

Research paper

Variation in leaf chlorophyll concentration from tropical to cold-temperate forests: Association with gross primary productivity



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ABSTRACT

Leaf chlorophyll is essential to harvest light energy to drive photosynthesis. Yet, most of studies of leaf chlorophyll concentration (Chl) have typically been limited to few species or sites, and there has been little understanding of its variation among species of contrasting environments, or across forests differing in gross primary productivity (GPP). We measured mass-based foliar Chl concentrations (Chl *a*, Chl *b*, and total Chl) for 937 species of nine Chinese forests ranging from tropical to cold-temperate regions. Total Chl varied by over ten-fold from 1.45 to 19.2 mg g⁻¹ (mean: 5.54 mg g⁻¹), and differed among plant functional groups. At the community level, total Chl decreased with increasing latitude. The ratio of Chl *a/b* decreased from trees to shrubs to herbs, consistent with adaptation to contrasting irradiance, i.e., overstory vs. understory. Total Chl was positively related to leaf N and P concentrations, as would be expected to optimize photosynthesis and carbon fixation. Across forests, GPP was positively correlated with community-averaged Chl ($r = 0.57$; $P < 0.01$), a new case of a linkage between leaf traits and ecosystem function at regional to continental scale. Our findings revealed variation in Chl from tropical to cold-temperate forests, and the linkage of leaf Chl with ecosystem function, enhancing our ability to parameterize vegetation models.

1. Introduction

Chlorophyll is an essential pigment for photosynthesis, utilizing the energy of photons for redox reactions (Mackinney, 1941; Baker, 2008). Thus, leaf chlorophyll concentration (Chl) may directly influence the photosynthetic capacity of plants to some extent (Croft et al., 2017). In past decades, many studies have focused on chlorophyll synthesis, fluorescence (Genty et al., 1989; Kooten and Snel, 1990; Maxwell and Johnson, 2000; Baker, 2008), and decomposition (Larkin et al., 2003; Pružinská et al., 2003; Zhang et al., 2006; Schelbert et al., 2009). However, most studies of Chl have been limited to specific species in a given region (Fleischer, 1935; Bojovic and Stojanovic, 2005; Luo et al., 2011; Schlemmer et al., 2013; Burzyński, 2014). Analyses across diverse species and communities may contribute to addressing a current major challenge for ecologists: how to use traits measured at organ or plant level to predict key ecosystem properties along environmental

gradients and under changing climate (Andersen et al., 2012; Garnier and Navas, 2012; Reichstein et al., 2014; Violle et al., 2014). Unfortunately, although Chl has been widely considered as an important trait there have not been data available for species across natural communities.

Leaf Chl mainly includes chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*). Both pigments have a strong light absorption capacity, but differ in their absorption peaks, with Chl *a* higher in the red band and Chl *b* higher in the blue violet band (Lichtenthaler and Buschmann, 2001). Therefore, variation in the Chl *a/b* ratio may represent an adaptive strategy of leaves to different light availability across different environments. Because the spectral composition of solar light does not differ across latitudes, the Chl *a/b* of dominant trees would be expected to unchangeable with latitude. However, the vertical canopy structure of plant community results in strong variation in the light environment, with greater availability of diffuse light and less blue light in the

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understory (Ostrom, 2005). Thus, understory plants might enhance Chl *b* concentration as part of their adaptation to their light environment, resulting in a lower Chl *a/b*. Further, given that the Chl *b* is more associated with photosystem II (PSII), the Chl *a/b* ratio may also reflect the ratio of investment in photosystem I (PSI) relative to photosystem II (PSII), leading to a lower ratio in shade, because shade leaves would allocate more strongly to PSII to widen the range of utilizable light wavelengths (Boardman, 1977; Kitajima and Hogan, 2003). Additionally, canopy structure may produce the shading effect on these understory plants, resulting in the less lower Chl *a/b* in the understory. These expectations have never been tested to date across natural forests at a large scale.

The relationship of Chl to foliar nutrient concentrations also requires examination across communities. Leaf nitrogen (N) and phosphorus (P) are essential elements for plant growth. N is important for the synthesis of chlorophyll and enzymes, and inorganic P is a major metabolite in photosynthetic reactions and other energetic reactions. Studies have demonstrated that Chl and leaf N concentration are strongly correlated across and within species (Sack et al., 2003), and based on conservation of stoichiometry for metabolic reactions, one might expect Chl to correlate with leaf P concentrations too. However, such scaling relationships might be weak, given that leaf Chl is located in the chloroplast-based thylakoid-based light reactions, and mostly in the light harvesting apparatus, rather than reaction centers (Horton et al., 2003), whereas N and P are mostly allocated to stroma-based carbon reactions.

Our study was intended to contribute to the global effort to linking plant traits with ecosystem functioning, a major priority that will improve our ability to explain and predict ecosystem assembly and responses to changing environments (Garnier and Navas, 2012; Reichstein et al., 2014). A major challenge is relating properties measured at the individual or organ level to the community scale (Houborg et al., 2013; Croft et al., 2015). Given that Chl is fundamentally important to photosynthesis, we hypothesized that Chl averaged for the forest community (Chl_{Commun}) would be positively correlated with GPP. Previous studies have suggested a link between Chl and GPP (Houborg et al., 2013; Croft et al., 2015), but no studies to our knowledge have tested this relationship, or the departure from this relationship that would be expected if communities vary in their Chlorophyll production efficiency (CPE = GPP/Chl_{Commun}), i.e., the relative productivity per unit of chlorophyll.

We measured Chl for 937 common plant species within 9 Chinese forests from tropical to cold-temperate regions. Our main objectives were 1) to analyze the variation in Chl across species, plant functional groups (PFGs), and communities; 2) to determine the variation in Chl *a/b*; 3) to test the hypothesis that Chl is positively correlated with N and P across species and forests to optimize photosynthesis; and 4) to establish the relationship between Chl and GPP across forest communities at a regional scale.

2. Materials and methods

2.1. Site description

The study was conducted along the North-South Transect of Eastern China (NSTEC), representing the 15th standard transect of the International Geosphere Biosphere Programme (IGBP). The transect ranged from 18.7° N to 51.8° N in latitude and from 108.9° E to 123.0° E in longitude. We selected 9 typical forests along the NSTEC, within experimental plots set up in the National Nature Reserve to minimize human disturbance. The plots ranged from −4.40 to 20.9 °C in mean annual temperature (MAT) and from 482 to 2449 mm in mean annual precipitation (MAP), which mainly occurred in the summer (Song et al., 2016). Detailed geographical information of the region is presented in Fig. 1 and Table S1 (Song et al., 2016).

2.2. Field sampling

Field sampling was conducted from July to August 2013, the period of highest growth rate for all of the selected forests. First, we set up four representative plots (30 m × 40 m) in each forest type, to survey trees, and smaller plots within to survey shrubs (5 m × 5 m) and herbs (1 m × 1 m). We recorded the height and diameter at breast height of each individual for all trees and shrubs and collected the above-ground parts of herbs.

We chose mature, healthy trees and collected fully expanded, sun-exposed leaves from four individuals of each plant species, and each of four individuals represented a replicate. Leaf samples were collected from branches in the upper part of trees by climbing or using pole pruners and placed in a plastic self-sealing bag (Tian et al., 2016; Zhao et al., 2016).

2.3. Measurement of chlorophyll concentrations

We weighed 0.1 g of fresh leaves to extract chlorophyll using 95% ethanol, with four replicates for each plant species. The Chl (Chl *a* and Chl *b*) of the filtered solution was measured using the classical spectrophotometric method with a spectrophotometer (Pharma Spec, UV-1700, Shimadzu, Japan) (Mackinney, 1941).

According to the Lambert-Beer law the relationship between concentration and optical density is:

$$D_{665} = 83.31 C_a + 18.60 C_b \quad (1)$$

$$D_{649} = 4.54 C_a + 44.24 C_b \quad (2)$$

$$G = C_a + C_b \quad (3)$$

where D_{665} and D_{649} are the optical densities of the chlorophyll solution at wavelengths 665 nm and 649 nm; C_a , C_b , and G are the concentrations of Chl *a*, Chl *b*, and total Chl, respectively (g L^{-1}); 83.31 and 18.60 are the specific absorption of Chl *a* and Chl *b* at a wavelength of 665 nm; and 4.54 and 44.24 are the specific absorption of Chl *a* and Chl *b* at a wavelength of 649 nm.

Based on the concentration of Chl *a*, Chl *b*, and total Chl, Chl was calculated (mg g^{-1} , leaf fresh mass, FM) as:

$$\text{Chl } a \text{ concentration}(\text{mg g}^{-1}) = C_a \times 50 / (1000 \times 0.1) \quad (4)$$

$$\text{Chl } b \text{ concentration}(\text{mg g}^{-1}) = C_b \times 50 / (1000 \times 0.1) \quad (5)$$

$$\text{Chl concentration}(\text{mg g}^{-1}) = G \times 50 / (1000 \times 0.1) \quad (6)$$

2.4. Measuring leaf nitrogen and phosphorus concentrations

After collection, the leaf samples were cleaned in the laboratory to remove soil and surface particles and oven-dried at 65 °C. The samples were then ground and an elemental analyzer (Vario MAX CN Elemental Analyzer, Elementar, Hanau, Germany) was used to measure the nitrogen (N) concentration. Phosphorus (P) was measured by the ammonium molybdate method using a continuous-flow analyzer (AutoAnalyzer3 Continuous-Flow Analyzer; Bran Luebbe, Hamburg, Germany) after $\text{H}_2\text{SO}_4\text{--HClO}_4$ digestion of plant samples (Zhao et al., 2014; Zhao et al., 2016).

2.5. Calculation of forest leaf biomass

Leaf biomass for each tree species was calculated using allometric equations, using the diameter at breast height (DBH) and height. Leaf biomass for each shrub species was also calculated using allometric equations, using ground-diameter and height. All equations were obtained from the Chinese Ecosystem Research Network (CERN) database (<http://159.226.111.42/pingtai/cernc/index.jsp>) or the published literature (Wang et al., 2015). The leaf biomass of grass species was

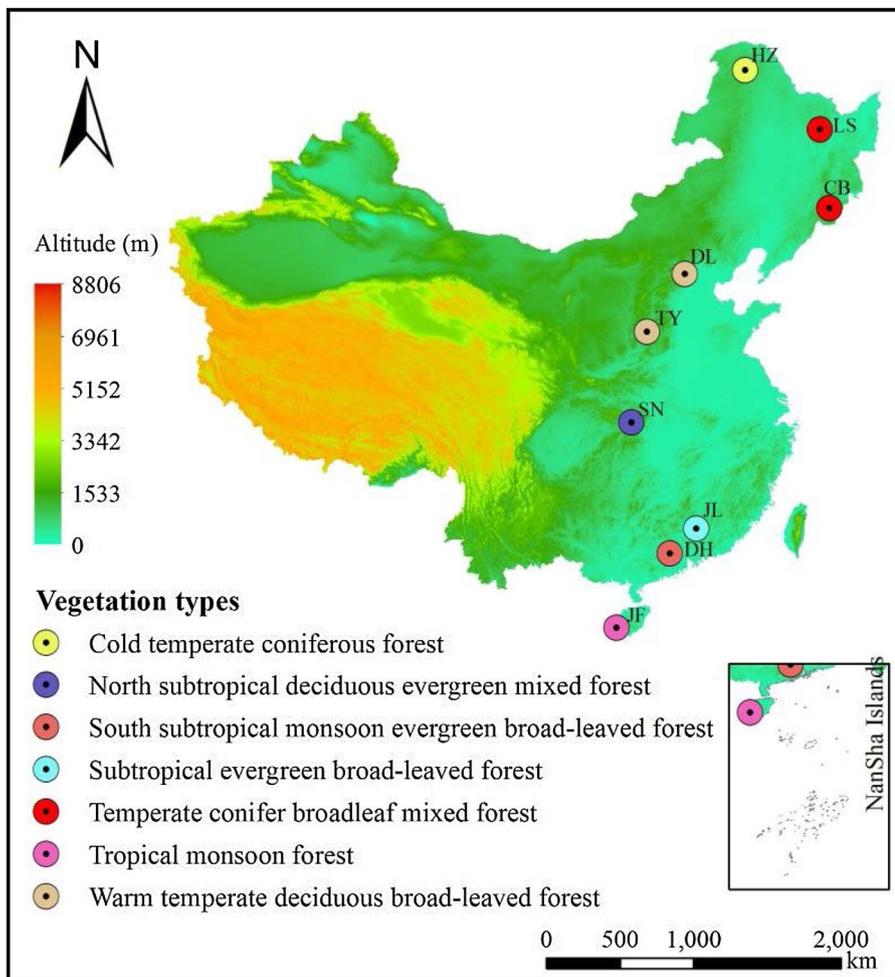


Fig. 1. Geographic locations of the nine selected forests in this study. HZ, Huzhong; LS, Liangshui; CB, Changbai; DL, Dongling; TY, Taiyue; SN, Shennongjia; JL, Jiulian; DH, Dinghu; JF, Jianfengling.

measured directly.

2.6. Data for gross primary productivity

The GPP for the nine selected forests was obtained from MODIS at a resolution of 1 km × 1 km (http://daac.ornl.gov/cgi-bin/MODIS/GLBVIZ_1_Glb/modis_subset_order_global_col5.pl). The GPP data for the nine forest communities were extracted from 2001 to 2010 based on the latitude and longitude. The 10-year average was used in this study.

2.7. Climate data

Data for mean annual precipitation (MAP, mm), and mean annual temperature (MAT, °C) for the nine forests were obtained from CERN. The drought index, which is negatively related to site aridity, integrates MAT and MAP, and is widely used as an indicator of dry climate. We used the drought index of de Martonne (1926):

$$DI = \frac{MAP}{MAT+10} \quad (7)$$

where DI is the drought index, MAP is the mean annual precipitation (mm), and MAT is the mean annual temperature (°C).

2.8. Analyzing chlorophyll data

We averaged Chl at the species-level, and then calculated the average Chl for each plant functional group (PFG), and for each community (Garnier et al., 2004):

$$Chl_{Tree} = \sum_{i=1}^n Biomass_i \times Chl_i \quad (8)$$

$$Chl_{Shrub} = \sum_{i=1}^n Biomass_i \times Chl_i \quad (9)$$

$$Chl_{Herb} = \sum_{i=1}^n Biomass_i \times Chl_i \quad (10)$$

$$Chl_{Commun.} = \sum_{i=1}^n Biomass_i \times Chl_i \quad (11)$$

where n is the species richness of the forest community, and $Biomass_i$ is the leaf biomass of the i th species in the specific PFG or community.

2.9. Chlorophyll production efficiency

We hypothesized that GPP and the total leaf Chl should be related across communities, given the central importance of chlorophyll in photosynthesis, the key biosynthetic reaction underlying plant and ecosystem productivity. Further, the peak period of plant growth (July and August), plant communities tend to have the highest leaf biomass and total amount of leaf Chl. We developed a new parameter for the leaf chlorophyll production efficiency (CPE, $kg\ C\ g^{-1}$) to test the variation across communities. The CPE was defined as the productivity per unit chlorophyll at the community level (Eq. (12)):

$$CPE = \frac{GPP}{\sum_{i=1}^n Chl_i \times Biomass_i} \quad (12)$$

where CPE is leaf chlorophyll product efficiency at the community level, GPP is the gross primary productivity of the forest community, n is the species richness of the forest community, Chl_i is the Chl of the i th species, and $Biomass_i$ is the biomass of the i th species.

2.10. Data analysis

Analyses of variance were used to test the importance of plant species, PFG, and communities as influences on Chl, with multiple comparisons (LSD). The relationships between Chl and latitude were analyzed using least squares linear regression at the species, PFG, and community levels.

The scaling relationship of Chl a and Chl b in leaves was quantified using the power law equation:

$$Y = b \cdot X^a \tag{13}$$

which was linearized by logarithmic transformation:

$$\log_{10}(Y) = \log_{10}(b) + a \cdot \log_{10}(X) \tag{14}$$

where X, Y, a, and b represent Chl a, Chl b, and the allometric slope and intercept respectively (Zhao et al., 2016). Standard major axes were used to analyze this scaling relationship at the species, PFGs, and community levels. Parameter estimation of the allometric equations was implemented by using the “lmodel2” function in the “lmodel2” package of R. The likelihood ratio test was used to test significant differences in Chl scaling among herbs, shrubs and trees by comparing regression slopes among the three PFGs in a pairwise manner using the “sma” function of the “smatr” program in R.

Other analyses were conducted using SPSS 20.0 (SPSS Inc. Chicago, IL, USA) and figures were produced with Sigma plot 10.0 (Washington, IL, USA, 2006).

3. Results

3.1. Variation across species, forests and growth forms in leaf chlorophyll concentration

The content of leaf Chl varied significantly across the 937 plant species. Total Chl ranged from 1.45 mg g⁻¹ (for *Gymnanthera gymnanther*) to 19.2 mg g⁻¹ (for *Lonicera lonicera*), with a mean of 5.54 mg g⁻¹. Total Chl also differed on average among the forests, from 4.02 mg g⁻¹ in Huzhong to 7.28 mg g⁻¹ in Liangshui (Fig. 2B; Table S2). Further, Chl varied among growth forms, with shrubs > herbs > trees (Fig. 2A).

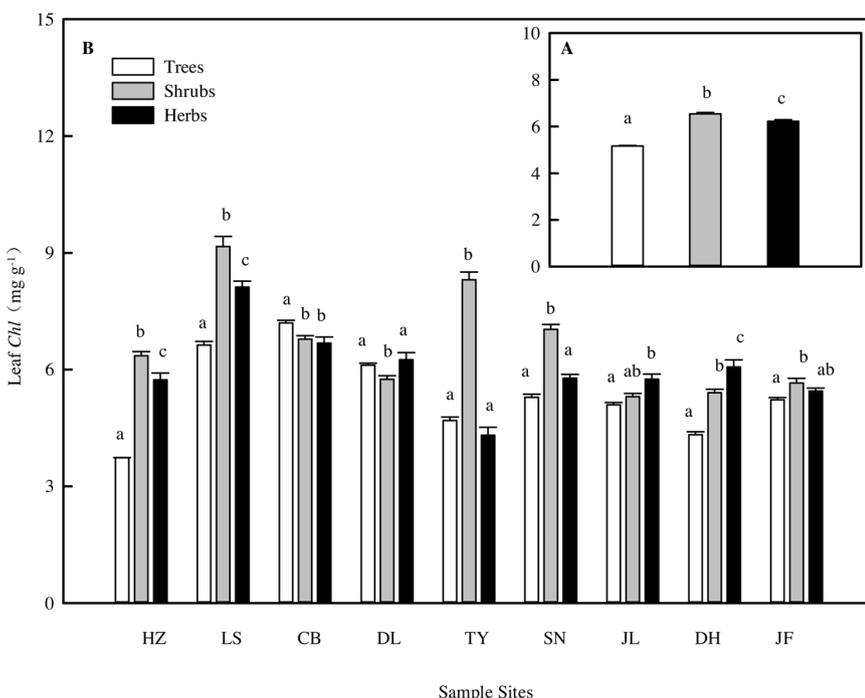


Fig. 2. Changes in leaf Chlorophyll concentration among different plant functional groups. HZ, Huzhong; LS, Liangshui; CB, Changbai; DL, Dongling; TY, Taiyue; SN, Shennongjia; JL, Julian; DH, Dinghu; JF, Jianfengling. Panel A and B were at total transect calculated and at each site, respectively. Data were represented mean ± S.E. Different letters indicated the significant difference among plant functional types ($P < 0.05$).

3.2. Latitudinal pattern of leaf Chl

The latitudinal patterns of leaf Chl were scale-dependent. Considering all species, leaf Chl showed no clear trend, or a minor increase, at increasing latitudes ($r = 0.24$, $P < 0.01$) (Fig. 3A). However, after weighting by community structure, Chl_{Tree} and Chl_{Commun} decreased at increasing latitudes ($r = -0.52$ and -0.54 respectively, $P < 0.01$) (Fig. 3B and C).

3.3. Distribution of Chl a and Chl b in leaves

From south to north along the transect, the Chl a/b ratio for trees showed no latitudinal pattern (Fig. S1). The Chl a was positively correlated with Chl b for trees, shrubs, and herbs (Fig. S2) and the values of the allometric exponent (a) were statistically similar across growth forms (Fig. 4). However, the Chl a/b ratio was higher for trees than herbs (Fig. 5; $P < 0.05$).

3.4. Relationships between leaf Chl and N and P concentrations

Total Chl was positively correlated with leaf N for all plant species ($P < 0.01$; Table S3), and, considering growth forms separately for trees ($r = 0.48$), shrubs ($r = 0.51$), and herbs ($r = 0.71$) ($P < 0.01$; Fig. 6A). Similarly, total Chl and P were correlated across all species ($P < 0.01$; Table S3) and separately for trees ($r = 0.21$), shrubs ($r = 0.38$), and herbs ($r = 0.15$), with a higher allometric slope for shrubs than for herbs (Fig. 6B). The Chl of trees ($r = 0.20$) and herbs ($r = 0.23$) was positively correlated with the N: P ratio ($P < 0.05$; Fig. 6C).

3.5. Relationship between leaf Chl and GPP in natural forest communities

Chl_{Commun} and GPP were positively associated across forest communities ($r = 0.57$, $P < 0.01$; Fig. 7). The CPE showed a hump-backed relationship with latitude ($r = 0.62$, $P < 0.01$; Fig. S3): at low latitudes, CPE decreased with increasing latitudes to the temperate zone, and then increased with latitude at higher latitudes (Fig. S3). CPE was positively correlated with the drought index ($r = 0.22$) indicating that a lower CPE occurs in more arid regions (Fig. S4).

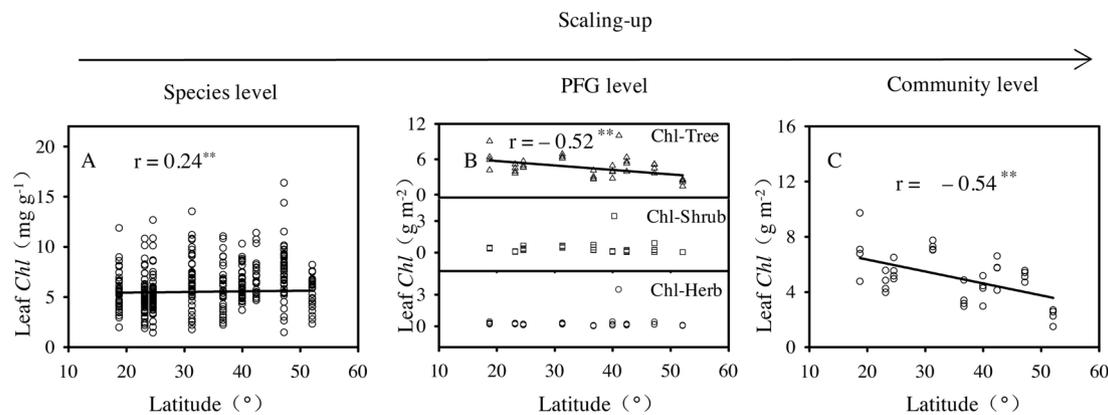


Fig. 3. Latitudinal trends of leaf Chlorophyll concentration at the species, plant functional group (PFG), and community levels in Chinese forests. Chl_{Trees}, Chl_{Shrub}, and Chl_{Herb} were Chl of the different functional groups of trees, shrubs and herbs, respectively. Panel A, B and C were the latitudinal trends of leaf Chlorophyll concentration at the species, plant functional group (PFG), and community levels respectively. Only significant regression ($P < 0.05$) being given. *, $P < 0.05$; **, $P < 0.01$.

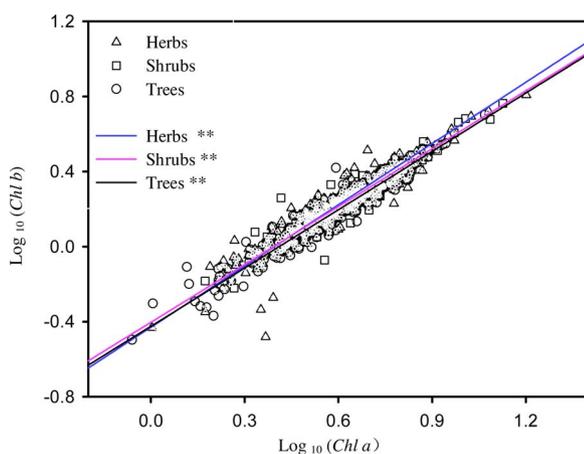


Fig. 4. Relationships of Chl *a* and Chl *b* across plant functional groups (PFGs). Only significant regressions ($P < 0.05$) are presented. *, $P < 0.05$; **, $P < 0.01$.

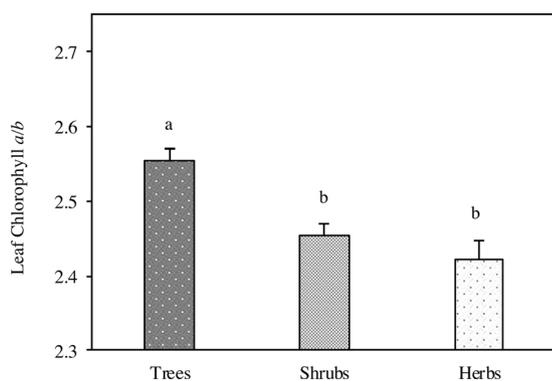


Fig. 5. Variation in leaf chlorophyll *a/b* ratio across plant functional groups (PFGs). Different letters indicated the significant difference among plant functional types ($P < 0.05$). Error bars represent standard error(SE).

4. Discussion

4.1. Regional variability of leaf Chl

The Chl of 937 plant species ranged from 1.45 to 19.2 mg g⁻¹, with an average of 5.54 mg g⁻¹. The strong species variation in Chl might represent a combination of genetic determination and plastic differences across growing conditions as individual species can show great plasticity in Chl. For example, in a study of saplings of *Betula papyrifera*, Chl varied from 0.04 to 3.87 mg g⁻¹ (Richardson et al., 2002).

Plasticity in Chl can arise from differences in concentrations of Chl within palisade and spongy mesophyll cells, the relative distributions of those cell types, and the numbers of layers of mesophyll cells (Sack et al., 2003).

Chl varied across growth forms, with greater concentrations for shrubs than herbs and trees. These differences may be linked with adaptation to light availability, such that higher Chl associated with deeper shade. Thus, shrubs, including many sub-canopy species, would be expected to have high abundance of Chl in the light harvesting antenna, for improved light capture, relative to canopy trees. The herbs included species of both understory and open habitats and thus on average would be intermediate.

Considering all species individually along the transect, total Chl showed a weak increasing trend with higher latitude. However, when weighted by community structure, Chl_{Trees}, Chl_{Shrub}, Chl_{Herb}, and Chl_{Commun} all decreased at higher latitude. Latitudinal patterns in Chl might reflect contrasting assembly processes, including adaptation and plasticity, at least in part forced by differences in the hydrological and thermal environments along the transect. In low latitude areas with higher precipitation and temperature, the forest community has a more complex structure including more deeply shaded environments in which greater Chl would be expected to aid in competition for light. This hypothesis needs additional testing in future studies.

4.2. Leaf Chl *a/b* ratio reflects an adaptation of plants to the light environment

The Chl *a/b* ratio was highest in trees, followed by shrubs and herbs. This result is consistent with plants adjusting to the light environment in their Chl *a/b* ratio given that light quality varies with the vertical structure of the forest community, such that the relative availability of red-light is greatest in the forest overstory, whereas the relative availability for blue light is more available in the understory. Chl *b* has a stronger absorption capacity for blue light, and therefore understory plants should increase their Chl *b* to improve photosynthesis in understory light environments. Our results extend previous work on given species grown in different light environments showing shifts to higher Chl *a/b* for plants grown under high light and shifts to lower Chl *a/b* under lower light (Chu et al., 1980). This trend has been shown both within species, e.g., for *Asplenium australasicum* (Leong et al., 1985), and in comparing sun versus shade adapted species (Johnson et al., 1993; Sack et al., 2003; Johnson et al., 1993).

There was no clear latitudinal pattern in the Chl *a/b* of trees along the transect for the 9 forest communities extending from tropical to cold-temperature zone. This invariance would be consistent with the evenness of spectral composition with increasing latitude and the canopy trees accessing the full available spectrum.

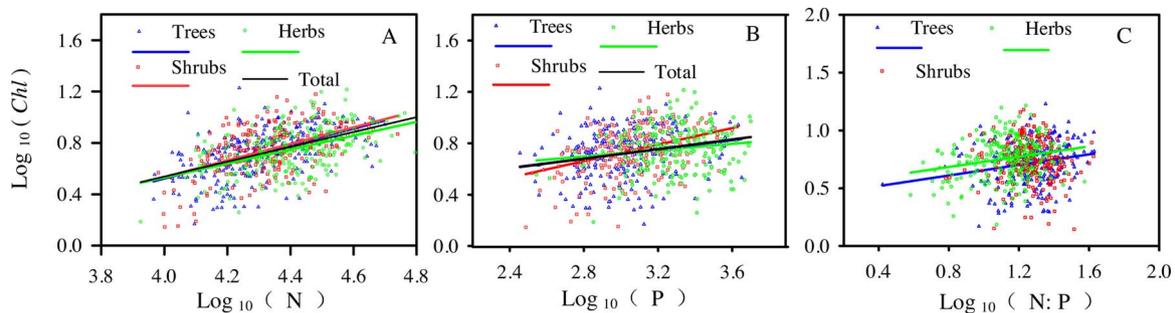


Fig. 6. Relationship between chlorophyll concentration and leaf nitrogen (N) and phosphorus (P). Panels A, B and C show the relationship between Chlorophyll content and N, P, and N: P respectively.

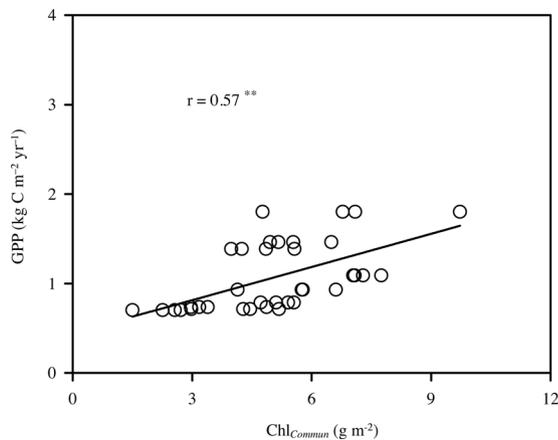


Fig. 7. Relationship between $Chl_{Community}$ and gross primary productivity (GPP). $Chl_{Community}$ was Chl of community. Only significant regression ($P < 0.05$) being given. *, $P < 0.05$; **, $P < 0.01$.

4.3. Chl is positively correlated with leaf N and P concentration

Chl was positively correlated with leaf N and P concentrations, consistent with the overall optimization of the photosynthetic machinery. First, N is an important component of chlorophyll, as each molecule contains four nitrogen atoms. Second, given that chlorophyll is a major component of the photosystems and light harvesting apparatus of the thylakoid-located light reactions, Chl should scale with N, which is a major component of the stroma-based Rubisco and other Calvin cycle enzyme. In this way, the scaling of light and carbon reactions would be expected from the coordination of rates of the reactions. Given the large variation in photosynthetic ecology across species, this scaling should hold even though the majority of Chl is in the light harvesting apparatus, and so, under high irradiance most of the chlorophyll is highly redundant with respect to the rates of photosystem reactions, decoupling the rate of photosynthesis from the chlorophyll concentration. Yet, Chl should increase with increasing leaf N concentration for a given species and across multiple plant species (Evans, 1989; Sack et al., 2003), and leaf N concentration is closely related to photosynthetic capacity (Evans, 1989; Reich et al., 1997). Addition, Kitajima and Hogan (2003) found there was a close relationship between Chl a/b ratio and nitrogen content, which was not considered in this paper, but it would be a good idea to do the further research exploring it in the future.

Similarly, Chl would be expected to relate to P concentration, given the roles of these components in the photosynthetic light reactions. In the electron transfer reactions, the chlorophyll absorbs sunlight and converts photon energy into reduction potential, leading ultimately to the conversion of nicotinamide adenine dinucleotide phosphate (NADP) to nicotinamide adenine dinucleotide phosphate (NADPH). In the photophosphorylation reactions, generating also a chemiosmotic

gradient that powers the conversion of inorganic phosphate (Pi) and adenosine diphosphate (ADP) to adenosine triphosphate (ATP) (Allen and Williams, 1998; Arnon, 1971; Farquhar et al., 1980). Given that NADP, Pi, and ADP all contain phosphorus (P) atoms, and low P may reduce the rate of photosynthetic reactions (Fredeen et al., 1990; Lapointe, 1987; Elser et al., 2000) a positive coordination of Chl and P would be expected, as found in this study.

4.4. Linkage between GPP and Chl in natural forest communities

Leaf chlorophyll is essential for plant photosynthesis and thus its concentration would be expected to scale with rate of forest productivity at the regional scale. This hypothesis has been supported at the individual species level, e.g., in a 10-year experiment, Chl in maize was closely related to GPP (Gitelson et al., 2014). However, this association has never been verified across natural forests. We found that at the community level, $Chl_{Community}$ was positively correlated with GPP along the tropical to cold-temperate forest transect. Previous studies have demonstrated that GPP depends strongly on stand biomass, leaf area index, and therefore, leaf biomass (Garnier and Navas, 2012; Reichstein et al., 2014). Our findings indicate that Chl is a key plant functional trait that influences ecosystem functioning.

Previous studies only considered $Chl_{Community}$ without considering the efficiency of chlorophyll investment at large scales. We showed that chlorophyll productivity efficiency (CPE) first decreased and then increased with increasing latitudes. Similarly, it was reported that stomatal density (d_{CWM}), stomatal area fraction (f_{CWM}) and the use efficiency of caloric value (CUE) all showed humpbacked shaped relationships with latitude as well (Liu et al., 2017; Song et al., 2016). Further, we found that the pattern of CPE was associated with site aridity (which depends on integrating the effects of MAP and MAT). This pattern is consistent with species of arid areas achieving greater maximum productivity by investing in greater stress causes the stomata to close, resulting in a decrease in the photosynthetic activity of mesophyll cells, one would expect that CPE should decline in the mid-latitude region of the NSTEC transect where the climate is drier. Therefore, the CPE should be influenced by community composition at a large scale. CPE would be well approximated by a straight line if we do not consider the measurements made at the last latitude in Huzhong. However, it would result in a misleading of other researchers. In Huzhong, the forest community was mainly dominated by *Larix gmelinii* (Dahurian larch and Pinaceae), which represented more than 90% of the community biomass, but it's a lower leaf biomass contributed to a higher CPE at these forests. Therefore, the bright coniferous forests in high-latitude should have its own unique photosynthetic strategy of Chl. Unfortunately, we have only one coniferous forest in this study, which require more works to verify these in future.

The linkage of Chl to GPP may contribute to efforts to remotely sense plant productivity. Our database of Chl will be useful in future development of approaches to scale from Chl to remotely-sensed variables for photosynthesis and productivity across communities and

regions.

5. Conclusions

This study reported the variation in leaf *Chl*, *Chl a/b* from tropical to cold-temperate forests, and the relationship of *Chl* with N and P at the species, PFG, and community levels. Furthermore, we established the linkage of *Chl* with GPP across natural forest communities. *Chl* varied strongly among species at each latitude, and showed little association with latitude at the species level, but the $Chl_{Community}$ of natural forests decreased with increasing latitude. The *Chl a/b* ratio was highest in trees, followed by shrubs and herbs, consistent with adjustment to the light environment. Leaf *Chl* averaged at the community level was positively correlated with GPP along the transect from tropical to cold-temperate forests. In conclusion, this study demonstrated new evidence linking leaf *Chl* with ecosystem function. These findings indicate the *Chl* has potential to improve the parameterization of models for photosynthesis and GPP across terrestrial ecosystems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2017.10.025>.

References

- Allen, J.P., Williams, J.C., 1998. Photosynthetic reaction centers. *FEBS Lett.* 438, 5–9.
- Andersen, K.M., Endara, M.J., Turner, B.L., Dalling, J.W., 2012. Trait-based community assembly of understory palms along a soil nutrient gradient in a lower montane tropical forest. *Oecologia* 168, 519–531.
- Arnon, D.I., 1971. The light reactions of photosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* 2883–2892.
- Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* 59, 89–113.
- Boardman, N.K., 1977. Comparative photosynthesis of sun and shade plants. *Annu. Rev. Plant Biol.* 28, 355–377.
- Bojovic, B., Stojanovic, J., 2005. Chlorophyll and carotenoid content in wheat cultivars as a function of mineral nutrition. *Arch. Biol. Sci.* 57, 283–290.
- Burzyński, M., 2014. Influence of lead on the chlorophyll content and on initial steps of its synthesis in greening cucumber seedlings. *Acta Soc. Bot. Pol.* 54, 95–105.
- Chu, Z.X., Xu, C.H., Mao, D.Z., Zhao, F.H., Dai, Y.L., 1980. Studies on plant chlorophyll-protein complexes. 1. The chlorophyll-protein complexes of the sun-loving plant sunflower and the shade-loving plant *Malaxis monophyllos* (L.) Sw. *Acta Phytophysiol. Sin.* 6, 163–172.
- Croft, H., Chen, J.M., Froelich, N.J., Chen, B., Staebler, R.M., 2015. Seasonal controls of canopy chlorophyll content on forest carbon uptake: implications for GPP modeling. *J. Geophys. Res.-Biogeol.* 1576–1586.
- Croft, H., Chen, J.M., Luo, X., Bartlett, P., Chen, B., Staebler, R.M., 2017. Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. *Glob. Change Biol.* <http://dx.doi.org/10.1111/gcb.13599>.
- Elsner, J.J., Sterner, R.W., Gorokhova, E., Fagan, W.F., Markow, T.A., Cotner, J.B., Harrison, J.F., Hobbie, S.E., Odell, G.M., Weider, L.W., 2000. Biological stoichiometry from genes to ecosystems. *Ecol. Lett.* 3, 540–550.
- Evans, J.R., 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78, 9–19.
- Farquhar, G.D., Von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. *Planta* 149, 78–90.
- Fleischer, W.E., 1935. The relation between chlorophyll content and rate of photosynthesis. *J. Gen. Physiol.* 18, 573–597.
- Fredeen, A.L., Raab, T.K., Rao, I.M., Terry, N., 1990. Effects of phosphorus nutrition on photosynthesis in *Glycine max* (L.) Merr. *Planta* 181, 399–405.
- Garnier, E., Navas, M.L., 2012. A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. *Agron. Sustain. Dev.* 32, 365–399.
- Garnier, E., Cortez, J., Billès, G., Navas, M.-L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., 2004. Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85, 2630–2637.
- Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *BBA-Gen. Subj.* 990, 87–92.
- Gitelson, A.A., Peng, Y., Arkebauer, T.J., Schepers, J., 2014. Relationships between gross primary production, green LAI, and canopy chlorophyll content in maize: implications for remote sensing of primary production. *Remote Sens. Env.* 144, 65–72.
- Horton, P., Ruban, A.V., Walters, R.G., 2003. Regulation of light harvesting in green plants. *Annu. Rev. Plant Biol.* 47, 655–684.
- Houborg, R., Cescatti, A., Migliavacca, M., Kustas, W., 2013. Satellite retrievals of leaf chlorophyll and photosynthetic capacity for improved modeling of GPP. *Agric. For. Meteorol.* 177, 10–23.
- Johnson, G., Scholes, J., Horton, P., Young, A., 1993. Relationships between carotenoid composition and growth habit in British plant species. *Plant Cell Environ.* 16, 681–686.
- Kitajima, K., Hogan, K.P., 2003. Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant Cell Environ.* 26, 857–865.
- Kooten, O., Snel, J.F., 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth. Res.* 25, 147–150.
- Lapointe, B.E., 1987. Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. *Mar. Biol.* 93, 561–568.
- Larkin, R.M., Alonso, J.M., Ecker, J.R., Chory, J., 2003. *GUN4*, a regulator of chlorophyll synthesis and intracellular signaling. *Science* 299, 902–906.
- Leong, T.Y., Goodchild, D.J., Anderson, J.M., 1985. Effect of light quality on the composition function, and structure of photosynthetic thylakoid membranes of *Asplenium australasicum* (Sm.) Hook. *Plant Physiol.* 78, 561–567.
- Lichtenthaler, H.K., Buschmann, C., 2001. Chlorophylls and carotenoids: measurement and characterization by UV-vis spectroscopy. *Curr. Protoc. Food Anal. Chem.* 39, 1230–1241.
- Liu, C., He, N., Zhang, J., Li, Y., Wang, Q., Sack, L., Yu, G., 2017. Variation of stomatal traits from cold-temperate to tropical forests and association with water use efficiency. *Funct. Ecol.* <http://dx.doi.org/10.1111/1365-2435.12973>. in press.
- Luo, L., Shen, G.Z., Xie, Z.Q., Yu, J., 2011. Leaf functional traits of four typical forests along the altitudinal gradients in Mt. Shennongjia. *Acta Ecol. Sin.* 21, 6420–6428.
- Mackinney, G., 1941. Absorption of light by chlorophyll solutions. *J. Biol. Chem.* 140, 315–322.
- Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* 51, 659–668.
- de Martonne, E., 1926. L'indice d'aridité. *Bull. de l'Association de géographes français* 3, 3–5.
- Ostrom, B.J., 2005. Effect of forest structure on the understory light environment and growth potential of Oka seedlings in a closed canopy riparian forest. A thesis of graduate faculty in Auburn University, Auburn, Alabama.
- Pružinská, A., Tanner, G., Anders, I., Roca, M., Hörtensteiner, S., 2003. Chlorophyll breakdown: pheophorbide a oxygenase is a Rieske-type iron-sulfur protein, encoded by the accelerated cell death 1 gene. *Proc. Natl. Acad. Sci. U. S. A.* 100, 15259–15264.
- Reich, P.B., Walters, M.B., Ellsworth, D.S., 1997. From tropics to tundra: global convergence in plant functioning. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13730–13734.
- Reichstein, M., Bahn, M., Mahecha, M.D., Kattge, J., Baldocchi, D.D., 2014. Linking plant and ecosystem functional biogeography. *Proc. Natl. Acad. Sci. U. S. A.* 111, 13697–13702.
- Richardson, A.D., Duigan, S.P., Berlyn, G.P., 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytol.* 153, 185–194.
- Sack, L., Grubb, P.J., Marañón, T., 2003. The functional morphology of juvenile plants tolerant of strong summer drought in shaded forest understories in southern Spain. *Plant Ecol.* 168, 139–163.
- Schelbert, S., Aubry, S., Burla, B., Agne, B., Kessler, F., Krupinska, K., Hörtensteiner, S., 2009. Pheophytin pheophorbide hydrolase (pheophytinase) is involved in chlorophyll breakdown during leaf senescence in *Arabidopsis*. *Plant Cell* 21, 767–785.
- Schlemmer, M., Gitelson, A., Schepers, J., Ferguson, R., Peng, Y., Shanahan, J., Rundquist, D., 2013. Remote estimation of nitrogen and chlorophyll contents in maize at leaf and canopy levels. *Int. J. Appl. Earth Obs.* 25, 47–54.
- Song, G.Y., Hou, J.H., Li, Y., Zhang, J.H., He, N.P., 2016. Leaf caloric value from tropical to cold-temperate Forests: latitudinal patterns and linkage to productivity. *PLoS One* 11, e0157935.
- Tian, M., Yu, G., He, N., Hou, J., 2016. Leaf morphological and anatomical traits from tropical to temperate coniferous forests: mechanisms and influencing factors. *Sci. Rep.-U.K.* 6, 19703.
- Violle, C., Reich, P.B., Pacala, S.W., Enquist, B.J., Kattge, J., 2014. The emergence and promise of functional biogeography. *Proc. Natl. Acad. Sci. U. S. A.* 111, 13690–13696.
- Wang, R., Yu, G., He, N., Wang, Q., Ning, Z., Xu, Z., Ge, J., 2015. Latitudinal variation of leaf stomatal traits from species to community level in forests: linkage with ecosystem productivity. *Sci. Rep.-U.K.* 5, 14454.
- Zhang, H., Li, J., Yoo, J.H., Yoo, S.C., Cho, S.H., Koh, H.J., Seo, H.S., Paek, N.C., 2006. Rice Chlorina-1 and Chlorina-9 encode ChlD and ChlL subunits of Mg-chelatase: a key enzyme for chlorophyll synthesis and chloroplast development. *Plant. Mol. Biol.* 62, 325–337.
- Zhao, N., He, N., Wang, Q., Zhang, X., Wang, R., Xu, Z., Yu, G., 2014. The altitudinal patterns of leaf C:N:P stoichiometry are regulated by plant growth form, climate and soil on Changbai Mountain, China. *PLoS One* 9, e95196.
- Zhao, N., Yu, G., He, N., Wang, Q., Guo, D., Zhang, X., Wang, R., Xu, Z., Jiao, C., Li, N., 2016. Coordinated pattern of multi-element variability in leaves and roots across Chinese forest biomes. *Glob. Ecol. Biogeogr.* 25, 359–367.