Title	Estimation of six leaf traits of East Asian forest tree species by leaf spectroscopy and partial least square regression
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Citation	Remote Sensing of Environment, 233, 111381 https://doi.org/10.1016/j.rse.2019.111381
Issue Date	2019-11
Doc URL	http://hdl.handle.net/2115/83371
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Type	article (author version)
File Information	2019RSE.pdf

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1   **Estimation of six leaf traits of East Asian forest tree species by leaf spectroscopy and**
2   **partial least square regression**

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Abstract

To elucidate the potential of hyperspectral remote sensing for estimating the functional leaf traits in East Asian forests, we investigated the utility of leaf spectroscopy and partial least square regression (PLSR) models for 141 tree species distributed widely across cool temperate to tropical climate zones in the Pacific region. In 14 forests in Japan, Thailand, and Malaysia, leaf samples at various developmental stages (young, mature, and senescent) were collected from two plant functional types (deciduous and evergreen species). The target leaf traits were leaf mass per unit area and concentrations of nitrogen (N), carbon (C), total phenol, cellulose, and lignin. The leaf reflectance at visible-short wave infrared spectral reflectance from 400 nm to 2,395 nm was measured at 3–10-nm intervals (351 bands) using a spectral radiometer. First, we compared the performance of the PLSR models in terms of dry mass (DM) -based

concentration and leaf area (LA) –based concentration. Then, we investigated the applicability of the models based on the different plant functional types and leaf developmental stages of the training dataset. Finally, we evaluated the minimum number of spectral bands needed for stable performance of the PLSR model by changing the used bands in accordance with the variable importance of projection (VIP) and equal interval resampling.

The coefficient of determination (R^2) was higher and the normalized root mean square error (NRMSE) tended to be lower for all chemical trait concentrations expressed based on LA than for those based on DM, except for the case of leaf N. Plant functional type in the training data affected the applicability of the estimation model strongly. The estimation error increased when the functional type in the training dataset differed from that of the target tree species. In the PLSR model calibrated using datasets of evergreen tree species, the NRMSEs of the six traits were 8.0–12.6% for evergreen tree species but 10.4–21.1% for deciduous tree species. A similar trend was observed in the model calibrated for deciduous tree species. The model calibrated using both functional types showed intermediate accuracy for both types of tree species (NRMSE = 5.3–13.5%). The developmental stage of training data also affected the model performance, and the model calibrated using all of the stages showed better estimation accuracies for young and senescent leaves than the model calibrated from the data of mature leaves alone.

The equal interval resampling provided better estimation accuracy than the case using the threshold of the VIP value although the model performance tended to be diminished with the

reduction of input waveband in both methods. Using at least 104 bands with equal interval removal at a 20-nm interval confers similar performance of the PLSR model compared to the model with full wavebands. This study is the first to describe the potential of leaf spectroscopy for trait estimation of East Asian forest tree species, and our findings suggest that training datasets of typical functional types and varied developmental stages are important for estimation of leaf traits through the several biomes distributed widely in East Asia.

Keywords: band selection, chemical content, developmental stage, leaf developmental stage, plant functional type

1. Introduction

Leaf traits such as thickness, nutrient content, and organic chemical content are crucial for understanding the functional status of vegetation, ecological interactions, and biogeochemical cycles in forest ecosystems. For example, the potential for photosynthetic activity (*i.e.*, light-saturated photosynthetic rate) generally increases with increasing leaf nitrogen (N) concentration and decreases with increasing leaf mass per unit area (LMA), which is an important leaf construction cost index related to leaf thickness and density (Wright *et al.*, 2004). Phenols, including tannins, can act as defensive compounds against herbivores (Ayres *et al.*,

1997; Rothstein *et al.*, 2004). Leaf concentrations of N, carbon (C), and secondary metabolites such as lignin and cellulose are also related to the nutrient cycle, mainly through the decomposition processes of leaf litter (Berg & Laskowski, 2006; Prescott, 2010). Because these traits are measured mainly by destructive sampling in the field and chemical analysis in the laboratory, development of nondestructive monitoring methods is desirable to evaluate their diversity and geographical variation.

Recently, the combined use of hyperspectral remote sensing and field spectroscopy techniques has been highlighted as one of the potential solutions to this problem (Asner & Martin, 2008; Asner *et al.*, 2011; Mitchell *et al.*, 2012; Lepine *et al.*, 2016; Yang *et al.*, 2016). Hyperspectral remote sensing generally measures the electromagnetic spectrum at a large number of consecutive wavebands (over 100 bands) with bandwidth usually < 10 nm full width at half maximum and sampling intervals from < 1 nm to 10 nm (Porcar-Castell *et al.*, 2015). The spectral reflectance data of wavebands from the visible to near-infrared (VNIR, 400–1,050 nm) are the most widely used in field research based on handheld sensors and airborne remote sensing, and the data from the visible to short-wave infrared (VSWIR, 400–2,500 nm) spectral region have also become easier to use in field research over the last few decades due to the development of handheld sensors. The leaf reflectance in these spectral ranges includes information on absorbance features caused by pigments, water, nitrogenous compounds such as protein, and secondary metabolites (Curran, 1989; Nagler *et al.*, 2000; Serrano *et al.*, 2002). Therefore, a large number of broad-band and narrow-band spectral vegetation indices derived

from multiband spectral reflectance in these regions have been studied to monitor the spatiotemporal variations in canopy leaf area index (LAI) (Zarco-Tejada *et al.* 2005; Brantley *et al.*, 2011), N (Serrano *et al.*, 2002; Chen *et al.*, 2010; Stagakis *et al.*, 2010; He *et al.*, 2016), pigments (Sims & Gamon, 2002; Moorthy *et al.*, 2008; Beck *et al.*, 2016), water (Champagne *et al.*, 2003; Colombo *et al.*, 2008; Casas *et al.*, 2014) and secondary metabolites (Nagler *et al.*, 2000; Curran *et al.*, 2001; Serrano *et al.*, 2002; Daughtry & Hunt, 2008). Since most spectral vegetation indices such as the normalized difference vegetation index (NDVI) (Tucker, 1979) can be obtained from calculations of wavebands related to the specific absorbance of target chemicals or structures, researchers have been able to estimate individual leaf traits of interest using the minimum number of spectral bands without measuring the entire range of hyperspectral features. However, in applications of these indices, estimation of traits with no clear absorbance features in the measured spectral range remains difficult. For example, robust spectral indices for total C and phenol have not been reported because the direct absorbance wavebands of these traits remain unclear.

The partial least square regression (PLSR) algorithm is based on canonical correlation analysis, and multiple regression between the target and optical information is performed using numerous training datasets after orthogonal decomposition of the explanatory and objective variables (Martens & Næs, 1992). Because of strong ability to extract information from large spectral bands, the PLSR model has the potential to estimate leaf traits even if the target traits do not have clear specific absorption features in the input spectral data. In recent decades,

several studies have used PLSR models to estimate single or multiple leaf traits, showing that these models can be used for remote sensing of leaf traits at the single-leaf and forest-canopy scales (Asner *et al.*, 2011). To date, field applications of PLSR models have been reported mainly for tropical forests (Asner & Martin, 2008, 2011; Asner *et al.*, 2011, 2014, 2015; Doughty *et al.*, 2011), grassland, and crop fields (Mitchell *et al.*, 2012; Zhai *et al.*, 2013; Serbin *et al.*, 2014, 2015). Recent studies have examined northern boreal tree species (Serbin *et al.*, 2014) and temperate forest species in the United States and Central Europe (Zhao *et al.*, 2013; Serbin *et al.*, 2015; Yang *et al.*, 2016). To our knowledge, there is little information on PLSR models of Asian forest tree species distributed in wide latitude ranges, although the PLSR models for estimating the leaf nutrient status of some tea, arbor and shrub species in China have been reported (Zhai *et al.*, 2013). Studies using airborne-mounted sensors carried out in North America investigated the performance of the PLSR model for estimations of the photosynthetic activity of nine evergreen broadleaved tree species growing within 35–37°N (Serbin *et al.*, 2015) and the leaf N content of evergreen coniferous forests within 29–45°N (Lepine *et al.*, 2016). Yang *et al.* (2016) studied the effect of the leaf phenology of four deciduous broadleaved tree species growing in temperate forests within 41–42°N, on PLSR model performance in terms of estimating three leaf traits. These studies demonstrated the good performance of the PLSR model for a few target species or leaf traits, but additional information about the utility of the PLSR model for species widely distributed from cool temperate to tropical climate zones is important for future development of hyperspectral remote sensing of leaf traits at the global

scale. East Asia is one of the most suitable regions for validation of the robustness of the model performance because many biomes with high species diversity are distributed widely from cool temperate to tropical zones. Furthermore, especially in temperate forests, the utility of PLSR models between plant functional types (*i.e.*, deciduous and evergreen tree species) and effects of seasonality on model performance should be confirmed, because both deciduous and evergreen tree species are distributed throughout Asia. To the best of our knowledge, few studies have examined the effects of seasonal variation on PLSR model performance except Yang *et al.* (2016). Because seasonal variation in the composition of leaf chemicals such as reductions in chlorophyll and N in senescent leaf, affects leaf reflectance (*e.g.*, Gitelson *et al.*, 2002, 2003, Sims & Gamon, 2002; Hesketh & Sánchez-Azofeifa, 2012), model calibration and validation should consider each plant functional type (deciduous or evergreen) and leaf developmental stage (young, mature, and senescent). Generally, PLSR models can estimate the sample characteristic variance if the variance has been included previously in the calibration process.

We investigated the performance of PLSR models established for estimation of leaf traits in 14 East Asian forests in the Pacific zone (3–45°N, 102–142°E). The research sites were located in Japan, Thailand, and Malaysia. The climate and phenology data show clear seasonality in temperate and cool temperate forests in northern regions. The datasets of optical properties and six leaf traits (LMA and concentrations of total N, total C, phenol, cellulose, and lignin) were collected at different developmental stages, including young, mature, and senescent leaves, from deciduous and evergreen species. Because the sampling interval varied across sites (from

half-month intervals in the entire leafy period to one sample for mature leaves), we investigated the effects of varied functional type on the PLSR models for mature leaves. Then we presented the fitness of the mature leaf model to estimate the other leaf developmental stages. Finally, we assessed the effects of reducing the analyzed wavebands and evaluated the possibility of remote estimation of leaf traits based on leaf spectroscopy.

2. Materials and Methods

2.1. Research site and field survey

A map of the research site and a summary of the climate conditions and collected samples are shown in Figure 1 and Table 1, respectively. In 2010, we selected 14 research sites in Japan, Thailand, and Malaysia. Between 2010 and 2014, we took field measurements of hyperspectral reflectance of leaf and collected leaf samples. The research sites were classified into five vegetation zones: cool temperate wet forest (six sites), warm temperate moist forest (one site), warm temperate wet forest (four sites), tropical dry forest (one site), and tropical moist forest (two sites) (Table 1). In total 141 species and 1,106 individual leaves were sampled (Table 1). Data were collected from deciduous (65%) and evergreen (35%) tree species, as well as young (10%), mature (77%), and senescent leaves (13%). We defined light-colored, juvenile leaves at full expansion as young leaves, and discolored and dead leaves immediately following natural

defoliation as senescent leaves. The timing of leaf expansion was previously investigated using time-lapse camera images at five sites in Nakagawa (NKG), Kuromatsunai (KUR), Tomakomai (TOM), Wakayama (WAK), and Pasoh (PSO) (Nakaji *et al.*, 2014; Nagai *et al.*, 2018) (Fig. 2), although we did not measure leaf age directly, as well as information obtained by researchers at nine additional sites. Most young leaves were collected during the 1 to 2 months following initiation of the expected leaf expansion. The list of collected tree species and the range of the measured leaf traits are shown in Appendix 1.

At each research site, we accessed the top closed canopy of target tree species using a scaffold, monitoring tower, and canopy crane to measure the hyperspectral reflectance of sunlit leaves. One leaf in the center position of a top branch was selected for sampling, and more than three replicates per tree species were collected. We measured the reflectance of the adaxial leaf surface at the VSWIR wavebands (350–2,500 nm) using a portable spectroradiometer with a plant probe and halogen light source (FieldSpec Pro FR with probe model A122317; ASD Inc., USA). The plant probe has a fixed geometry and takes into account one angular illumination and viewing angle. The angles of light source and probe to clipped leaf surface are 78° and 55°, respectively. In this study, we used this plant probe system to measure the leaf reflectance of broad leaf in the field because of high portability and rapid measurement on the top canopy. Because the main purpose was to clarify the effects of varying plant functional type, leaf age and band number on the model performance, we did not compare the reflectance data obtained from plant probe and those from integrating sphere in this study. The center area of the

individual leaf without the central nerve was set in the leaf clip of plant probe, and the reflectance signal was observed during rotation of the two background plates affixed to the instrument, a black low-reflectance standard and white high-reflectance standard. The materials of the surface of the black and white background plates were adhesive plastic label sprayed with matte black paint and Gore-Tex label without paint, respectively. The background plates had spectral reflectance factors of < 0.04 and about 0.98. In this study, the Spectralon reflectance standard (Labsphere Inc., USA) was used to calibrate these affixed background plates periodically during the leaf measurement process in the field to remove any effect of dirt on the reflectance measurement. The leaf spectral reflectance factor (R_{leaf}) was calculated by the method of Miller et al. (1992) (Equation 1):

$$R_{\text{leaf}} = (R_w * B - R_b * W) / (B - W - W * B * (R_b - R_w)) \quad (1),$$

where R_b and R_w are the spectral reflectance factors from the leaf adaxial surface measured with the black and white background plates (range of factors: 0.03–0.88), respectively, and B and W are the measured spectral reflectance factors of the black and white background plates, respectively. In the case of one conifer species (*Chamaecyparis obtusa*), the spectral reflectance of needle leaves was measured using an integrating sphere (1800-12S; Li-Cor Inc., USA) according to the method of Noda *et al.* (2013). Because the needle leaves of *Chamaecyparis* are relatively flat, the spectral features of this species at 350–2,500 nm were also measured using a

plant probe with a black background plate. The reflectance factor obtained using an integrating sphere was not available at wavelength < 440 nm and $> 1,770$ nm due to large noise. Therefore, we estimated the reflectance at these spectral regions by multiplying the data obtained from the plant probe and the ratio at the overlapped wavebands between both methods.

Reflectance was measured on intact leaves in most cases, but detached leaves were used in some cases. In the latter case, the leaves were detached from the branch with scissors, and the collected leaves were stored under wet, cool conditions until measurement to minimize the effect of dryness on the spectral characteristics and chemical traits, which was determined within 1–3 h of detaching the leaves. For each tree, we checked for natural defoliation on the ground and collected senescent leaves immediately after defoliation.

2.2. Trait analyses

After reflectance measurement, color photographs of the leaves were taken to calculate the projected leaf area (LA). Leaves were then stored in silica gel or zeolite and dried for 10 days at room temperature. The temperature was controlled at $< 40^{\circ}\text{C}$ to avoid reduction in the extraction efficiency of polyphenols in the chemical analysis (Constantinides & Fownes, 1994). We measured six leaf traits (LMA and concentrations of N, C, phenol, cellulose, and lignin). All dried leaf samples were weighed and then ground using a micro-smasher (Multi-Beads Shocker; Yasui Kikai Co., Japan). The LMA was calculated as the leaf dry weight divided by the projected LA. The concentrations of total N and C in the powdered samples were measured by

combustion with an NC analyzer (Sumigraph NC-900; Sumika Chemical Analysis Service, Ltd., Japan).

The analysis of lignin and cellulose followed the methods of Osono and Takeda (2005), with some modifications. In a polypropylene test tube, 20 mg of dried sample powder was shaken with 12 ml 50% (v/v) methanol at 70°C for 1 h. After centrifugation at $12,000 \times g$ for 10 min at room temperature, the extract solution was analyzed for condensed tannin and total phenols. The residue was rinsed with 50% methanol and centrifuged again. We repeated this rinsing process three times. Then, the residue was dried and mixed with 0.75 ml 72% (v/v) H_2SO_4 solution at room temperature for 2 h. The mixture was diluted to 2.5% H_2SO_4 with distilled water and autoclaved at 121°C for 1 h. After cooling, the mixture was passed through a 10- μ m-pore glass filter to separate the H_2SO_4 solution and residue. The concentration of carbohydrates in the filtrate was measured following the phenol–sulfuric acid method (Dubois *et al.*, 1956). The cellulose concentration was estimated using this carbohydrate concentration. The filtered residue was rinsed with distilled water and dried at 105°C. After weighing the dry mass (DM), the residue was combusted in a muffle furnace at 600°C for 4 h and weighed. The lignin concentration was calculated from the weight difference between the dried residue and ash. Although this fraction included other acid-insoluble (lignin-like) organic components, we considered this concentration to represent lignin content. The concentration of total phenols was measured as a gallic acid equivalent using the Folin–Ciocalteu method (Singleton *et al.*, 1999). Differences in leaf traits across plant functional type and leaf developmental stage were

investigated using two-way analysis of variance (ANOVA) together with power analysis. These analyses were performed using SPSS ver. 11.0.1J (SPSS Inc., Tokyo, Japan).

2.3. Leaf trait estimation

To avoid the effects of noise on the analysis, we used the reflectance data at wavelengths between 400 nm and 2,400 nm. Since the full width at half maximum of the used spectral radiometer was about 3 nm and 10 nm in the ranges of 400–980 nm and 980–2,400 nm, respectively, we used the binned spectral reflectance at 3 nm intervals (400–979 nm) and 10 nm intervals (985–2,395 nm). As a result, we included 336 bands in the VSWIR spectral region for all of the analyses. The PLSR analysis was conducted using Pirouette ver. 4.5 (Infometrix Co., USA). The raw spectral reflectance factors were used for the PLSR analysis since processing the derivative reflectance and log transformation offered no improvement in regression performance. To avoid overfitting of the regression factor, the optimal number of factors was selected by minimizing the prediction residual error of squares statistic (Haaland & Thomas, 1988; Osten, 1988; Chen *et al.*, 2004). For each leaf trait, the PLSR model was calibrated using the entire dataset of leaf traits and spectral reflectance factors (*i.e.*, training data). The calibrated model was evaluated by cross-validation with the leave-one-out method. R^2 , RMSE and NRMSE (Asner *et al.*, 2011; Cheng *et al.*, 2014; Yang *et al.*, 2016) were calculated as indices of model performance. The relative importance of reflectance at each wavelength was investigated by calculating the variable importance of projection (VIP) (Wold *et al.*, 2001). In the band

selection analysis, we tested two methods, the VIP value threshold method and the equal interval removal method. In the method using the VIP value, the spectral bands in which the VIP value for any one of the six traits exceeded the threshold value were selected from among all 336 bands. We tested eight threshold VIP values, varying from 0.4 to 1.8, with an interval of 0.2. During removal, the spectral bands were resampled at intervals of 10 nm, 15 nm, 20 nm, 25 nm, and 30 nm from the original reflectance factor data without any binning treatment. The starting wavelength of the collection was 400 nm. In this analysis, the reflectance data were binned after resampling at 5–10 nm, similar to the other analysis.

3. Results

3.1. Leaf traits and spectral reflectance features

Table 2 shows the leaf trait properties in each of the six categories (two functional types × three developmental stages) of the collected leaves. We assessed the leaf chemical traits in terms of DM- and LA-based concentration. Although the statistical powers were sufficiently high in most cases (power of analysis: > 0.8), a few non-significant effects may be explained by low statistical power (*e.g.*, effect of developmental stage on LA-based cellulose concentration) (Table 2). The ranges of most data overlapped between plant functional type; however, all leaf traits showed significant differences between the deciduous and evergreen tree species ($P < 0.05$,

Table 2). The LMA and all of the chemical traits expressed as LA-based concentrations were higher in evergreen species than in deciduous species (Table 2). When the mature leaves were considered, LMA and LA-based chemical concentrations were 5.5% (N) to 42% (C) higher in evergreen species than deciduous species. In contrast, for the DM-based concentrations of mature leaves, N and phenol were lower, and the other traits were only 1.9% (C) to 3.3% (lignin) higher in evergreen species than deciduous species (Table 2). The differences between the functional types are larger for LA-based concentrations than DM-based concentrations, and that higher chemical concentrations in evergreen species are linked to a higher LMA. High LMAs tend to be observed in evergreen tree species often grown under warm and wet conditions at the regional scale (Wright *et al.*, 2004; Ordoñez *et al.*, 2009; Meng *et al.*, 2015) although the LMA of an individual tree is affected by environmental factors such as light, water, and tree height (Larcher 2003; Markesteijn *et al.*, 2007; Nabeshima & Hiura, 2008; Miyata *et al.*, 2011). In this study, the mean annual temperature (MAT) at the research sites ranged widely, from 6.0°C to 27.0°C (Table 1), and most evergreen leaf samples were collected at warmer sites with MATs $\geq 17.7^{\circ}\text{C}$ (Table 1) (sites: TAN, YAK, SAK, LAM, and PSO; Appendix 1). All of the leaf traits except the LA-based cellulose concentration significantly differed among the leaf developmental stages (Table 2). In deciduous tree species, the LMA and all LA-based concentrations of chemical traits increased with leaf development, and the concentrations, except for lignin, were reduced with leaf senescence (Table 2). In evergreen species, LMA and LA-based concentrations tended to be reduced with leaf senescence, but LMA of young leaves

showed the highest value and the concentrations of chemical traits in these leaves were highly similar to those of the mature leaves (Table 2). This high LMA of young leaves of evergreen tree species has been reported as “delayed greening” (Miyazawa et al., 1998; Numata et al., 2004), which is a common phenomenon in evergreen broadleaved forests in warm temperate and tropical regions.

Figure 3 shows the leaf spectral reflectance features in each plant functional type and leaf developmental stage. In both functional types, the mean leaf NDVI was similar between young and mature leaves, and was reduced by senescence. The NDVIs of mature and senescent leaves were 0.82–0.84 (Figs. 3a, b, d, e) and 0.49–0.52 (Figs. 3c, f), respectively. As shown in Figures 3a, b, d, and e, young and mature leaves had typical spectral features such as green peaks (550 nm), valleys before the red edge (680 nm), plateaus (850–950 nm), deep valleys due to water absorption (1,450 nm and 1,940 nm), and shallow valleys related to lignin and cellulose (1,680–1,780 nm and 2,100–2,300 nm) (Curran, 1989; Nagler *et al.*, 2000; Serrano *et al.*, 2002) irrespective of functional type. In these developmental stages, reflectance factors of evergreen species were significantly higher in NIR–SWIR spectral regions at 712–1,255 nm but lower in SWIR spectral regions at 1,865–2,395 nm than those of deciduous species although the range in variation (*i.e.*, 25th–75th percentile) of the reflectance factor was not largely different between the deciduous and evergreen species (Figs. 3a, b, d, e). Meanwhile, in senescent leaves, a green peak around 500–650 nm and shallow valley before the red edge tended to be rounded or lacking, together with discoloration in both functional types (Figs. 3c, f). These features around

the visible spectral region are common due to seasonal changes in the balance of pigments such as anthocyanin, carotenoids, and chlorophylls (Sims & Gamon, 2002; Gitelson & Merzlyak, 2004) although we did not measure foliar pigments in this study. The reflectance features of senescent leaves showed a clear valley for the lignin and cellulose absorption bands at around 2,100 nm, especially for evergreen tree species (Fig. 3f). For deciduous tree species, the same trend appeared in the 75th percentile of the spectral feature (Fig. 3c). The reflectance factor of senescent leaves was significantly higher at most wavebands in evergreen tree species than in deciduous tree species (Figs. 3c, f).

3.2. Difference in sensitivity of the PLSR model between DM- and LA-based traits

Figure 4 shows the performances of PLSR models compared between DM-based and LA-based trait concentrations except LMA. As shown in Figures 4a–e, the R^2 values of C, phenol, cellulose, and lignin tended to be higher for the LA-based concentrations, and the R^2 value of the pooled traits was significantly higher for the LA-based (mean: 0.71) than the DM-based (mean: 0.43) concentrations (Fig. 4f). Similarly, the NRMSEs of the chemical leaf traits, except N, tended to be lower in the model constructed using the LA-based trait concentrations, and the mean NRMSE for all of the traits was marginally lower for the LA-based trait concentrations (7.6%) than for the DM-based concentrations (10.7%) ($P = 0.09$; Fig. 4f).

On the other hand, a similar trend of higher model performance for LA-based traits compared

to DM-based traits was also observed in PLSR models by reference to the VNIR spectral region (data not shown). The performance in the case of VNIR was lower than in the case of VSWIR. For example, the R^2 and NRMSE values of the pooled LA-based traits were 0.38% and 11.3%, respectively. Although we also tested the first and second derivative reflectance factors and log-transformed reflectance factors for calibrating the PLSR model, clear improvement compared to the normal spectral reflectance factor was not found (data not shown). Based on these results, we hereafter report the validation results for the PLSR model that was calibrated for the LA-based concentrations using the leaf spectral reflectance factor in the VSWIR spectral range.

3.3. Interchangeability of the PLSR model between functional type and leaf developmental stage

We first tested the PLSR models using training datasets with four cases: mature leaves of deciduous tree species, evergreen tree species, both functional types, and leaves in all of the developmental stages and functional types (*i.e.*, all samples). Because of the large sample size, the models based on mature leaf data (*i.e.*, first three cases) were used to test the interchangeability of the PLSR model between the two functional types. Then, by using the PLSR models calibrated for mature leaves and for all developmental stages, the estimation accuracy for young leaves and senescent leaves was investigated.

Table 3 shows the results of the calibration and cross-validation of the developed PLSR models. In all four cases, the estimation accuracy differed depending on the leaf traits.

Relatively high R^2 (> 0.79) and low NRMSE ($< 9.6\%$) values at validation were obtained for LMA, C concentration and phenol concentration (Table 3). The accuracies for cellulose and lignin were not high in any test cases, and the lowest R^2 (0.40) and highest NRMSE (12.6%) were observed in the model that estimated the cellulose in evergreen tree species (Table 3).

The compatibility of the PLSR model based on mature leaves was evaluated by changing the combination of calibration and validation data by functional type. Table 4 shows the matrix of the validation results. For all leaf traits, the highest R^2 and lowest NRMSE values were recorded when the same functional type was used for calibration and validation (Table 4). In contrast, the estimation accuracy was reduced when the functional type differed. For example, in the model calibrated using deciduous leaf samples, the NRMSEs of six traits were 4.8–11.3% when validating deciduous leaf samples, but were 9.0–17.5% when validating evergreen leaf samples (Table 4). The increase in error was larger in the model calibrated for evergreen species than in that for deciduous species. The range in NRMSEs for the evergreen species increased from 8.0–12.6% to 10.4–21.1% when the functional type was changed (Table 4). The PLSR model calibrated by using both functional types showed intermediate accuracy in the validation for deciduous species (NRMSE = 5.3–12.3%) and evergreen tree species (NRMSE = 8.0–13.5%) (Table 4). When both functional types were validated, the highest accuracy was observed in this model (NRMSE = 6.4–10.8%) compared to the other two models (NRMSE = 7.1–17.9%) (Table 4). These results indicate that a training dataset that includes both functional types is suitable for estimating the leaf traits of both deciduous and evergreen tree species. Hereafter,

this model is used in the investigation of sensitivity to varying leaf developmental stages. Table 5 shows the results of the PLSR model validation using the datasets of young and senescent leaves. When the model was calibrated by using only mature leaf data, the model showed a lower R^2 (0.01–0.86) and higher NRMSE (7.9–39.5%) than the model using the datasets including all developmental stages ($R^2 = 0.62$ – 0.91 , NRMSE = 6.7–13.7%) (Table 5).

3.4. Estimation of important wavebands

To understand which wavebands in the spectral data would be effective for use in the PLSR model, we calculated the VIP values of the two PLSR models calibrated using mature leaf data and all leaf data (*i.e.*, all developmental stages and functional types) (Figs. 5a, b). In the model based on mature leaves, the VIP values at wavebands from 733–2,395 nm outside two deep valleys representative of water absorption (1,450 nm and 1,940 nm) showed similar relatively high values in all leaf traits (Fig. 5a). In particular, in the SWIR spectral region, two broad peaks around 1,580 nm and 1,800 nm (width $\approx$ 300 nm) were observed for LMA, N, C, cellulose, and lignin (Fig. 5a). In addition, two sharp peaks at 550 nm and 700 nm and one small peak around 570 nm were observed for N and phenol, respectively (Fig. 5a). Conversely, in the PLSR model calibrated for all leaf developmental stages, the high-VIP wavebands were shifted towards longer wavelengths around the SWIR region, except for that of lignin (Fig. 5b). In the case of N, the peak at 700 nm remained, although the importance of the green peak was lower than that for mature leaves (Fig. 5b).

We assessed the efficacy of extracting important wavebands from hyperspectral data based on VIP values and the combing removal method. Figure 5c shows an example of the tested band filter at the threshold VIP of 0.8 for the PLSR model using all plant functional types and leaf developmental stages in this study. In this case, a score of 1 indicates the wavebands with VIP > 0.8, and the wavebands showing a score of 1 in any trait were within 622–715 nm and 739–2,395 nm (255 bands). We selected not only the wavebands with VIP in excess of 0.8, but also those with VIP in excess of the threshold (between 0.4 and 1.8) according to a 0.2 interval step (total of eight cases). The numbers of selected wavebands exceeding the VIP threshold of 0.4–1.8 were 292, 277, 254, 229, 218, 185, 151, and 64. On the other hand, the numbers of wavebands in the equal interval resampling method at the 30-, 25-, 20-, 15-, and 10-nm intervals were 66, 85, 104, 134, and 207, respectively. Figure 6 indicates how the changes in the accuracy parameters of PLSR model responded to the variation in the used wavebands. In both methods, a reduction in R^2 and increment in NRMSE were observed with a reduction in the number of wavebands (Fig. 6). However, the slope of the response curve of these parameters differed among the band selection methods, and a reduction in model accuracy occurred in a larger number of wavebands with VIP-based selection than with the equal interval resampling. We evaluated the necessary minimum number of wavebands as the number keeping relative changes of R^2 and NRMSE within 5% compared to the case using original waveband. According to this definition, the necessary minimum numbers of wavebands were less in the equal interval resampling (66–104) than in those in VIP method (151–185) (Fig. 6). Maximum number in the

equal interval resampling, 104 was corresponded to 20-nm interval sampling.

4. Discussion

4.1. Performance of PLSR model for Asian forest tree species

In this study, we chose six leaf traits, LMA and concentrations of N, C, phenol, cellulose, and lignin, which are related to vegetation production (Larcher, 2003; Wright *et al.*, 2004), plant defenses to insects and pathogens (Rothstein *et al.*, 2004; Bidart-Bouzat & Imeh-Nathaniel, 2008), and the metabolic cycle (Berg & Laskowski, 2006; Prescott, 2010). It has been reported that leaf phenolic metabolites and the balance between leaf C and N tend to vary due to global warming and elevated atmospheric concentrations of CO₂ and air pollutants (Zvereva and Kozlov, 2006; Bidart-Bouzat & Imeh-Nathaniel, 2008). Increasing annual temperature and climate extremes of temperature and precipitation are ongoing in East Asian region (IPCC, 2012, 2013), and the concentration of gaseous pollutant such as tropospheric O₃ is also still high (Hatakeyama, 2017). Therefore, remote sensing of these traits would provide us with important information for understanding the current state and responses of East Asian forests to ongoing climate change and anthropogenic disturbances.

The PLSR models in this study showed performances similar to those reported for humid tropical forest tree species based on large datasets collected globally (Asner *et al.*, 2011). For

example, the NRMSE values of the six leaf traits (LMA and DM-based concentration) of mature leaves in this study were 4.8–12.8% (data not shown), similar to the range of 5.9–10.0% reported for a leaf-level analysis of humid tropical forests (Asner *et al.*, 2011). Additionally, in the cases of temperate forests, R^2 values of DM-based leaf N in this study (0.62–0.66) were close to the value of oak and birch in United States (0.63) (Yang *et al.*, 2016) and the range of tea, arbor and shrub species (0.25–0.66) in China (Zhai *et al.*, 2013). Although the estimation accuracy of cellulose in this study was low among the five tested traits (the reason for which remains unclear), the range of R^2 values for mature leaves (0.40–0.50) was also similar that of deciduous and evergreen temperate forests in the United States (0.45–0.47) (Singh *et al.*, 2015). This indicates that the PLSR model has similar potential for application to East Asian forests, and this fact could be very important for informing future studies on satellite monitoring of forest responses in not only East Asia but also in global scale.

4.2. Comparison between LA-based and DM-based trait concentration

Higher model performance for LA-based trait concentrations than DM-based trait concentrations was observed, especially for total C and cellulose (Figs. 4c, d). This improvement in interpretability can be explained by reference to the methodology and spatial distribution of chemical traits in plant leaves. Generally, leaf reflectance measurements record the reflected radiation within a constant target surface area (i.e., field of view) with no adjustment for target weight. The intensity of radiance is expressed by energy per surface area,

and the reflectance is the ratio. This suggests that traits estimated based on reflectance data may correspond to LA-based leaf traits irrespective of leaf thickness, if the DM-based concentration is not largely affected by leaf thickness. The main component of leaf C is cellulose, which is generally contained in the cell walls at a high ratio of < 40% in higher plants (Fry *et al.*, 1989). Because cell walls are distributed uniformly throughout the whole leaf lamina, except for the leaf vein, the DM-based concentration does not vary with leaf thickness. In this study, when we compared the chemical concentration of these traits between deciduous species with low LMA and evergreen species with high LMA (Table 2), DM-based concentrations of C and cellulose differed only < 3% among these functional types (*e.g.*, cellulose in mature leaves: 153 mg g⁻¹ vs. 158 mg g⁻¹), but LA-based concentrations showed a large difference, ranging from 40–42% (cellulose in mature leaves: 13.9 mg m⁻² vs. 19.5 mg m⁻²). In addition, the relationship between LMA and DM-based cellulose concentrations was not significant ($R = 0.009$, $P = 0.746$) but was significant for LA-based concentrations ($R = 0.98$, $P < 0.001$). Although LMA is affected by both leaf thickness and bulk density (Lamont *et al.* 2015), this result supports that leaf thickness affects LA-based chemical concentrations but does not strongly impact DM-based concentrations. Although the results regarding of the VIP features indicated that wavebands in the NIR–SWIR spectral regions (739–1,135 nm and 1,395–2,395 nm) are important for estimating concentrations of chemical traits in the PLSR model (Fig. 5c), the NIR spectral region generally relates to leaf thickness and the developmental status of the leaf (Slaton *et al.*, 2001); therefore, if the PLSR model estimates these traits based on leaf thickness

indirectly via NIR reflectance, expecting better performance for LA-based versus DM-based cellulose and total C concentration estimates is reasonable. In contrast, the model accuracy with respect to leaf N did not vary by the calculation method (Fig. 4a). The DM-based N concentration was approximately 21% lower in evergreen tree species with high LMA (Table 2). Because the major nitrogenous components, such as proteins and photosynthetic pigments, are distributed mainly in chloroplasts in palisade tissue below the epidermis and cuticle, DM-based concentrations may be diluted in thicker leaves if the leaves have thick spongy mesophyll cells. Wold *et al.* (2001) proposed a VIP threshold of 0.8 to evaluate the importance of spectral data in each waveband. Since wavebands at both the red-edge and NIR–SWIR regions were identified as important bands (VIP > 0.8; Fig. 5c), estimations by PLSR would be able to reflect both the surface pigment concentration and leaf thickness, resulting in similar estimation accuracy between LA- and DM-based N concentration.

4.3. Effect of functional type and leaf developmental stage on PLSR model performance

The performance of the PLSR model was relatively worse when the plant functional type and leaf developmental stage of interest were not included in the training dataset. This result suggests that we should consider the differences in the effectiveness of the PLSR models across plant functional type and leaf developmental stage when applying this estimation method in any wide area of East Asian forest. In this study, both data of the leaf traits and spectral features

showed significant differences between functional types and among leaf developmental stages (Table 2 and Fig. 3). For example, the LMA was higher in evergreen species than deciduous species (Table 2), and the spectral reflectance at the plateau region in the NIR around 750–950 nm was also higher in evergreen species irrespective of the leaf’s developmental stage (Fig. 3). The wavebands at this spectral region showed $VIP > 0.8$ in LMA estimation (Fig. 5c); therefore, we expected that the PLSR models of either deciduous or evergreen species would be able to extrapolate the LMA for both functional types. However, in the result, the PLSR models based on the training data of one functional type were unable to accurately estimate the LMA of the other functional type (Table 4). A reduction in accuracy caused by mismatch of the training data was observed not only for LMA, but for all measured traits. Although further studies are needed to clarify the reason for this observation, the fact indicates a limitation of the empirical approach using the PLSR model. On the other hand, the test case using training datasets of both functional types indicated that the PLSR model could be improved by using mixed datasets collected from several biomes in cool temperate, temperate and tropical forests.

Statistical estimation models such as PLSR generally require large amounts of training data. According to the case of tropical tree species reported by Asner *et al.* (2011), the required sample number varied among leaf traits, and a stability test of PLSR models with random sampling showed that $> 1,250$ samples yielded stable calibrations for C, N, phenol, and cellulose. Because we measured a maximum of 1,106 leaves, it may be necessary to increase the sample size to improve the robustness of the PLSR estimation for East Asian forests. Because of

the sample size limitation, we calibrated the PLSR model without random sampling, and validated the model with the leave-one-out method. In addition, we tested the calibration using 75% of the entire data and performed the validation with the remaining data with selection by stratification, but the resulting accuracy did not differ substantially from the data presented in this paper (data not shown). Moreover, a larger sample size of evergreen species, including more coniferous species, will be necessary to improve the robustness of the PLSR estimation model for mixed forests in East Asia. For future work, although it would be a labor-intensive method requiring great effort, sample collection from typical biomes may be a method facilitating the development of a higher-accuracy estimation model of leaf traits. Since certain large databases of leaf traits and/or spectral characteristics, such as the TRY Plant Trait Database (Future Earth and the Max Planck Institute for Biogeochemistry; <https://www.try-db.org/TryWeb/Home.php>) and the Spectranomics (Carnegie Institution For Science, <https://cao.carnegiescience.edu/spectranomics>) database, have been developed, future linkage of datasets based on field surveys of other biomes would be helpful for producing integrated studies pertaining to the development of robust remote sensing models.

Regarding the effects of leaf phenology on PLSR model performance, Yang *et al.* (2016) reported the potential for using the PLSR model to track seasonal variations in LMA, pigments (chlorophylls and carotenoids), and mass-based N in two temperate forests in the United States. The authors performed field measurements biweekly and found that the relationships among

leaf spectra traits varied over time. Therefore, they proposed that calibrating PLSR models using data sampled throughout the season on a biweekly basis would be suitable to trace seasonal variations in leaf traits in deciduous temperate forest (Yang *et al.*, 2016). In our study, although we did not conduct periodic sampling, with particularly low periodicity in the evergreen forests, the improved performance of the PLSR model calibrated using all developmental stages (Table 5) partially supports their findings, underlining the necessity of including training data from various developmental stages of both deciduous and evergreen forest species.

4.4. Necessary minimum number for PLSR model

Our test about band selection indicates that the reduced VSWIR wavebands filtered by combing removal with an interval < 20 nm have potential for stable estimation of the six leaf traits within $< 5\%$ error range compared to the model using full band dataset. The analysis of VIP provided valuable information about the most important wavebands, but filtering based on a VIP threshold (0.4–1.8) showed similar or reduced model performance compared to the cases using the combing removal method (Fig. 6). Regarding leaf N and LMA, the trends of the peak around the red-edge (N) and relatively high VIP in the NIR and SWIR spectral region (LMA) (Fig. 5a) were similar to the VIP values in other studies of temperate forest tree species (Singh *et al.*, 2015; Yang *et al.*, 2016). In this study, the spectral region remaining after filtering by the VIP threshold of 0.8 included absorption bands related to N (700 nm for chlorophyll, 1,510 nm

and 2,180 nm), lignin, and cellulose (1,420 nm, 1,940 nm, etc.) (Curran *et al.*, 2001; Serrano *et al.*, 2002) (Fig. 5c). In the cases of total C and LMA, because these traits are related to NIR reflectance and are also strongly correlated with N ($R = 0.62\text{--}0.64$, $P < 0.001$) and lignin ($R = 0.70\text{--}0.74$, $P < 0.001$), these traits might be estimated indirectly by PLSR model even though the reflectance in specific absorbance wavebands could not be measured. Similarly, the concentration of phenol was correlated positively with total C ($R = 0.69$, $P < 0.001$).

As for the minimum necessary band number for PLSR model, Lepine *et al.* (2016) reported that reducing the spectral resolution from 10 nm to 90 nm did not affect PLSR model performance for estimating leaf N concentrations in canopy scale observations of boreal and temperate forests in North America. In this study, the NRMSE of leaf N estimation tended to increase with resampling, and relative increase in NRMSE at the 5% level was found for the interval > 20 nm (Fig. 6). Because the spatial scale of reflectance monitoring and vegetation type are quite different, comparison of suitable band numbers is difficult between the cases in North America and our study. However, at least in the case of our research, a sampling interval < 20 nm with enough spectral resolution is recommended for future remote sensing of leaf traits in East Asian forests. Because there are plans for satellite-mounted hyperspectral imagers in the United States, Europe, and Japan (Tachikawa *et al.*, 2012; Lee *et al.*, 2015), we believe that this basic information about band selection will support the development plan of monitoring devices.

5. Conclusion

Based on leaf spectroscopy and the analysis of the PLSR model using leaves of East Asian forest tree species, we found that (1) the PLSR model showed good performance for estimation of LMA and LA-based concentrations of N, C, phenol, cellulose, and lignin; (2) the performance of the PLSR model was relatively worse when the plant functional type and leaf developmental stage of interest were not included in the training dataset; and (3) wavebands in the VSWIR spectral region (400–2,395 nm) selected with a less than 20-nm interval (> 104 bands) provided stable model performance for estimating these leaf traits. These findings are fundamental information for developing trait estimation model for diverse biomes distributed widely from cool temperate to tropical region. Especially, accuracy difference between LA-basis and DM-basis trait concentration and importance of plant functional type in model calibration will be valuable knowledge. Several studies on hyperspectral remote sensing of leaf traits in other regions have applied PLSR modeling at both the single-leaf and canopy scales, in combination with radiative transfer modeling (Asner *et al.*, 2011, 2015; Lepine *et al.*, 2016). Because all of our findings are based on leaf-level observations, the scaling up of monitoring and validation in East Asian forest is next important work.

Acknowledgements

We are greatly indebted to Mrs. Saori Yoshida (Hokkaido Univ.) for technical support of chemical analysis. We also thank to all peoples who supported our leaf sampling in the research sites; Dr. Yoshihisa Suyama (Tohoku Univ.), Dr. Naoki Yasumura (Univ. Tokyo), Dr. Junji Sano (Tottori Univ.), Dr. Masahiro Takagi (Miyazaki Univ.), Dr. Ayumi Katayama, Dr. Tsutomu Enoki (Kyusyu Univ.), Dr. Noriyuki Osada (Hokkaido Univ.), Dr. Shin Nagai (JAMSTEC), Dr. Tomonori Kume (National Taiwan Univ.) and Mr. Satoshi Ichikawa. The field research in Yakushima National Park, Pasoh forest reserve, Lambir Hills National Park and Sakaerat research site were permitted by Kagoshima Prefecture, Forest Research Institute Malaysia, Forest Department in Sarawak and National Research Council of Thailand, respectively. This work was supported by a JSPS Grant-in-Aid for Scientific Research (KAKENHI: 24255014 and 26304015), by the Environmental Research and Technology Development Fund (S-9-3) of the Ministry of the Environment and by GRENE-ei from MESSC, Japan.

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Figure legends

Figure 1. Locations of the research sites.

Figure 2. Sample images of phenology monitoring digital camera. The fish-eye image of forest canopy in Tomakomai site (top) and samples of target tree (*Acer mono*) at different phenological stages (bottom) are shown. The timing of leaf sampling was planned based on similar photo images in six sites. The images in Tomakomai have been published in website of Phenological Eyes Network (http://pen.agbi.tsukuba.ac.jp/index_e.html). The enclosing lines and each of abbreviations in top image indicate the canopy of individual tree and name of tree species, respectively.

Figure 3. Spectral reflectance factors of (a–c) deciduous and (b–d) evergreen tree leaves in East Asian forests. The solid lines, dark-gray areas, and light-gray areas indicate the median, 25th–75th percentile range, and minimum–maximum range, respectively. The solid- and dotted-horizontal lines above the reflectance factor of evergreen species indicate respectively significant higher- and lower-reflectance factors

compared to deciduous species ($P < 0.05$). The effects of leaf developmental stage and interaction with plant functional type on the reflectance factors were significant in all measured wavebands (two-way ANOVA, $P < 0.05$). The NDVI was calculated from the leaf spectral reflectance at 850 nm (R_{850}) and 650 nm (R_{650}) as follows:

$$\text{NDVI} = (R_{850} - R_{650}) / (R_{850} + R_{650}).$$

Figure 4. Performance of the chemical trait estimation models based on either DM-basis or LA-basis concentrations. The PLSR models were calibrated to estimate five chemical traits in each of six leaf categories (two functional types $\times$ three developmental stages) All 1,106 samples were used for the analysis (number in each category is shown in Table 2). The R^2 and NRMSE were calculated from the model cross-validation. The quartiles in the five chemical traits (a–e) indicate the variance in the parameters between the six test cases ($n = 6$). Panel (f) shows the variance for all of the data ($n = 30$). Significance differences of parameters between DM-basis and LA-basis concentration are represented by the P value (t -test).

Figure 5. Spectral features of VIP values of the models calibrated using the data of mature leaves (a) and all leaves (b). Dotted horizontal lines in left figures indicate the VIP value 0.8, and wavebands with $\text{VIP} > 0.8$ is shown as score of 1 in right figures (c). Filter scores in (c) are result in PLSR model using all data (b).

941

942 Figure 6. The performance of the PLSR model which used the varied number of spectral band.

943 The selections of spectral wavebands were based on the threshold of VIP score (▲)

944 or combing removal at equal wavelength interval (○). The PLSR models were

945 calibrated using dataset of all plant functional types and developmental stages.

946 Number of spectral band in the case of original dataset (●) was 351. In the VIP score

947 method, the data at VIP threshold of 1.8 (64 bands), 1.6 (151 bands), 1.4 (185 bands)

948 and 0.8 (254 bands) were plotted. Number in the case of combing removal method

949 (interval) were 66 (30 nm), 85 (25 nm), 104 (20 nm), 134 (15 nm) and 207 (10 nm),

950 respectively. The response curve was shown as logarithmic regression curve. Gray

951 colored zones indicate the range of 5% variance of the original dataset.

Table 1. Description of the leaf data collection sites.

Country / Site name	Location (degree)	MAT ^a (°C)	MAP ^b (mm)	Holdridge life zone	Vegetation type ^c	No. of samples	Dominant tree species	References ^d
<i>Japan</i>								
Nakagawa (NKG)	N 44.81, E 142.08	5.4	1449	Cool temperate wet forest	DBF	40	<i>Betula ermanii</i>	Nakamura et al. 2016
Tomakomai (TOM)	N 42.71, E 141.57	6.7	1112	Cool temperate wet forest	DBF	494	<i>Quercus crispula</i> , <i>Acer mono</i>	Hiura, 2005; Ishihara et al., 2011
Kuromatsunai (KUR)	N 42.68, E 140.26	7.6	1523	Cool temperate wet forest	DBF	17	<i>Fagus crenata</i>	Tateishi et al., 2010
Hiyama (HYM)	N 41.75, E 140.15	10.4	1228	Cool temperate wet forest	DBF, ENF	4	<i>Fagus crenata</i> , <i>Chamaecyparis obtusa</i>	AMeDAS (http://www.jma.go.jp/en/warm/) Station: Esashi-sho
Kawatabi (KTB)	N 38.76, E 140.75	10.6	1684	Cool temperate wet forest	DBF	4	<i>Quercus serrata</i> , <i>Quercus crenata</i>	AMeDAS (http://www.jma.go.jp/en/warm/) Station: Osaki-shi Seibu
Hiruzen (HRZ)	N 35.31, E 133.60	11.5	1526	Cool temperate wet forest	DBF	28	<i>Quercus serrata</i>	AMeDAS (http://www.jma.go.jp/en/warm/) Station: Shinjo-son
Tanashi (TNS)	N 35.74, E 139.54	14.8	1610	Warm temperate moist	MBF	23	<i>Celtis sinensis</i> , <i>Quercus myrsinifolia</i>	Shi et al., 2015
Wakayama (WAK)	N 33.66, E 135.67	14.9	3877	Warm temperate wet	MBF	33	<i>Quercus acuta</i> , <i>Neolitsea aciculata</i>	Ohta et al., 2014
Shiiba (SIB)	N 32.38, E 131.16	13.0	3045	Warm temperate wet	MBF	34	<i>Fagus crenata</i> , <i>Quercus acutissima</i>	Ishihara et al., 2011
Tano (TAN)	N 31.87, E 131.30	17.7	2720	Warm temperate wet	MBF	34	<i>Castanopsis sieboldii</i> , <i>Symplocos prunifolia</i>	Ishihara et al., 2011
Yakushima (YAK)	N 30.32, E 130.64	19.7	4656	Warm temperate wet	MBF	48	<i>Vernicia cordata</i> , <i>Cinnamomum tenuifolium</i>	Ishihara et al., 2011
<i>Thai</i>								
Sakaerat (SAK)	N 14.51, E 101.94	24.0	1200	Tropical dry forest	DDF, DEF	121	<i>Shorea siamensis</i> , <i>Lagerstroemia dupperreana</i>	Lamotte et al., 1998; Asiaflux website (http://asiaflux.net/index.php?page_id=102)
<i>Malaysia</i>								
Lambir (LAM)	N 4.20, E 114.04	27.0	2740	Tropical moist forest	MDF	58	<i>Shorea beccariana</i> , <i>Dryobalanops aromatica</i>	Kumagai et al., 2005; Asiaflux website (http://asiaflux.net/index.php?page_id=76)
Pasoh (PSO)	N 2.97, E 102.30	25.3	1865	Tropical moist forest	LDF	119	<i>Dipterocarpus sublamellatus</i> ,	Nakaji et al., 2014; Asiaflux website (http://asiaflux.net/index.php?page_id=93)

^a MAT: mean annual temperature

^b MAP: mean annual precipitation

^c DBF: deciduous broadleaf forest, DDF: dry dipterocarp forest, DEF: dry evergreen forest, ENF: evergreen needleleaf forest, MBF: mixed broadleaf forest, MDF: mixed dipterocarp forest, LDF: lowland

^d AMeDAS (Automated Meteorological Data Acquisition System) is based on the widely distributed weather stations that developed by Japan Meteorological Agency.

Table 2. Properties of the analyzed leaf traits. The average and range (minimum–maximum) are shown for each category. The leaf concentrations of chemical traits (N, C, phenol, cellulose, and lignin) were calculated based on both LA and DM. Differences by plant functional type (FT), leaf developmental stage (DS), and their interaction were assessed with analysis of variance and power analysis.

	Deciduous				Evergreen				2-way ANOVA ^a				Power analysis			
	Young		Mature		Senescent		Young		Mature		Senescent		FT		FT	
	13 sp., <i>n</i> = 50	62 sp., <i>n</i> = 538	13 sp., <i>n</i> = 134	11 sp., <i>n</i> = 39	72 sp., <i>n</i> = 318	9 sp., <i>n</i> = 27	134	(57-308)	127	(36-240)	120	(70-155)	***	***	***	***
LMA (g m ⁻²)	59	(31-108)	91	(26-247)	73	(24-140)	134	(57-308)	127	(36-240)	120	(70-155)	***	***	***	1.00 1.00 1.00
Chemical traits (LA basis)																
N _{area} (g m ⁻²)	1.6	(0.85-2.56)	1.8	(0.43-3.37)	1.1	(0.31-2.59)	1.9	(0.93-4.68)	1.9	(0.57-3.78)	1.5	(0.89-2.33)	***	***	*	1.00 1.00 1.00
C _{area} (g m ⁻²)	27.1	(13.1-49.1)	43.3	(12.8-127.2)	33.5	(9.5-60.7)	62.3	(23.8-148.9)	61.5	(19.4-122.4)	57.4	(35.5-80.6)	***	***	***	1.00 1.00 1.00
Phenol _{area} (g m ⁻²)	6.7	(1.1-22.4)	12.1	(0.5-41.5)	7.6	(0.1-28.3)	14.9	(2.2-31.3)	15.3	(0.5-58.8)	5.6	(0.9-14.1)	**	***	***	0.97 1.00 1.00
Cellulose _{area} (g m ⁻²)	6.7	(2.4-20.1)	13.9	(2-46.5)	10.6	(3.4-24.3)	24.7	(7-57.7)	19.5	(3.3-59.5)	25.2	(15.4-34.4)	***	ns	***	1.00 0.40 1.00
Lignin _{area} (g m ⁻²)	8.6	(3.4-17.7)	14.5	(0.7-81.4)	14.9	(1.5-33.8)	24.4	(4.3-62.5)	25.0	(2-74.3)	40.6	(18-73.7)	***	***	***	1.00 1.00 1.00
Chemical traits (DM basis)																
N _{mass} (mg g ⁻¹)	29.5	(9.8-47.6)	20.3	(9.3-41.9)	14.9	(7.9-25.7)	14.6	(9.9-19.8)	15.9	(7.6-39.7)	13.3	(9.1-24)	***	***	***	1.00 1.00 1.00
C _{mass} (mg g ⁻¹)	462	(420-518)	472	(310-583)	456	(308-569)	463	(403-512)	481	(319-592)	479	(411-552)	**	**	ns	0.84 0.94 0.47
Phenol _{mass} (mg g ⁻¹)	110	(22-250)	128	(6-366)	91	(1-289)	112	(30-248)	114	(5-300)	49	(7-129)	*	***	*	0.85 1.00 0.64
Cellulose _{mass} (mg g ⁻¹)	105	(56-206)	153	(20-433)	148	(39-229)	182	(71-321)	158	(24-433)	212	(158-278)	***	***	***	1.00 0.97 1.00
Lignin _{mass} (mg g ⁻¹)	148	(64-221)	158	(7-587)	220	(15-495)	186	(75-414)	193	(19-461)	333	(163-498)	***	***	***	1.00 1.00 0.99

^a Significance level: * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001, ns not significant

Table 3. Results of calibration and cross-validation of the PLSR models developed to estimate six leaf traits in East Asian tree species. Validation was performed using the leave-one-out method. In total, 538 data points for mature deciduous species, 318 data points for mature evergreen species, and 856 data points for mature leaves in both functional types (deciduous + evergreen) were included. The PLSR model for all of the developmental stages and functional type used all 1,106 samples.

Trait	Training data		No. of factor	Calibration			Validation		
	Developmental stage	Functional type		R^2	RMSE (g m ⁻²)	NRMSE (%)	R^2	RMSE (g m ⁻²)	NRMSE (%)
LMA	Mature	Deciduous	14	0.84	9.7	4.4	0.81	10.6	4.8
	Mature	Evergreen	14	0.80	19.1	9.4	0.79	19.5	9.6
	Mature	Deciduous + evergreen	14	0.85	14.1	6.4	0.83	14.9	6.7
	All	Deciduous + evergreen	16	0.87	14.2	5.0	0.85	14.9	5.2
N _{area}	Mature	Deciduous	15	0.71	0.27	9.1	0.65	0.30	10.1
	Mature	Evergreen	15	0.69	0.34	10.7	0.61	0.39	12.2
	Mature	Deciduous + evergreen	15	0.63	0.33	10.0	0.57	0.36	10.8
	All	Deciduous + evergreen	18	0.70	0.33	7.6	0.67	0.35	7.9
C _{area}	Mature	Deciduous	15	0.84	5.19	4.5	0.79	5.83	5.1
	Mature	Evergreen	15	0.87	7.65	7.4	0.83	8.89	8.6
	Mature	Deciduous + evergreen	13	0.86	7.14	6.2	0.84	7.46	6.5
	All	Deciduous + evergreen	16	0.87	7.13	5.1	0.86	7.45	5.3
Phenol _{area}	Mature	Deciduous	14	0.84	2.70	6.6	0.81	2.96	7.2
	Mature	Evergreen	10	0.83	4.34	7.4	0.80	4.68	8.0
	Mature	Deciduous + evergreen	14	0.82	3.59	6.2	0.81	3.76	6.4
	All	Deciduous + evergreen	15	0.81	3.72	6.3	0.79	3.87	6.6
Cellulose _{area}	Mature	Deciduous	14	0.56	4.63	10.4	0.50	5.01	11.3
	Mature	Evergreen	16	0.53	6.18	11.0	0.40	7.09	12.6
	Mature	Deciduous + evergreen	13	0.48	5.94	10.3	0.45	6.12	10.6
	All	Deciduous + evergreen	14	0.55	5.83	10.1	0.52	5.98	10.4
Lignin _{area}	Mature	Deciduous	16	0.65	4.62	5.7	0.57	5.14	6.4
	Mature	Evergreen	18	0.73	7.21	10.0	0.64	8.41	11.6
	Mature	Deciduous + evergreen	15	0.66	6.77	8.4	0.63	7.16	8.9
	All	Deciduous + evergreen	17	0.66	7.01	8.7	0.62	7.38	9.1

Table 4. Effects of plant functional type training data on PLSR model performance. The model validation was performed by changing the functional type. Characters in bold indicate that the same functional type was used for model calibration and validation. All of the tests were performed for mature leaves.

Trait	Training data (Functional type)	Validation					
		Deciduous ($n = 538$)			Evergreen ($n = 318$)		
		R^2	RMSE (g m^{-2})	NRMSE (%)	R^2	RMSE (g m^{-2})	NRMSE (%)
LMA	Deciduous	0.81	10.6	4.8	0.75	21.83	10.7
	Evergreen	0.32	31.11	14.0	0.79	19.53	9.6
	Deciduous + evergreen	0.77	11.66	5.3	0.80	19.13	9.4
N_{area}	Deciduous	0.65	0.30	10.1	0.44	0.48	14.8
	Evergreen	0.45	0.43	14.7	0.61	0.39	12.2
	Deciduous + evergreen	0.62	0.31	10.6	0.52	0.43	13.5
C_{area}	Deciduous	0.79	5.83	5.1	0.79	10.14	9.8
	Evergreen	0.44	11.87	10.4	0.83	8.89	8.6
	Deciduous + evergreen	0.77	6.23	5.4	0.82	9.19	8.9
$\text{Phenol}_{\text{area}}$	Deciduous	0.81	2.96	7.2	0.76	5.26	9.0
	Evergreen	0.29	7.50	18.3	0.80	4.68	8.0
	Deciduous + evergreen	0.80	3.08	7.5	0.80	4.68	8.0
$\text{Cellulose}_{\text{area}}$	Deciduous	0.50	5.01	11.3	0.16	9.86	17.5
	Evergreen	0.12	7.46	16.8	0.40	7.09	12.6
	Deciduous + evergreen	0.40	5.46	12.3	0.38	7.10	12.6
$\text{Lignin}_{\text{area}}$	Deciduous	0.57	5.14	6.4	0.44	10.96	15.2
	Evergreen	0.01	17.06	21.1	0.64	8.41	11.6
	Deciduous + evergreen	0.47	5.69	7.1	0.58	9.13	12.6
Mean	Deciduous			7.5		12.9	9.0
NRMSE (%)	Evergreen			15.9		10.4	12.7
	Deciduous + evergreen			8.0			8.3

Table 5. Effects of leaf developmental stage training data on PLSR model performance. The models were validated using the datasets for young leaves and senescent leaves.

Trait	Training data (Developmental stage)	Validation			
		Young leaves ($n = 113$)		Senescent leaves ($n = 137$)	
		R^2	RMSE (g m^{-2})	R^2	NRMSE (%)
LMA	Mature	0.86	22.0	0.69	37.39
	All developmental stages	0.91	18.45	0.85	11.39
N_{urea}	Mature	0.66	0.40	0.02	0.76
	All developmental stages	0.73	0.34	0.75	0.26
C_{urea}	Mature	0.84	11.26	0.74	10.91
	All developmental stages	0.90	8.92	0.84	6.02
Phenol _{urea}	Mature	0.72	4.44	0.11	11.17
	All developmental stages	0.83	3.35	0.68	3.80
Cellulose _{urea}	Mature	0.74	7.26	0.47	10.72
	All developmental stages	0.79	6.68	0.69	4.06
Lignin _{urea}	Mature	0.58	9.29	0.01	26.24
	All developmental stages	0.62	8.12	0.73	6.79
Mean	Mature			11.7	31.3
NRMSE (%)	All developmental stages			9.9	10.7

Figure

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Figure 1. Locations of the research sites.

Figure 2. Sample images of phenology monitoring digital camera. The fish-eye image of forest canopy in Tomakomai site (top) and samples of target tree (*Acer mono*) at different phenological stages (bottom) are shown. The timing of leaf sampling was planned based on similar photo images in six sites. The images in Tomakomai have been published in website of Phenological Eyes Network (http://pen.agbi.tsukuba.ac.jp/index_e.html). The enclosing lines and each of abbreviations in top image indicate the canopy of individual tree and name of tree species, respectively.

Figure 3. Spectral reflectance factors of (a–c) deciduous and (b–d) evergreen tree leaves in East Asian forests. The solid lines, dark-gray areas, and light-gray areas are the median, 25–75th percentile range, and minimum–maximum range, respectively. The solid- and dotted-horizontal lines above the reflectance factor of evergreen species indicate respectively significant higher- and lower-reflectance factors compared to deciduous species ($P < 0.05$). The effects of leaf developmental stage and interaction with plant functional type on the reflectance factors were significant in all measured wavebands (two-way ANOVA, $P < 0.05$). The NDVI was calculated from the leaf spectral reflectance at 850 nm (R_{850}) and 650 nm (R_{650}) as follows: $NDVI = (R_{850} - R_{650}) / (R_{850} + R_{650})$.

Figure 4. Performance of the chemical trait estimation models based on either DM-basis or LA-basis concentrations. The PLSR models were calibrated to estimate five chemical traits in each of six leaf categories (two functional types $\times$ three developmental stages). All 1,106 samples were used for the analysis (number in each category is shown in Table 2). The R^2 and NRMSE were calculated from the model cross-validation. The quartiles in the five chemical traits (a–e) indicate the variance in the parameters between the six test cases ($n = 6$). Panel (f) shows the variance for all of the data ($n = 30$). Significance differences of parameters between DM-basis and LA-basis concentration are represented by the P value (t -test).

Figure 5. Spectral features of VIP values of the models calibrated using the data of mature leaves (a) and all leaves (b). Dotted horizontal lines in left figures indicate the VIP value 0.8, and wavebands with VIP > 0.8 is shown as score of 1 in right figures (c). Filter scores in (c) are result in PLSR model using all data (b).

Figure 6. The performance of the PLSR model which used the varied number of spectral band. The selections of spectral wavebands were based on the threshold of VIP score ($\blacktriangle$) or combing removal at equal wavelength interval ($\circ$). The PLSR models were calibrated using dataset of all plant functional types and developmental stages. Number of spectral band in the case of original dataset ($\bullet$) was 351. In the VIP score method, the data at VIP threshold of 1.8 (64 bands), 1.6 (151 bands), 1.4 (185 bands) and 0.8 (254 bands) were plotted. Number in the case of combing removal method (interval) were 66 (30 nm), 85 (25 nm), 104 (20 nm), 134 (15 nm) and 207 (10 nm), respectively. The response curve was shown as logarithmic regression curve. Gray colored zones indicate the range of 5% variance of the original dataset.

Figure 1 (tif)

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Expansion
DOY 120

Young
DOY 133

Mature
DOY 153

Senescent
DOY 185

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