respiratory metabolism, little attention has been paid to how variation in R_{dark25} is linked to differences in Mg, Mn, Ca, K and/or S abundance in leaves across terrestrial forest ecosystems.

Additionally, large-scale surveys of R_{dark25} variability and mechanistic understanding of its driving factors are also greatly limited by traditional R_{dark25} measurement (Garcia *et al.*, 2022), determined by measuring the changing flux rate of oxygen or carbon dioxide concentrations in dark-adapted leaves (Long & Bernacchi, 2003; Coast *et al.*, 2019; Lamour *et al.*, 2021), which is often time-consuming and labor-intensive, and may be limited by canopy access. Therefore, an efficient, rapid and accurate alternative for characterization of R_{dark25} variability remains particularly imperative. Leaf reflectance spectroscopy has shown a substantial potential in multi-scale monitoring of leaf–trait variations within and across terrestrial ecosystems (Asner *et al.*, 2016; Fu *et al.*, 2020; Serbin & Townsend, 2020; Lamour *et al.*, 2023; Liu *et al.*, 2024). This potential arises primarily from the strong connection between the emergent continuous leaf reflectance spectrum with respect to solar radiation and a broad suite of underlying leaf structural, biochemical and nutritional characteristics that drive the reflectance signatures through the interaction of radiation within internal leaf electronic and vibrational absorption properties (Curran, 1989; Elvidge, 1990; Kokaly *et al.*, 2009). By employing statistical modeling methods such as partial least squares regression (PLSR) that harness the full-band leaf reflectance spectra, key signal bands related to target traits can be identified (Ollinger & Smith, 2005; Serbin *et al.*, 2014) and the primary mechanisms underlying spectral predictions can be verified (Dechant *et al.*, 2017; Chavana-Bryant *et al.*, 2019; Meacham-Hensold *et al.*, 2019). Accordingly, a multitude of studies has confirmed that leaf reflectance spectroscopy can estimate a wide range of leaf morphological, biochemical to physiological traits. Leaf spectroscopy should also be efficient to estimate R_{dark25}, as it can effectively predict LMA and V_{cmax25} and empirically estimate leaf nutrients (N, P, K, Ca, Mg, S and Mn) concentrations, all of which are loosely associated with R_{dark25} (Asner *et al.*, 2016; Serbin *et al.*, 2019; Yan *et al.*, 2021; Kothari *et al.*, 2023). Recent studies have indeed revealed the potential capacity of leaf spectroscopy to predict

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variation in R_{dark25} in wheat (Coast *et al.*, 2019) and in a tropical forest ecosystem (Lamour *et al.*, 2021). Therefore, we suspect that spectroscopy is likely to be a promising alternative approach for characterizing how R_{dark25} varies across a large breadth of trait and spectra space and across several terrestrial ecosystems. However, the prior studies of spectra–R_{dark25} models are constrained to a limited number of species and forest types, and the generalizability of spectra–R_{dark25} models remains unknown across diverse forest ecosystems.

The goal of this study was to explore linkages between R_{dark25} and leaf traits both within and across diverse forest types, and evaluate the performance of leaf reflectance spectroscopy as a more efficient alternative for predicting R_{dark25}. Specifically, we addressed the following two questions:

(**1**) How do the empirical relationships of R_{dark25} with V_{cmax25} and with leaf morphological and biochemical traits vary within and across forest types?

(**2**) Can leaf reflectance spectroscopy provide an efficient and robust alternative for predicting R_{dark25} within and across forest types?

To address these questions, we collected a dataset of R_{dark25}, V_{cmax25}, LMA, leaf N, P, K, Ca, Mg, S and Mn concentrations, and leaf reflectance spectra of canopy trees from three different forest sites. These sites are located across a large latitudinal gradient, and include a temperate, mixed needle-/broad-leaved forest, a subtropical evergreen broad-leaved forest and a tropical evergreen broad-leaved forest, hence achieving a broader comparison of species and forest types than incorporated in previous studies. Through addressing these questions, we hope this work can foster the mechanistic understanding and effective monitoring of R_{dark25} variability across forest types, and improve the representation of leaf R_{dark25} in TBMs to better model terrestrial plant respiration and the C cycle.

Materials and Methods

Study sites and plant materials

This study was conducted at three forest sites in China (Fig. 1): (1) Mountain Changbai (CB; 42°24′N, 128°06′E), a temperate, mixed needle-/broad-leaved forest with a mean annual temperature (MAT) of 2.8°C and a mean annual precipitation (MAP) of 691 mm (He *et al.*, 2019); (2) Mountain Gutian (GT; 29°15′N, 118°07′E), a subtropical evergreen broad-leaved forest with a MAT of 15.3°C and a MAP of 1963.7 mm (Ning *et al.*, 2013); and (3) Xishuangbanna (XSBN; 21°47′N, 101°03′E), a tropical evergreen broad-leaved forest with a MAT of 21.8°C and a MAP of 1493 mm (Shen *et al.*, 2018). The three sites are representative of the range of forests found in China, and span a great diversity of biotic (tree species and forest types) and abiotic (temperature, precipitation and soil properties) conditions (detailed information on soil pH, organic C, total N and total P are provided in Supporting Information Table S1). This large range in species and abiotic environmental conditions create the opportunity to investigate trait and spectral variation and evaluate more generalizable approaches (Guo *et al.*, 2022).

In the three forest sites, the Chinese Academy of Sciences operates a tower crane that, we used/accessed to collect sunlit leaves from the representative canopy trees. The tower crane facilities enabled access to a 1-ha area of each forest site, and the crane tower was 40 m high at CB, 60 m at GT and 81 m at XSBN. Specifically, 80 trees from 9 dominant canopy tree species in CB (Yan *et al.*, 2021; Liu *et al.*, 2023), 93 trees from 17 dominant canopy tree species in GT (Hu *et al.*, 2005) and 95 trees from 39 dominant canopy tree species in XSBN (Shen *et al.*, 2018; Liu *et al.*, 2023) were selected as shown in Table S2. Sunlit branches in the upper canopy of these trees were sampled during the peak growing season (July–August) of 2023. We excised branches from the trees before dawn. The excised branches were immediately put in water and re-cut 10 cm away from the initial cut, ensuring that the branches were always immersed during the sampling and measurements. These precautions were taken to avoid xylem embolisms and water stress (Wu *et al.*, 2016) and limit the impact of excision on leaf physiology (Verryckt *et al.*, 2020; Akalusi *et al.*, 2021; Ferguson *et al.*, 2023) and spectral reflectance (Haynes *et al.*, 2024; Fig. S1). On each branch, we selected mature leaves to measure leaf gas exchange, leaf reflectance spectra, and leaf morphological and biochemical traits (i.e. LMA and leaf N, P, K, Ca, Mg, Mn and S concentrations). The statistical results of these measurements are summarized in Table S2. The detailed protocols of measurement are described as follows.

Field measurements

Leaf gas exchange Branch samples were stored in individual buckets and placed in the shade until used for leaf gas exchange measurements. We used six portable Li-Cor gas exchange systems (two Li-6400XTs and four Li-6800s; Li-Cor Inc., Lincoln, NE, USA) simultaneously to measure the response of net assimilation rate (A) to intercellular carbon dioxide concentration (C_i) (commonly known as an A-C_i curve) and instantaneous leaf respiration in dark-adapted leaves (R_{dark}) between 06:00 and 17:00 h each day. Our previous study has demonstrated that the type of Li-Cor gas exchange system did not affect the results (Liu *et al.*, 2023).

Measurement of A-C_i curves closely followed Rogers *et al.* (2017) and Yan *et al.* (2021) with details shown in Methods S1. Measurements of R_{dark} followed established protocols (Rowland *et al.*, 2016; Lamour *et al.*, 2021). The leaf was dark-adapted to eliminate the influence of light on leaf respiration (Shapiro *et al.*, 2004) for a period of 25 min, with the light source off and a dark cloth positioned on the instrument to avoid diffusion of light inside the chamber. The chamber conditions were set as follows: reference CO_2 concentration at 400 ppm, exchanger temperature set at the outside temperature to prevent the risk of condensation in the instrument and flow rate at 350 μmol s^{-1} to maximize the signal : noise ratio. After the dark adaptation period, CO_2 exchange rates and chamber conditions were checked for stability over a 5 min window before measurements occurred. Once stable, gas exchange was measured every 5 s for 1 min to constitute one measurement of R_{dark}. To

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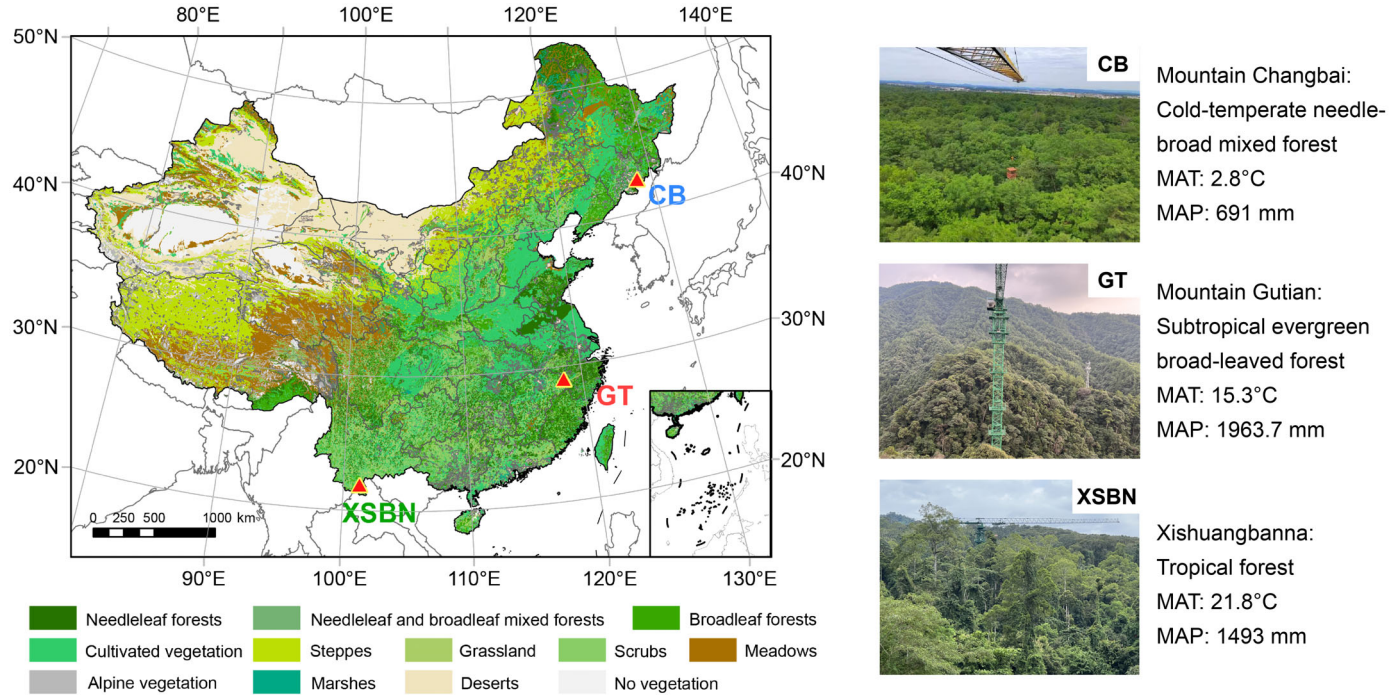

Fig. 1 Location and basic information of the three typical forest sites in China. The three forest sites span a large latitudinal gradient, including a temperate forest in mountain Changbai (CB), a subtropical forest in mountain Gutian (GT) and a tropical rainforest in Xishuangbanna (XSBN). All the three forest sites have tower crane facilities enabling to access to the sunlit canopy leaves. The background shows a map of the 1 : 1000 000 scale vegetation distribution of China provided by 'Environmental & Ecological Science Data Center for West China, National Natural Science Foundation of China' (http://westdc.westgis.ac.cn). MAP, mean annual precipitation; MAT, mean annual temperature.

enable temperature-standardized comparisons of respiration rate, we cross-compared two approaches to adjust R_{dark} to a reference temperature of 25°C (R_{dark25}). One method used an inverse Arrhenius equation (Davidson *et al.*, 2023b), while the other method used a temperature-dependent Q_{10} based on a known rate at measured temperature (as used in Atkin *et al.*, 2015). We found that R_{dark25} calculated by these two approaches was almost identical (Fig. S2), therefore chose the widely used method described by Davidson *et al.* (2023b) to calculate R_{dark25} (Bernacchi *et al.*, 2001, 2013; Von Caemmerer, 2013).

Leaf reflectance spectra After completing the gas exchange measurements, we promptly measured the leaf reflectance spectra. A portable handheld contact-type spectrometer QualitySpec Trek (PANalytical, Boulder, CO, USA; spectral full-range: 400–2500 nm; spectral resolution: ≤ 3 nm at 700 nm, ≤ 9.8 nm at 1500 nm, ≤ 8.1 nm at 2100 nm; sampling internal: 1 nm through the linear interpolation) with a leaf clip was used to measure the spectral reflectance of leaves. At instrument startup, a 99% reflective Spectralon white reference disk (Labsphere Inc., North Dutton, NH, USA) was placed on the outside of the sampling window to calibrate the reflectance. The instrument was also calibrated automatically every half hour during operation using an internal white reference on the inside of the sampling window. Leaf spectral reflectance was calculated following the protocol by Wu *et al.* (2019) and Yan *et al.* (2021). Depending on leaf size, measurements were taken at three to six different positions on the adaxial side of each leaf, and the average reflectance for each wavelength was calculated as the reflectance spectrum of the leaf. During the collection of spectral data, issues such as spectral response saturation, instrument overheating and abnormal spectral responses were addressed promptly according to prompts from the Trek instrument and the user manual.

Leaf biochemical and morphological traits Following spectral measurements, the leaves without petiole were sampled for eight morphological and biochemical traits, including LMA, leaf N, P, K, Ca, Mg, Mn and S concentrations. The measurements were performed via protocol in Methods S2, and they provided mass-based estimates of elemental concentrations. To make it comparable with the area-based R_{dark25}, we convert them to area-based units for subsequent analysis.

Data analysis

Exploring trait–R_{dark25} relationships within and across forest types To investigate the relationships between R_{dark25} and other leaf traits within and across the three forest sites, we conducted four analyses. First, to examine the differences in leaf traits across forest types, we employed a one-way analysis of variance (ANOVA) with the least significant difference (LSD) *post hoc* test for multiple comparisons. To assess the individual contribution of each trait to the prediction of R_{dark25} within and across

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forest types, we performed ordinary least squares (OLS) regression and slope tests via R (v.4.3.2; R Core Team, 2013) package SMATR on the relationships between R_{dark25} and other leaf traits. To rank the relative importance (RI) of each of the eight relatively easy-to-measure traits (i.e. LMA, leaf N, P, K, Ca, Mg, Mn and S concentrations) in predicting R_{dark25}, the R package RELAIMPO (Wang *et al.*, 2021) was used to analyze based on data from all three forest sites. We explored the collective contribution of the eight relatively easier-to-measure traits to predicting R_{dark25} through multivariate linear regression. Specifically, we employed multiple linear regressions as: lm (R_{dark25} ~ *trait variables*) to evaluate the predictive capability of these traits for R_{dark25} within and across the forest sites. A backward stepwise regression based on the Akaike information criterion (AIC) was used to determine whether traits with low RI could be excluded from the model. To ensure the validity and comparability, we implemented a repeated double cross-validation (rdCV) (Filzmoser *et al.*, 2009; see the subsequent section on PLSR modeling) consisting of 10-fold cross-validation with 200 repetitions. This method maintains consistency with the validation strategy employed in spectroscopy modeling (Fig. S3).

Developing spectral models of R_{dark25} and leaf morphological and biochemical traits To establish the spectra–trait models, we followed existing protocols (Dechant *et al.*, 2017; Burnett *et al.*, 2021; Yan *et al.*, 2021). We adopted the PLSR method (Wold *et al.*, 2001) in conjunction with a rdCV, implemented using the PYTHON library SCIKIT-LEARN (Pedregosa *et al.*, 2011), to develop the spectra–trait models. The PLSR method, similar to principal component analysis (PCA), reduces the number of predictor variables down to a set of orthogonal latent variables (Liu *et al.*, 2023) and accommodates scenarios where the number of predictor variables greatly exceeds the number of response variables (Yan *et al.*, 2021). This enables PLSR to summarize complex spectral data, solve the problem of collinearity of variables and achieve direct interpretation with linear regression, capturing the relationship between reflectance and physiological traits (Burnett *et al.*, 2021). Given these advantages, PLSR has been extensively applied in spectroscopic and chemometric analyses (Ollinger & Smith, 2005; Serbin *et al.*, 2014, 2019). The rdCV method separates the data repeatedly and randomly through cross-validation procedures into a calibration subset (containing training and test components) and an independent validation subset, and evaluates models were conducted on independent validation subsets generated from numerous possible random splits. This method thereby possesses the advantage of decreasing the odds of good or bad outcomes solely due to chance (Wu *et al.*, 2019). The modeling procedures have been shown by Dechant *et al.* (2017), Yan *et al.* (2021) and Liu *et al.* (2023), and further modeling details are shown in Methods S3 and Figs S3–S5.

Exploring the generalizability of spectra–R_{dark25} relationships under different spectral modeling scenarios To test the generalizability of spectra–R_{dark25} model, we cross-compared PLSR model performance under two modeling scenarios: 'site-specific' and 'cross-site' scenario, and summarized the model performances in Table 1. In the 'site-specific' scenario, the spectra–R_{dark25} model was developed and evaluated using the data from each single forest site only. Each resulting PLSR model was then applied to the left-out data from the two other sites. With the 'cross-site' scenario, one spectra–R_{dark25} model was developed and evaluated using full dataset from the three sites. These modeling tests allowed us to assess the generalizability of spectra–R_{dark25} relationships, and reveal potential scenarios and reasons for the deterioration of spectra–R_{dark25} relationships.

Table 1 Performance of the two types of spectra–R_{dark25} models: 'site-specific model' (trained and evaluated using the data from the single forest site, respectively, and then applied to the other two independent sites), and 'cross-site model' (trained and evaluated using the data from all the three forest sites).

		R_{dark25} (μmol CO_2 m^{-2} s^{-1})		
Scenarios	Site	*n*	r^2	RMSE
CB model	CB	146	0.67	0.16
	GT	173	0.06	0.46
	XSBN	151	0.07	0.35
	All	470	0.23	0.35
GT model	CB	146	0.19	0.31
	GT	173	0.58	0.15
	XSBN	151	0.19	0.28
	All	470	0.28	0.25
XSBN model	CB	146	0.33	0.25
	GT	173	0.46	0.18
	XSBN	151	0.76	0.13
	All	470	0.56	0.19
Cross-site model	CB	146	0.51	0.20
	GT	173	0.57	0.16
	XSBN	151	0.71	0.16
	All	470	0.65	0.17

The repeated double cross-validation method was used for training and evaluating all the models. The three diverse forest sites span large latitudinal gradients, including the temperate forest in mountain Changbai (CB), the subtropical forest in mountain Gutian (GT) and the tropical forest in Xishuangbanna (XSBN). *n*, sample size; r^2, the coefficient of determination; R_{dark25}, leaf dark respiration standardized to 25°C; RMSE, the root mean square of error.

Cross-comparison of field-measured and spectra-modeled R_{dark25} variability in relation to leaf traits To evaluate the influence of spectral model performance on trait–R_{dark25} relationships and the drivers of R_{dark25} variability, we explored the relationships between R_{dark25} and values of the other eight leaf morphological and biochemical traits predicted by the 'cross-site' spectral models. We then cross-compared these relationships with those derived from field measurements. Furthermore, we analyzed the inter- and intraspecific variation of leaf traits within each forest site, as well as in predicted values and direct field measurements across all sites following the method proposed by Guillén-Escribà *et al.* (2021). We used an ANOVA-based general linear model: R_{dark25} ~ *trait* + *species*, and partitioned the total variance among

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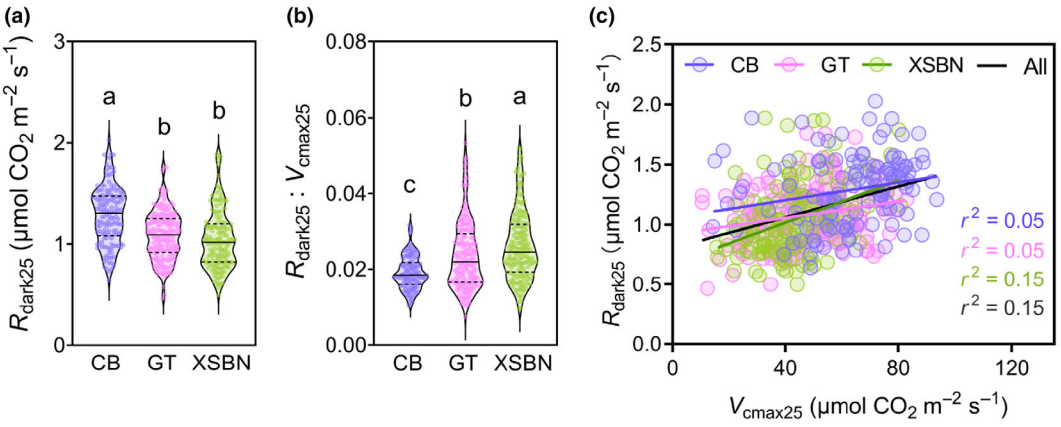

Fig. 2 Variability of R_{dark25} and its relationship with V_{cmax25} within and across forest sites. (a) The differences in R_{dark25} among diverse forest sites. (b) The differences in the ratio of R_{dark25} to V_{cmax25} (R_{dark25} : V_{cmax25}) among diverse forest sites. (c) R_{dark25}–V_{cmax25} relationships within and across forest sites. These values are gas exchange measurements. One-way ANOVA with the least significant difference *post hoc* test was used for the comparisons among the three forest sites. Different lower-case letters adjoining the violin plots indicate the significant difference ($P < 0.05$) among different groups. The fitted lines were determined by ordinary least squares regressions, showing significantly different slopes among the Changbai (CB), Gutian (GT) and Xishuangbanna (XSBN) sites, which represent temperate, subtropical and tropical forests in China, respectively. The black line represents regression fit for all sites. r^2 is the coefficient of determination and the relationships are all significant ($P < 0.05$). Horizontal solid and dotted lines in violin plots are the median and quartiles, respectively.

individual leaves by using the percentage of variance explained by the species term to represent the interspecific components, with the residuals representing the intraspecific components.

Results

Trait–R_{dark25} relationships and the relative importance of leaf traits for predicting R_{dark25} across forest types

To investigate our first question regarding trait-based methods for R_{dark25} predictions, we first analyzed the variations in R_{dark25}, V_{cmax25}–R_{dark25} relationships, and leaf morphological and biochemical traits within and across different forest types. We observed similar R_{dark25} in tropical and subtropical forest, but significant higher R_{dark25} in temperate forest compared with the other two forest types ($P < 0.001$) (Fig. 2a). Subsequently, we found that R_{dark25} : V_{cmax25} ratio was 0.023 across the three forest sites and exhibited significant differences in these sites ($P < 0.001$; Fig. 2b), with higher R_{dark25} : V_{cmax25} ratio at tropical forest in Xishuangbanna (0.026), followed by subtropical forest in GT (0.024), and temperate forest in CB (0.019). In addition, we found that LMA and multi-elemental (except Mg) concentrations showed large variability within and across the three forest sites, with Ca concentration exhibiting the similar trends as R_{dark25} (Fig. S6). In addition, there were covariations among biochemical traits (Fig. S7).

To further explore the ability of traits to predict R_{dark25} at our sites, we conducted both univariate and multiple linear regressions (multiple traits have no collinearity effect on R_{dark25}; VIF < 5; Figs 3, 4; Table S3). We found that univariate trait–R_{dark25} relationships were weak ($r^2 \leq 0.13$), and that the slope and intercept of the linear regression were site dependent (Fig. 3). Both the univariate and multivariate analyses suggested that the best predictor of R_{dark25} variation was leaf Mg concentration ($r^2 = 0.13$, $P < 0.001$; RI = 23%), followed by leaf Ca ($r^2 = 0.13$, $P < 0.001$; RI = 21%), N ($r^2 = 0.06$, $P < 0.001$; RI = 17%), Mn ($r^2 = 0.06$, $P < 0.001$; RI = 15%), S ($r^2 = 0.06$, $P < 0.001$; RI = 10%) and P ($r^2 = 0.06$, $P < 0.001$; RI = 8%) concentrations (Figs 3b,c,e–h, 4b). Leaf K concentration ($r^2 = 0.02$, $P < 0.001$; RI = 4%) and LMA ($r^2 = 0.00$, $P > 0.05$; RI = 2%) were relatively poor predictors of R_{dark25} across forest types (Fig. 3a,d). Leaf K was removed from stepwise regression with AIC = 22.20. Multiple linear regression models exhibited better R_{dark25} prediction ($r^2 = 0.30$; RMSE = 0.25 μmol CO_2 m^{-2} s^{-1}) than univariate linear models across forest types (Figs 3a–h, 4b). In opposite to other nutrients, leaf Mn concentration showed a significantly negative relationship with R_{dark25} across diverse forest types (Fig. 3g).

Leaf reflectance spectroscopy outperforms leaf–trait relationships in predicting R_{dark25} across forest types

We next examined the question of whether leaf reflectance spectra are sufficient to estimate R_{dark25} and retrieve the trait–R_{dark25} relationships across forest types. We found that our 'cross-site' spectral models were the most robust for R_{dark25} prediction ($r^2 = 0.65$, RMSE = 0.17 μmol CO_2 m^{-2} s^{-1}) and performed better than the multivariate model based on leaf traits ($R^2 = 0.30$, RMSE = 0.25 μmol CO_2 m^{-2} s^{-1}) (Figs 4b, 5). Meanwhile, the 'cross-site' spectral models captured the variations in all the other leaf traits including LMA ($r^2 = 0.96$, RMSE = 6.78 g m^{-2}), leaf Mn ($r^2 = 0.79$, RMSE = 0.03 g m^{-2}), Mg ($r^2 = 0.77$, RMSE = 0.04 g m^{-2}),

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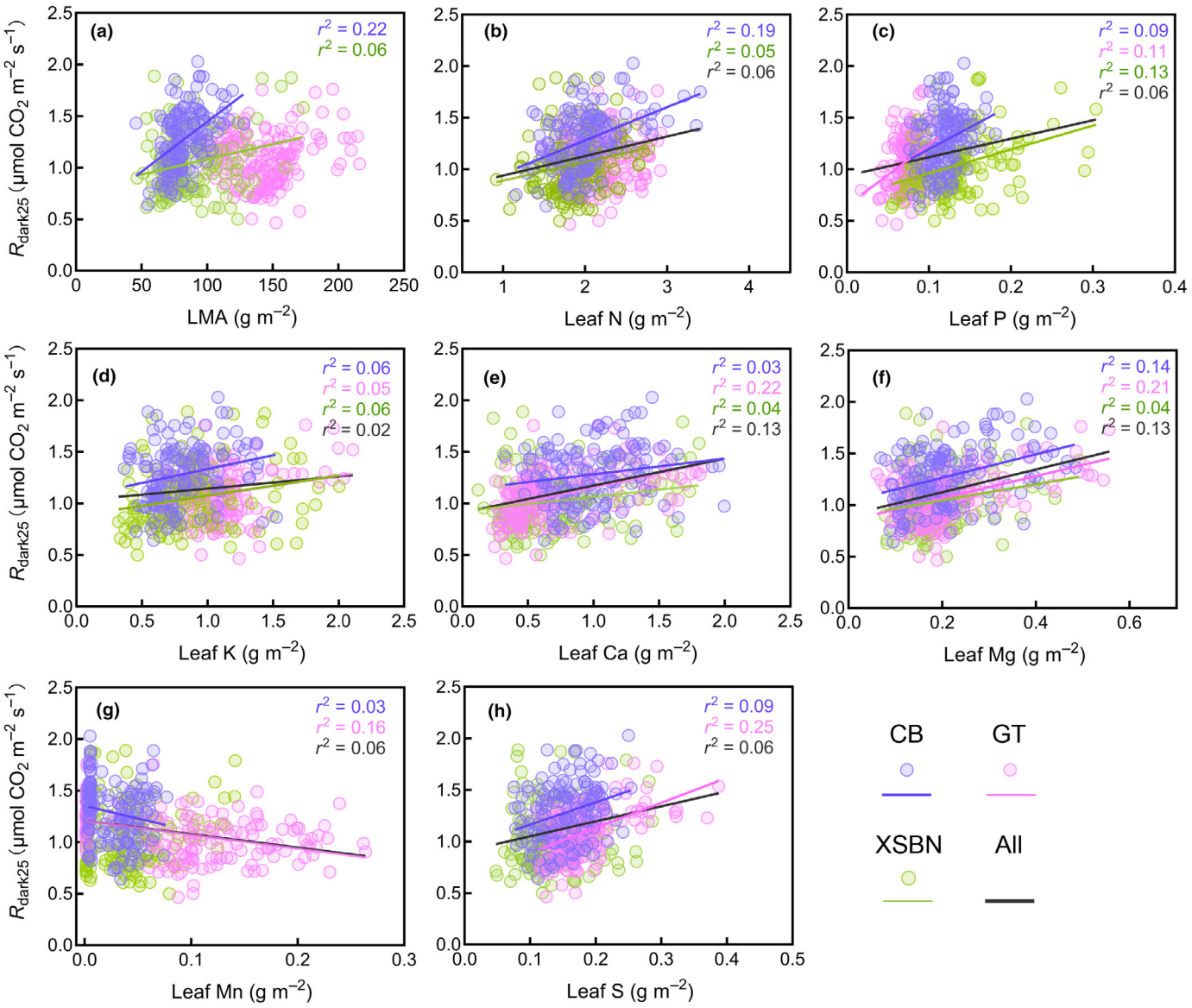

Fig. 3 Trait–R_{dark25} relationships within and across forest sites. (a–h) Pairwise relationships between R_{dark25} and eight leaf traits (i.e. leaf mass per area (LMA), and leaf N, P, K, Ca, Mg, Mn and S concentration). The fitted lines are determined by the ordinary least squares regression, with colored lines representing regression fits for specific sites and black lines representing regression fit for all sites. r^2 is the coefficient of determination. All metrics and lines shown are statistically significant ($P < 0.05$). CB, mountain Changbai; GT, mountain Gutian; XSBN, Xishuangbanna.

N (r^2 = 0.74, RMSE = 0.20 g m^{-2}), P (r^2 = 0.72, RMSE = 0.02 g m^{-2}), Ca (r^2 = 0.71, RMSE = 0.21 g m^{-2}), K (r^2 = 0.71, RMSE = 0.18 g m^{-2}) and S (r^2 = 0.70, RMSE = 0.03 g m^{-2}) concentrations across forest types (Fig. S8a–h). The linear relationships between spectrally derived traits and spectrally derived R_{dark25} were highly consistent with those obtained from field measurements, with commensurable r^2, slopes and intercepts for these two types of leaf–trait relationships (Fig. S9a–h). In addition, we found that while the contributions of inter- and intraspecific variation to R_{dark25} variability were similar between the spectra-modeled and field-measured methods, the interspecific variation was slightly greater in the spectrally modeled approach (Fig. 6a–d). These results verified that leaf reflectance spectroscopy provided an accurate alternative for inferring R_{dark25} and trait–R_{dark25} relationships across diverse forest types.

To further test the generalizability of spectra–R_{dark25} relationships, we cross-compared the spectra–R_{dark25} models under 'site-specific' and 'cross-site' scenarios. As shown in Fig. 5 vs Fig. 7 and Table 1, the 'cross-site' model outperformed 'site-specific' models across different forest types (r^2 = 0.65, RMSE = 0.17 μmol CO_2 m^{-2} s^{-1}) (Fig. 5), followed by the 'site-specific' XSBN model (r^2 = 0.56, RMSE = 0.19 μmol CO_2 m^{-2} s^{-1}) (Fig. 7c), GT model (r^2 = 0.28, RMSE = 0.25 μmol CO_2 m^{-2} s^{-1}) (Fig. 7b) and CB model (r^2 = 0.23, RMSE = 0.35 μmol CO_2 m^{-2} s^{-1}) (Fig. 7a).

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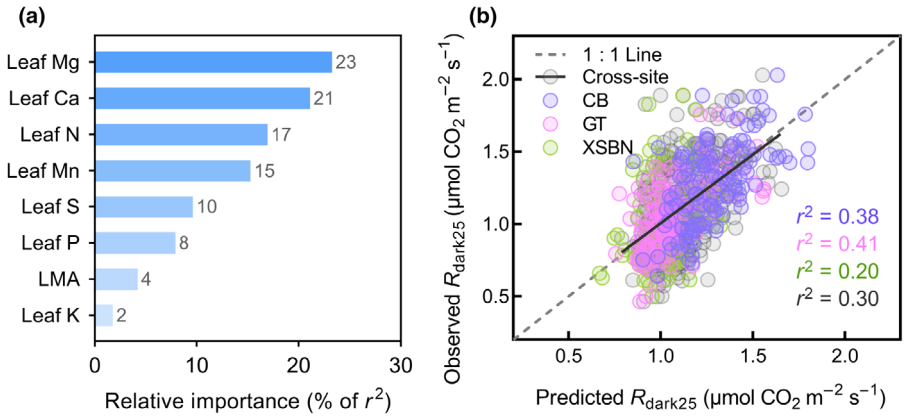

Fig. 4 Performance of multiple linear models for predicting R_{dark25} using all the other eight easy-to-measure leaf traits as predictor variables, and the relative importance of these predictor variables. (a) The relative importance of these traits on R_{dark25} prediction across forest sites, derived from their proportions of the explanation of the variance (r^2) indicated by the number adjoining the bars. (b) The performance of site-specific and cross-site model using multiple leaf traits (i.e. LMA, leaf N, P, Ca, Mg, Mn and S concentrations) for predicting leaf R_{dark25}. The colored circles in the figure represent site-specific predictions (with mountain Changbai (CB) in mediumslateblue, mountain Gutian (GT) in orchid and Xishuangbanna (XSBN) in green), the gray circles represent the predictions of the cross-site multivariable linear model, and black line is the ordinary least squares fit of the cross-site model.

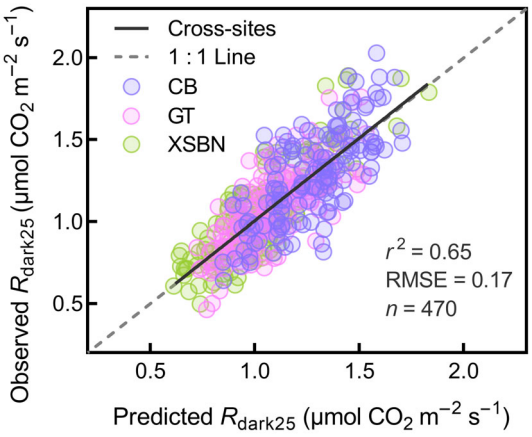

Fig. 5 Accuracy assessment for the cross-site spectra–R_{dark25} relationship. The cross-site model used the whole data set of leaf R_{dark25} and reflectance spectra from the three forest sites, and was trained and evaluated using the repeated double cross-validation method. The black line is the ordinary least squares fit; the gray line indicates the 1 : 1 line. The colored points represent the predictions of the cross-site PLSR model for each forest site (with mountain Changbai (CB) in mediumslateblue, mountain Gutian (GT) in orchid and Xishuangbanna (XSBN) in green). n, sample size; r^2, the coefficient of determination; RMSE, the root mean square of error.

Importantly, as the number of species or range of R_{dark25} included in the data of 'site-specific' model training subset decreased, the models exhibited worse performance and increased bias (as indicated by the RMSE and slopes) when applied to predict R_{dark25} for the sites not involved in the spectral modeling (Fig. 7). These results suggest that an accurate and transferable spectra–R_{dark25} model could be developed only when sufficient ranges of R_{dark25} variability and species diversity were encompassed in the PLSR modeling.

To untangle the underlying mechanism of the cross-site spectral–R_{dark25} model, we identified key band for prediction by analyzing the patterns in the PLSR variable importance in projection (VIP) metrics and coefficients (Fig. 8). Specifically, our 'cross-site' spectra–R_{dark25} model revealed the important bands as follows (VIP > 1, Liu *et al.*, 2023): (1) 400–427, 519–575 and 694–700 nm in the visible band; (2) 700–800 nm in the red-edge band; (3) 800–924 nm in near-infrared band; and (4) short-wave infrared band of 1374–1428, 1645–1672, 1860–1887, 2156–2221 and 2253–2315 nm (Fig. 8a).

Discussion

Leaf R_{dark25}, an important, complex but difficult-to-measure eco-physiological trait, significantly contributes to the whole-plant net C exchange, and is an important physiological parameter in many TBMs to estimate ecosystem respiration and global C cycling (Atkin *et al.*, 2015; Huntingford *et al.*, 2017; Butler *et al.*, 2021; Ren *et al.*, 2023). However, a coherent understanding and efficient monitoring of fine-scale R_{dark25} variability across forest types remain elusive. Here, we revealed the relationships of R_{dark25} with usual suspects leaf traits and leaf reflectance spectra across diverse forest types spanning large environmental gradients. We made two important findings. First, we found that leaf Mg, Ca and Mn concentrations were important in explaining R_{dark25}, but the relationships between R_{dark25} and other leaf traits were relatively weak ($r^2 \leq 0.13$) and forest type-specific. Second, leaf reflectance spectroscopy could be used to create a single robust model of R_{dark25} predictions across forest types ($r^2 = 0.65$), with the transferability of the spectra–R_{dark25} model dependent on the trait range and spectral diversity in the trait training data. Taken together, our study expands on the key determinants of R_{dark25}, and highlights the substantial potential of reflectance spectroscopy in fast, reliable and high-throughput monitoring of plant eco-physiological traits and C cycling.

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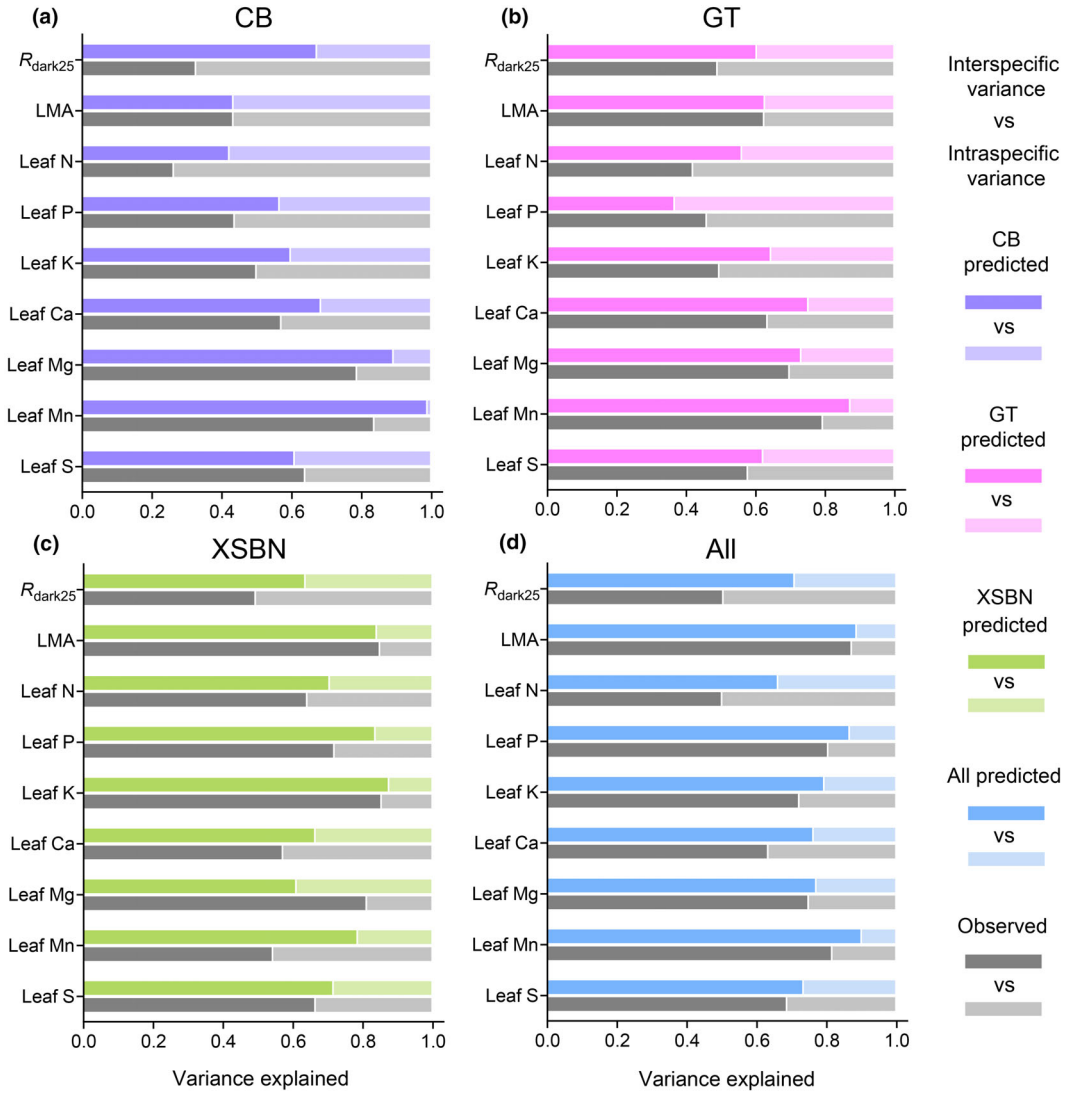

Fig. 6 Variance partitioning of leaf R_{dark25} and leaf morphological (i.e. LMA) and biochemical (i.e. leaf N, P, K, Ca, Mg, Mn and S concentrations) traits within (a–c) and across forest sites (d). The forest sites include the temperate forest in mountain Changbai (CB), the subtropical forest in mountain Gutian (GT), and the tropical forest in Xishuangbanna (XSBN) in China. The total variability for each trait was partitioned into intraspecific and interspecific components. The gray bars denote the variance partitioning from field-observed leaf traits, while the colored bars indicate the variance partitioning results from leaf traits predicted from the cross-site spectra–trait models.

Variations in R_{dark25} and R_{dark25} : V_{cmax25} ratio across diverse forest types

Our results revealed substantial variation in leaf R_{dark25} and the R_{dark25} : V_{cmax25} ratios within and across forest types in China spanning large environmental gradients. The observations agree with previous field-based or global-scale synthesis studies regarding the latitudinal pattern of leaf respiratory and photosynthetic traits (Reich *et al.*, 1998b; Wright *et al.*, 2006; Atkin *et al.*, 2015). While the large within-site variation may have weakened the effect of climate gradients and make R_{dark25} : V_{cmax25} lower in temperate forests than in tropical forests, which is different from previous studies (Atkin *et al.*, 2015). This variation may have several possible explanations, including the thermal acclimation responses of respiratory metabolism, contrasting species survival strategies and nutrient availability, which all affect the cost of investments in the respiratory apparatus and the balances between respiratory and photosynthetic metabolism (Lambers, 1985; Reich *et al.*, 1998a; Atkin & Tjoelker, 2003; Ren *et al.*, 2023). As a result, despite the strong functional coupling between R_{dark25} and V_{cmax25} underpinned by chloroplast-mitochondrion interdependence, the proportionality of the two traits varies across species types and environmental conditions (Reich *et al.*, 1998b; Atkin *et al.*, 2015). However, despite the dynamic nature of both R_{dark25} and R_{dark25} : V_{cmax25} observed here and previously, a PFT-specific R_{dark25} value or constant R_{dark25} : V_{cmax25} ratio still remains widely used in TBMs to simulate plant respiration (Schwalm *et al.*, 2010; Clark

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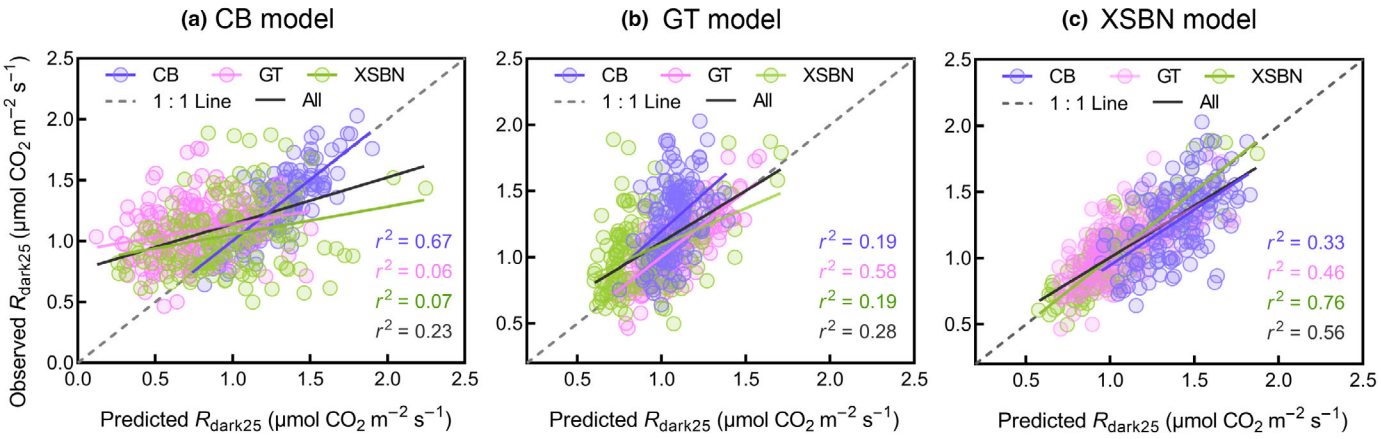

Fig. 7 Accuracy assessment for the spectra–R_{dark25} relationship under site-specific modeling scenarios. (a) CB model; (b) GT model; and (c) XSBN model. The site-specific models used specific data sets of leaf R_{dark25} and reflectance spectra within each of the three forest sites, was trained and evaluated using repeated double cross-validation method and then was applied to the other two independent sites. The colored points and lines represent the predictions of the PLSR model and ordinary least squares fit for each forest site (with mountain Changbai (CB) in mediumslateblue, mountain Gutian (GT) in orchid and Xishuangbanna (XSBN) in green). The number of species in the CB model, GT model and XSBN model are 10, 16 and 33, respectively.

et al., 2011; Fan *et al.*, 2021). Our results show that compared with what is in some TBMs, R_{dark25} at a given V_{cmax25} is higher in our forest types (R_{dark25} : V_{cmax25} ratio ranging from 0.019 to 0.026) than commonly assumed (0.015), which means the TBMs would underestimate R_{dark}. Therefore, we suggest incorporating more flexible parameterization schemes in TBMs to better capture the variation in large-scale terrestrial respiration, and model the associated C fluxes under the current and changing climate (Kyker-Snowman *et al.*, 2022).

Biochemical traits reveal additional sources of leaf R_{dark25} variability

Leaf trait–R_{dark25} relationships are widely used to derive R_{dark25} from other morphological and biochemical traits, and formulate the empirical equations to facilitate the representation of R_{dark25} in TBMs (Atkin *et al.*, 2015; Rowland *et al.*, 2016). Among those traits, LMA, leaf N and P concentrations are often used, given their important roles in leaf construction costs, protein turnover, N assimilation, mitochondrial electron transport and glycolysis, which are functionally inter-dependent with leaf respiratory metabolism (Meir *et al.*, 2001; Tjoelker *et al.*, 2002; Fernie *et al.*, 2004; Turnbull *et al.*, 2005). Our results demonstrated weak ($r^2 \leq 0.06$) and forest type-specific relationships of R_{dark25} with these three traits (Fig. 3a–c), which are consistent with previous studies that reported the moderate-to-weak trait–R_{dark25} relationships across different PFTs, growth environments and biogeographical regions (Wright *et al.*, 2006; Reich *et al.*, 2008; Atkin *et al.*, 2015; H. Wang *et al.*, 2020). These observed weak and dynamic trait–R_{dark25} relationships within and across sites might be attributed to the differences in nutrient allocation to metabolic vs structural components, distinct species compositions and the interactions among leaf traits (Millar *et al.*, 2011; Atkin *et al.*, 2015; O'Leary *et al.*, 2018; Rowland *et al.*, 2018). Our study, together with previous findings, therefore suggests that there is no single universal scaling relationship accounting for R_{dark25} over large biogeographical extents. In other words, some prevailing relationships between traits and R_{dark25} observed at the global scale have limited predictability at fine scale. This highlights the uncertainty of leveraging the conventional leaf–trait relationships for characterizing cross-site R_{dark25} variability.

In addition to the aforementioned three leaf traits, we further analyzed multiple leaf morphological and biochemical traits as determinants of R_{dark25}, and found the best predictor of cross-site R_{dark25} variability was leaf Mg concentration, followed by leaf Ca, N, Mn, S and P concentrations, with minor roles of LMA and leaf K concentration (Figs 3, 4). Despite this order, together they control much of R_{dark25} variability. This study quantified the relationships between multiple leaf elemental concentrations and R_{dark25} over a large biogeographical scale, and highlights the important but previously unexpected roles of leaf Mg, Ca, Mn and S concentrations in explaining variability in R_{dark25}. Given the physiological functions of Mg, Ca and S in mitochondrial activities (Millar *et al.*, 2011; Bhosale *et al.*, 2015; Rueda *et al.*, 2016; Chen *et al.*, 2018; Fratte *et al.*, 2021), this may be the main reason for their positive relationship with leaf dark respiratory flux across forest types. It is worth noting that Mg and Ca, as the two most important elements affecting R_{dark25}, also have strong covariation (Fig. S6), suggesting that there may be a pathway between them connected through respiratory metabolism. In contrast with Mg, Ca and S, Mn was negatively correlated with R_{dark25} (Fig. 3g), which has also been described in previous studies (Li *et al.*, 2010; Takagi *et al.*, 2021). The negative correlation of Mn and Mg shows that there is also a competitive relationship between the utilization of the two elements by leaves not only on cell scale but on a large scale (Fig. S6), resulting in their opposite regulations on leaf respiratory metabolism (Bloom & Kameritsch, 2017; Bloom & Lancaster, 2018). The Mn-induced decrease in dark respiration may suggest that the Mn concentrations observed in this study have reached a

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certain range of toxicity, because excessive Mn inhibits NAD-malic enzyme activity (Takagi *et al.*, 2021) or alters stomatal and leaf anatomical development, causing stomatal dysfunction, and thus inhibit the activities of both C anabolism and catabolism (Li *et al.*, 2010). Collectively, all of the examined eight leaf traits jointly contribute to 30% of cross-site R_{dark25} variability, which still leaves a large proportion of unexplained R_{dark25} variance, which might be associated with many other unconsidered factors, such as temperature acclimation, drought, leaf ontogeny, phylogeny, and leaf metabolic traits and metabolic status (Atkin & Macherel, 2009; Atkin, 2011; Reich *et al.*, 2016; O'Leary *et al.*, 2017; Yan *et al.*, 2023). Further studies are thereby needed to reveal the mechanisms underlying the R_{dark25} variability across forest types with the integration of more relevant abiotic and biotic sources.

Spectroscopy is an effective alternative for monitoring and understanding cross-site R_{dark25} variability

Our results showed that leaf reflectance spectroscopy outperformed traditionally used leaf–trait relationships in predicting R_{dark25} across sites (r^2 = 0.65 vs 0.30, RMSE = 0.17 vs 0.25 μmol CO_2 m^{-2} s^{-1}; Figs 4a, 5). To the best of our knowledge, this is the first demonstration of R_{dark25} prediction across forest types using hyperspectral spectra, although previous studies have also shown the efficiency of spectral models in a limited number of species or vegetation types (with relatively few species and limited size of the overall dataset) or of a single species, wheat (Doughty *et al.*, 2011; Coast *et al.*, 2019; Lamour *et al.*, 2021). Our findings of the accurate (r^2 = 0.70–0.96) cross-site spectral modeling of other eight traits also consolidated the recent studies in which leaf reflectance spectroscopy could accurately infer important leaf biochemical, morphological and physiological traits across different plant functional groups and ecosystems (Ely *et al.*, 2019; Nakaji *et al.*, 2019; Kothari *et al.*, 2023). These results therefore reinforce that leaf reflectance spectroscopy offers a viable alternative for monitoring multiple trait dimensions across diverse plant species and growth environments, and can particularly enrich trait databases to fill in the key observational gaps in those difficult-to-measure physiological traits.

Given the likely lack of direct spectral absorption features by components in the respiratory systems, the spectra–R_{dark25} model may rely on the absorption features that are not causally associated with the target trait, but rather indirectly via other covarying traits such as pigments, leaf structure and water content driving the spectral changes ('constellation effects'; Nunes *et al.*, 2017; Chadwick & Asner, 2018). The identified R_{dark25}-sensitive spectral bands are similar to the findings from a previous study on tropical forests (Lamour *et al.*, 2021) and are often shown to be sensitive to leaf biochemical traits (leaf Mg, Ca, N, Mn, S and P) (Kokaly *et al.*, 2009; Osco *et al.*, 2020; Liu *et al.*, 2023; also see Fig. 8). The appearance of similar VIP peaks for leaf traits could be reflective of the shared functional roles. While leaf biochemical traits exhibit similar reflectance patterns to R_{dark25}, their weak explanatory power shown highlights the complexity of these relationships and their potential influence by additional factors. Meanwhile, these identified spectral bands are related to other leaf biochemical and physiological properties. The visible range has been connected with cell pigments; for example, Chl absorbs red and blue light while reflecting green, which gives leaves their characteristic color and is closely linked to leaf N levels (Ustin *et al.*, 2009; S. Wang *et al.*, 2020). The red-edge range is associated with Chl, Chl fluorescence and V_{cmax25} (Zarco-Tejada *et al.*, 2000; Yan *et al.*, 2021), while the detected NIR and SWIR bands are tightly associated with water content, lignin, cellulose, and the amount of starch or lipid, which are substrates for respiration (Kokaly *et al.*, 2009). These suggest that other unmeasured traits or processes might also participate in the indirect prediction of R_{dark25}, and spectroscopy may have potential for simultaneously monitoring a full suite of leave traits. However, when comparing observed and spectrally predicted trait relationships, we observed that the r^2 values were generally higher for spectrally predicted relationships (Fig. S9), likely due to the nonindependence of the estimates, as both traits and R_{dark25} are derived from spectral measurements. This may indicate that the spectra-modeled trait relationships tend to compress the variation and reduce large residuals, suggesting potential biases in the strength of the estimated trait covariance.

Another important finding was that the cross-site general spectra–R_{dark25} model can be built only when leaf samples covering sufficient variability in both R_{dark25} and leaf reflectance spectra are incorporated into the model development. This finding is supported by two aspects: (1) the spectra–R_{dark25} model under 'cross-site' scenario largely outperformed those under the 'site-specific' scenario (Figs 5, 7a–c) and (2) the spectra–R_{dark25} model under the 'site-specific' scenario showed much higher accuracy for its own forest site in contrast with the other two sites not involved in the model development (Table 1). Previous studies have observed that both leaf reflectance spectra and R_{dark25} change remarkably with ecosystems, plant functional groups, climate conditions and leaf ages (Reich *et al.*, 1998b; Atkin *et al.*, 2015; Smith & Dukes, 2018; O'Leary *et al.*, 2023). However, most of these drivers of R_{dark25} are still not sufficiently captured in our study. Notably, most of the variation in spectrally predicted traits appears to be interspecific rather than intraspecific (Fig. 6). This finding underscores the necessity of considering trait variability at larger taxonomic scales when developing spectra-based models (Kothari *et al.*, 2023). Although site-specific models effectively capture variations driven by the local environmental and biological factors of specific forest types (Lamour *et al.*, 2021), they lack the ecological heterogeneity necessary to generalize across greater biogeographical gradients. In cross-site modeling, while some precision at individual sites may be sacrificed, the model's adaptability across different biogeographic regions is significantly enhanced. These results suggested that a more general and transferable spectra–R_{dark25} model would require significantly broader and more diverse datasets, covering a wide range of leaf traits and spectra across diverse plant functional types and ecosystems (Serbin *et al.*, 2019; Burnett *et al.*, 2021; Ji *et al.*, 2024). However, while this step is promising, we still have some distance from being able to rely solely on

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spectroscopy to predict complex traits like R_{dark25} with high accuracy. Achieving this will likely involve trade-offs, such as accepting some reduction in site-specific precision in exchange for broader applicability. To effectively use spectroscopy at large scales, establishing a robust validation framework is also essential to ensure reliable predictions across diverse ecosystems.

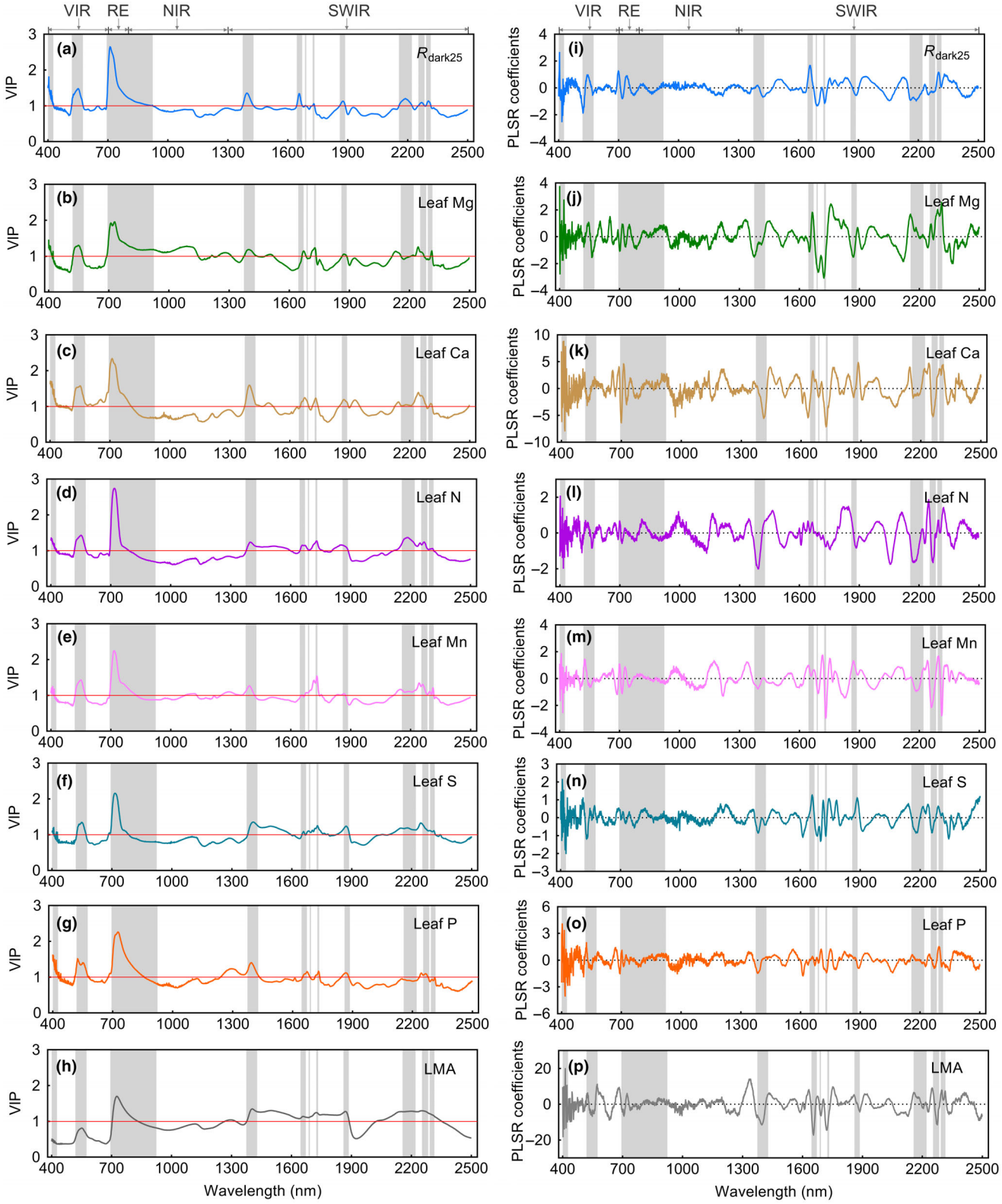

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Fig. 8 Assessing the contributions of reflectance bands to the spectral models of leaf R_{dark25}, and the important R_{dark25} predictors (i.e. leaf Mg, Ca, N, Mn, S and P concentrations following the order of relative importance in predicting R_{dark25}) and leaf mass per area (LMA) under the 'cross-site' scenario. The left panels (a–h) present the partial least squares regression (PLSR) variable importance in projection (VIP), and the right panels (i–p) present the PLSR regression coefficients. The mean values and 95% confidence interval of the PLSR VIP spectrum and regression coefficients were indicated by central-colored lines and shaded regions, respectively. On the left panel, the spectral regions with VIP ≥ 1 are those important for the spectral modeling of R_{dark25} and the important predictors (Lamour *et al*., 2021; Liu *et al*., 2023). The corresponding important spectral bands for R_{dark25} predictions are identified by the shaded gray regions across all subpanels. VIR, visible range (450–700 nm); RE, red-edge range (700–800 nm); NIR, near-infrared range (800–1300 nm); SWIR, short-wave infrared range (1300–2500 nm).

Caveats and future directions

This work identifies two important next steps that need to be considered for future advances. First, our study collected leaf samples only from the top-canopy stratum of three typical forest sites at the peak of the growing season, which cover just a small fraction of Earth's vast plant diversity and omit many other important biomes, such as tundra, grasslands, shrublands and wetlands. Therefore, to test the robustness and generalizability of the mechanistic linkages of R_{dark25} to multiple leaf traits (particularly Mg, Ca, Mn and S) and leaf reflectance spectra, additional efforts would be needed to cover broader breadths of leaf–trait and spectral variations (Kothari *et al*., 2023; Ji *et al*., 2024). Meanwhile, R_{dark25}, leaf traits and reflectance spectra vary remarkably throughout the vertical profiles within the canopy (Niinemets *et al*., 2015; Lamour *et al*., 2023), and across seasonal changes with different leaf developmental stages and environmental factors (Chen *et al*., 2022; Davidson *et al*., 2023a). Therefore, a comprehensive understanding and spectra-model evaluation of the R_{dark25} across more representative field sites, vertical structural gradients and full growing season are still imperative. The continued combination of traditional leaf gas exchange measurements and reflectance spectroscopy as part of global efforts are needed to further enable the development of generalized spectra–R_{dark25} model that can be applied in broad conditions. This can help incorporate a spectra-based data assimilation module into ecosystem models, and revolutionize the parameterization approach by directly integrating trait information rather than relying on predefined empirical relationships between traits (Fu *et al*., 2020). Notably, currently a fully open database (Global Spectra-Trait Initiative) for leaf-level physiological and spectral data is being assembled to enable the continued development of spectra–trait models (https://github.com/plantphys/gsti/tree/main).

Second, given the sampling difficulties caused by canopy access have limited the measurement of R_{dark25} over large spatial extents, an alternative and rapid remote sensing method based on imaging spectroscopy technology is needed to monitor large-scale R_{dark25}. Canopy spectra can be collected using a range of platforms including unoccupied aerial systems (UAS; Yang *et al*., 2022), piloted airborne sensors and spaceborne satellites (Serbin & Townsend, 2020; Liu *et al*., 2024), and it has been demonstrated to be effective in monitoring leaf traits associated with R_{dark25} (e.g. V_{cmax25}, leaf N, P concentrations and LMA) (Liu *et al*., 2023). Moreover, as the spectral variability of the canopy monitored by imaging spectroscopy mainly comes from the leaf spectrum (Asner, 1998), understanding the leaf-level spectra–R_{dark25} relationships is beneficial to the spectral modeling and potential vegetation indices developing of R_{dark25} at larger spatial scales (Serbin & Townsend, 2020). Therefore, future studies should explore R_{dark25} using various imaging spectroscopy platforms and try to extend leaf spectral models to canopy and ecosystem scales. This will require the development of appropriate hybrid modeling and validation approaches (Fu *et al*., 2020). During the upscaling process across different spatial resolutions, issues including the effects of canopy structure (Liu *et al*., 2023) and environmental noise (Asner, 1998; Ollinger, 2011; Cimoli *et al*., 2024) need to be attention. As the community addresses challenges, spectroscopy will further contribute to the explanation of more detailed scale-dependent mechanisms and monitoring of terrestrial plant respiration and C uptake capacities over large spatiotemporal extents (Serbin *et al*., 2015; Jetz *et al*., 2016; Yan *et al*., 2021; Liu *et al*., 2024). Our ultimate hope is that by combining field data with cutting-edge remote sensing technology, we will not only expand our understanding of knowledge of plant physiological traits but also help us better diagnose the role and fate of terrestrial ecosystems under climate change.

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Competing interests

None declared.

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Author contributions

ZY and FW planned and designed the research. FW, NY, TD, WX, GD and JD performed experiments and conducted fieldwork. FW and ZY analyzed data with inputs from SL, JW and HW. FW drafted the manuscript with constructive input from ZY. FW, SL, JL, OKA, NY, TD, WX, NG Smith, ZW, HW, Y Su, XL, Y Shi, AX, GD, JD, NG Swenson, JK, PBR, SPS, AR, JW and ZY contributed to interpreting the results and editing the manuscript.

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Data availability

The data supporting the results of the manuscript and the primary gas exchange measurements can be accessed via figshare dataset using the following link: https://doi.org/10.6084/m9.figshare.27641136.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Comparison of the leaf spectral reflectance from leaves on the tree and excised branch.

Fig. S2 Cross-comparison of the leaf R_{dark25} derived using the temperature response functions in Atkin *et al.* (2015) and Davidson *et al.* (2023b).

Fig. S3 Schematic illustration of the repeated double cross-validation method for multivariate linear regression and partial least squares regression.

Fig. S4 The selection criterion for the optimal number of latent components in the spectral model of leaf traits under the cross-site scenario.

Fig. S5 Histogram distribution of the coefficient of determination (r^2) for the PLSR spectral models over the 200 permutations under the cross-site scenario.

Fig. S6 Exploring the variabilities of leaf morphological and biochemical traits within and across forest sites.

Fig. S7 Pearson's correlation analysis of leaf biochemical traits across the three forest sites.

Fig. S8 Accuracy assessment for the cross-site spectral models of leaf morphological and biochemical traits.

Fig. S9 Cross-comparisons between the observed and spectra-modeled trait–R_{dark25} relationships across the three forest sites.

Methods S1 Protocol of AC_i curves measurement.

Methods S2 Protocol of the measurements of morphological and biochemical traits, including LMA, leaf N, P, K, Ca, Mg, Mn and S concentrations.

Methods S3 Protocol of PLSR modeling with repeat double cross-validation.

Table S1 Soil information for the three forest sites in China.

Table S2 Summary of species, leaf traits and sample size of representative canopy trees across the three forest sites in China.

Table S3 Statistical summary of the relationships between R_{dark25} and the eight leaf morphological and biochemical traits across the three forest sites.

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