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Development of models for estimating leaf chlorophyll and nitrogen contents in tree species with respect to seasonal changes

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Abstract

Models were developed to estimate nondestructively chlorophyll (Chl) content per unit of leaf area (Chl_{area}) and nitrogen content per unit of leaf area (N_{area}) using readings of two optical meters for five warm-temperate, evergreen, broad-leaved tree species (*Castanopsis sieboldii*, *Cinnamomum tenuifolium*, *Eurya japonica*, *Machilus thunbergii*, and *Neolitsea sericea*). It was determined whether models should be adjusted seasonally. Readings (were obtained six times during a year period and Chl_{area} and N_{area} were determined using destructive methods. Bayesian inference was used to estimate parameters of models that related optical meter readings to Chl_{area} or N_{area} for each species. Deviance information criterion values were used to select the best among models, including the models with seasonal adjustment. The selected models were species-specific and predicted Chl_{area} accurately (R^2 = 0.93–0.96). The best model included parameters with seasonal adjustments for one out of five species. Model-based estimates of N_{area} were not as accurate as those for Chl_{area}, but they were still adequate (R^2 = 0.64–0.82). For all species studied, the best models did not include parameters with seasonal adjustments. The estimation methods used in this study were rapid and nondestructive; thus, they could be used to assess a function of many leaves and/or repeatedly on individual leaves in the field.

Additional key words: *Agriexpert PPW-3000*; Bayesian statistics; evergreen broad-leaved species; leaf chlorophyll content; leaf nitrogen; optical meter; seasonal change; *SPAD-502*.

Introduction

Quantification of leaf Chl content is important for understanding adaptations and acclimations of photosynthesis to environments with limited supplies of light and nitrogen (Niinemets 2010). For instance, foliage Chl content per unit of dry mass decreases with increasing light availability as much as nitrogen (N) is invested in other photosynthetic processes, including carbon fixation and electron transport (Ellsworth and Reich 1992, Hikosaka and Terashima 1996). Chl content has been used as an indicator of N status (Neilsen *et al.* 1995, Schlemmer *et al.* 2005) and various kinds of biotic and abiotic stresses (Carter 1993, Carter and Knapp 2001).

Quantifying leaf N content is also important because it shows close intra- and interspecific relationships with parameters describing leaf functions, such as light-saturated photosynthetic capacity (Field and Mooney 1986, Ellsworth and Reich 1992, Reich *et al.* 1997, Wright *et al.* 2004) and respiration (Reich *et al.* 1997, Lusk and Reich 2000, Wright *et al.* 2004). The close relationships between N content and leaf functions exist, because a large portion of N in leaves occurs in enzymes in chloroplasts and mitochondria, which catalyze biochemical processes that support these functions (Kozlowski and Pallardy 1997).

Conventional methods for determining leaf Chl and N contents are destructive: leaves are sampled and destroyed when measured (Porra *et al.* 1989, Cornelissen *et al.* 2003). Moreover, this type of analysis is time- and money-consuming and the methods do not allow repeated measurements of individual leaves. Therefore, rapid, nondestructive methods with optical meters have been developed for determining leaf Chl and N contents (*e.g.*, Yadava 1986, Campbell *et al.* 1990, Ichie *et al.* 2002). These methods allow to collect large data sets rapidly in

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Abbreviations: CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; Chl – chlorophyll; Chl_{area} – chlorophyll content per unit of leaf area; DIC – deviance information criterion; EuJ – *Eurya japonica*; MaT – *Machilus thunbergii*; N_{area} – nitrogen content per unit of leaf area; NeS – *Neolitsea sericea*; R^2 – coefficient of determination; RMSE – root mean squared error.
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the field and to detect changes in leaf Chl and N contents in individual leaves (Yadava 1986, Yamamoto *et al.* 2002), even throughout the entire leaf life span.

An important feature of the relationships between optical meter readings and leaf Chl or N contents is that they are species- or cultivar-specific (Jifon *et al.* 2005, Marenco *et al.* 2009, Coste *et al.* 2010) and they are affected by growth conditions (Campbell *et al.* 1990, Martínez *et al.* 2004, Pinkard *et al.* 2006, Marenco *et al.* 2009). Therefore, different models need to be established for different species.

Another related concern about estimation models is that the relationships between optical meter readings and leaf Chl or N contents may change seasonally. To date, inconsistent results have been obtained regarding seasonal changes in these relationships. Neilsen *et al.* (1995) established relationships between SPAD values and leaf N content for some apple cultivars on four occasions from May to July and they observed temporal effects. Chang and Robison (2003) also found that the relationship between SPAD values and leaf Chl content changed seasonally in a deciduous tree species (*Liquidambar styraciflua*). In contrast, Eguchi *et al.* (2006) found that in deciduous tree species (*Betula platyphylla* and *Betula maximowicziana*), the relationships of *Agriexpert* readings and leaf N content did not differ between maturing (sampled in May and June) and mature leaves (sampled in July and August). However, in these studies, the effect of season could not be separated from the effect of leaf age because the target species were deciduous.

Leaf life spans are generally longer in evergreen species than in deciduous ones (Kikuzawa and Lechowicz 2011) and leaf functions change temporally during their life span (Niinemets 2010, Kikuzawa and Lechowicz 2011). Given their long life spans and considerable changes in functions, nondestructive measurements of leaf traits are especially useful for evergreen species by enabling assessments of leaf functions throughout their life span. For evergreen species in particular, examination of possible seasonal changes in the relationships between optical meter readings and leaf Chl or N contents is important, because these relationships are used year-round. Moreover, the effect of season can be separated from the effect of leaf age for evergreen species.

The main objective of this study was to develop models for the estimation of Chl_{area} and N_{area} from optical meter readings in five evergreen, broad-leaved, tree species [*C. sieboldii* (Makino) Hatus. (CaS), *C. tenuifolium* (Makino) Sugim. ex H.Hara (CiT), *E. japonica* Thunb. (EuJ), *M. thunbergii* Siebold *et* Zucc. (MaT), and *N. sericea* (Blume) Koidz. (NeS)] growing in the warm-temperate zone in Japan. Based on model selection results, the necessity of seasonal adjustments of the models was also examined.

Materials and methods

Target species: Five tree species were selected as the target species, for which models were developed to estimate Chl_{area} and N_{area} from optical meter readings, because they are major components of evergreen, broad-leaved forests in the warm-temperate region of Japan. They are shade-tolerant canopy (CaS and MaT) or subcanopy species (CiT, EuJ, and NeS). Although they are not very important commercially, they are sporadically used for timber and other wood products (CaS, MaT, and NeS) or as ornamental trees (CiT and EuJ) (Hotta *et al*. 1989).

Sampling: Saplings, from which leaves were sampled, were selected under the canopy of a forest stand on the campus of Chiba University, Japan (35˚46’ 58’’N, 139˚54’ 2’’E, 20 m a.s.l.). The selected saplings regenerated naturally, they were 0.3–3 m high, and their age was from 4 to15 years according to their sizes at the time of sampling. The saplings were shaded by the canopy trees. No fertilizer was applied to the stand. Leaf samples were collected six times within a year (June, August, October, and December 2007, and March and April 2008). Sampling was mostly random, but a wide range of leaves of different ages and colors was collected at all occasions.

Measurements: *SPAD-502* (*Konica-Minolta*, Osaka, Japan; hereafter SPAD) and *Agriexpert PPW-3000* (*Satake Corp*., Hiroshima, Japan; hereafter Agriexpert) were used as optical meters for estimating Chl_{area} and N_{area}, respectively. SPAD is a widely used instrument for assessing leaf Chl content in trees (*e.g*., Campbell *et al.* 1990, Markwell *et al.* 1995, Martínez and Guiamet 2004, Coste *et al.* 2010). In recent years, Agriexpert was developed as a tool to assess leaf N content (Ichie *et al.* 2002, Eguchi *et al.* 2006, Kitahashi *et al.* 2008). Both meters use light absorption rates at selected wavelengths: SPAD measures the absorption rates of light at two wavelengths (650 and 940 nm) and calculates SPAD values (Uddling *et al.* 2007). Agriexpert measures absorption rates of light at four wavelengths (560, 660, 900, and 950 nm; Ichie *et al.* 2002, Eguchi *et al.* 2006). Estimation models, which relate the outputs of these optical meters to leaf Chl or N contents, must be established by users of the meters.

SPAD values and Chl_{area} were measured on 18 or 20 leaves from each species during each sampling event. Because SPAD values may change temporally within a day (Martínez and Guiamet 2004, Nauš *et al.* 2010), SPAD measurements were taken between 10:00 and 12:00. Sampled leaves were transferred to the laboratory and one leaf disc (0.785 cm^2 per disc) was punched out from each specimen. For each disc, SPAD was measured three times and an average value was calculated. After measuring SPAD, leaf disc was extracted destructively using N,N'-dimethylformamide as a solvent and Chl content was determined according to Porra *et al.* (1989). Since evergreen, broad-leaved trees have a solvent-resistant structure, Chl cannot be extracted by simple soaking in the solvent; therefore, a homogenizer was used to crush a disc immersed in solvent. Then, the solution was centrifuged, and Chl content was measured spectrophotometrically (*V-550*, *JASCO Corporation*, Tokyo, Japan). Chl_{area} was calculated based on leaf disc area.

N_{area} was measured on 9–18 leaves from each species during each sampling event. Light absorption rates at 560, 660, 900, and 950 nm were recorded five times on each leaf using Agriexpert. Then, the leaves (excluding the petioles) were scanned with a digital scanner (*PM-A850*, *Epson*, Tokyo, Japan) to determine the area of each individual leaf. Leaves were dried for more than 72 h at 80ºC and the dry masses were determined using an analytical balance. Dried leaves were ground and N content was estimated using a CN-coder (*MT-700*; *Yanaco*, Kyoto, Japan). Then, N content was calculated on a unit area basis (N_{area}).

Statistical analyses: Models to estimate Chl_{area} and N_{area} were generated using a model selection method based on Bayesian statistics. The best models were selected among the following models using deviation information criterion (DIC) values (Spiegelhalter *et al.* 2002). Some of the models included terms for seasonal adjustments. If a best model included seasonal adjustment terms, this was taken as an indication that the relationship between the optical meter readings and Chl_{area} or N_{area} values changed seasonally.

Outline of models: Four classes of models with different levels of complexity were used to relate readings of optical meters and Chl_{area} or N_{area}. Only outlines of models were given here; details are presented in Appendix 1. The 1st class was basic and related SPAD values and Chl_{area} by quadratic or cubic equations, and Agriexpert readings and N_{area} by linear or quadratic equations. The 2nd class (models with seasonal effects) took into account possible seasonal changes in the relationships between readings of optical meters and Chl_{area} or N_{area}. Seasonal effects were incorporated by assuming that coefficients of basic models changed seasonally according to a cosine curve with a year cycle, with a flexible amplitude and a phase shift. The 3rd class (mixed-effects models) considered random effects associated with sampling events. A term for random effects, which had a hierarchical structure, was introduced into these models. Finally, the 4th class (mixed-effects models with seasonal effects) considered both seasonal effects and random effects associated with sampling events.

Bayesian inference: Parameters in the models were estimated by Bayesian inference. The model that related best to the readings of the optical meters to Chl_{area} or N_{area}, was selected among eight models (two basic models *vs*. [with or without seasonal effects] *vs*. [with or without random effects]) for each species. The model with the lowest DIC value was considered as the best one. Coefficients of determination (R^2) were calculated as a fitness criterion in a relative sense and root mean squared error (RMSE) was used to express the accuracy of model prediction in an absolute sense. Details of Bayesian inference are given in Appendix 2.

Results

Models for Chl_{area}: The lowest DIC values (Fig. 1) were produced by the mixed-effects, quadratic models for CiT and NeS, the cubic model for CaS and MaT, and the quadratic model with seasonal effects for EuJ. The difference in DIC between the best and the second best model was negligible for EuJ.

There were similarities and differences in the patterns of DIC values among species (Fig. 1). For all species but CaS, DIC values were generally lower for models with a random-effect term than for the corresponding models without a random-effect term. For CaS, MaT, and NeS, DIC was lower for the models without seasonal effects than for the corresponding ones with seasonal effects. For CiT and EuJ, the reverse pattern was observed.

Although the same model was selected as the best for CaS and MaT, several estimated parameters differed significantly between these two species (Table 1). For example, the 95% credible interval of b_1 for MaT did not include the posterior mean of b_1 for CaS.

The R^2 for the best Chl_{area} models were higher than 0.9 for all of the species studied (Fig. 2). RMSE values for estimation models ranged from 3.90 to 5.99 µg cm^{-2}.

Models for N_{area}: The lowest DIC values were produced by the quadratic model for CaS, the mixed-effect, linear model for CiT and MaT, and the mixed-effect, quadratic model for EuJ and NS (Fig. 2). For CaS and EuJ, the differences between the best and the second-best models (with or without random-effect terms, respectively) were negligible. The models with the seasonal effects were not selected as the best ones for any of the species.

Patterns of DIC values fell into two species groups (Fig. 2). The first group included CaS and EuJ, which tended to have higher DIC values for the models with the seasonal effects than for the corresponding ones without the seasonal effects. For these species, DIC values were similar for the models with a random-effect term and the corresponding ones without a random-effect term. The second group included CiT, MaT, and NeS, which tended to have lower DIC values for the models with

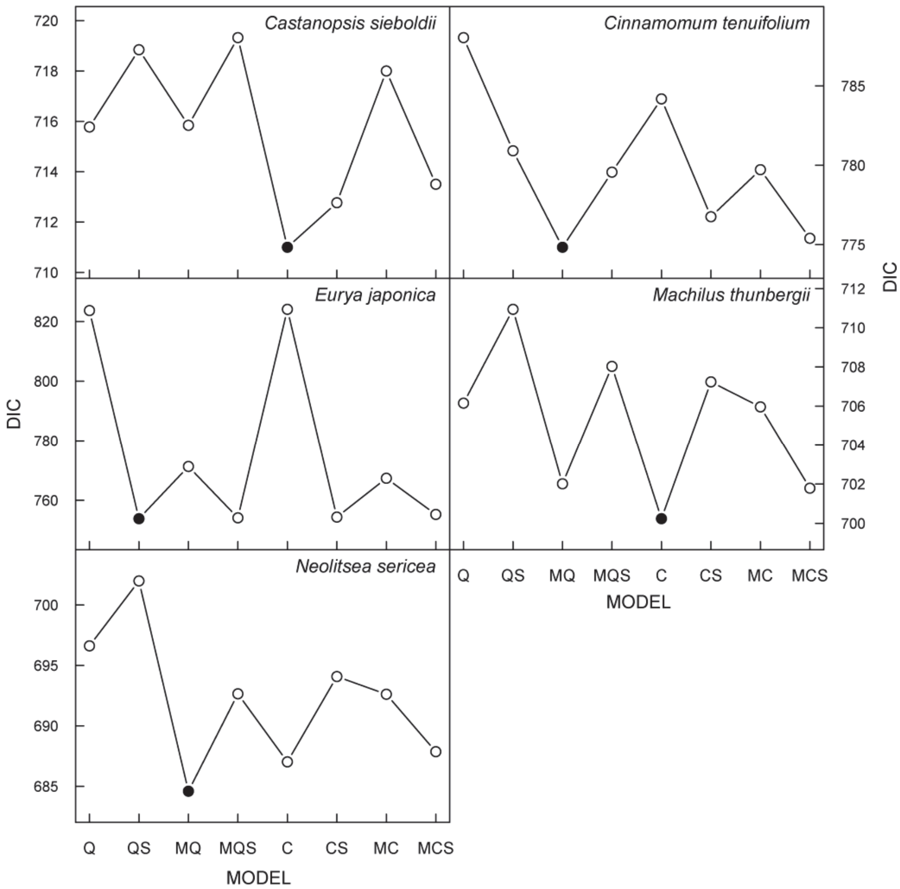

Fig. 1. Deviance information criterion (DIC) values for the models used to estimate leaf chlorophyll content per unit area (Chl_{area}) using SPAD-502 readings. Q – quadratic model, QS – quadratic model with seasonal effects, MQ – mixed-effects quadratic model, MQS – mixed-effects quadratic model with seasonal effects, C – cubic model, CS – cubic model with seasonal effects, MC – mixed-effects cubic model, MCS – mixed-effects cubic model with seasonal effects.

a random-effect term than for corresponding ones without a random-effect term. For these species, the impacts of the seasonal effects differed considerably depending on the presence/absence of random effects.

Among species, for which the same model was selected as the best, most of the estimated parameters were similar; the 95 % credible interval of a parameter for one species included the posterior means of the same parameter for other species (Table 2), but this was not always the case. For example, the 95% credible interval of b_0 for CiT did not include the posterior mean of b_0 for MaT, and *vice versa*. The R^2 for the selected models for N_{area} ranged from 0.64 to 0.82 (Fig. 4). RMSE values for the estimation models ranged from 13.7 to 22.6 µg cm^{-2}.

Discussion

The R^2 values for the best Chl_{area} models were higher than 0.90 for all of the species studied, indicating that these models could be used reliably. SPAD has been proven to be reliable for assessing Chl_{area} in various plant species ranging from annual crops to evergreen, broad-leaved trees (*e.g.*, Yadava 1986, Campbell *et al.* 1900, Ichie *et al.* 2002, Yamamoto *et al.* 2002, Haripriya Anand and Byju 2008, Marenco *et al.* 2009). Although the R^2 values were high, estimation errors should be pointed out because the RMSE ranged from 3.90 to 5.99 µg cm^{-2}. Differences in estimated Chl_{area} were essentially meaningless when they were smaller than these RMSE values. For EuJ, the second best model was also usable, because the difference in DIC between the best model and the second best model was negligible.

Although the R^2 values for the best models for N_{area} were lower than the R^2 values for the Chl_{area} models, indicating that estimates were not as accurate, the estimates were still acceptable. The R^2 values calculated in this study (0.64–0.82) were within the range of R^2 values reported by Ichie *et al.* (2002) and Eguchi *et al.* (2006) for deciduous and evergreen, broad-leaved tree species in temperate and tropical regions. Although only linear models have been previously used to estimate N content (Ichie *et al.* 2002, Eguchi *et al.* 2006), the present work showed that models with quadratic terms might be better for some species. The estimation errors should be pointed out, because RMSE ranged from 13.7 to 22.6 µg cm^{-2}. For CaS and EuJ, the second-best models were also usable, because the differences in DIC between the best and the second best model were negligible.

The results of the study showed that relationships between optical meter readings and Chl_{area} or N_{area} were highly species-specific at multiple levels, including

Table 1. Posterior means and 95% credible intervals (in brackets) for the parameters of models that best estimated leaf chlorophyll content using *SPAD-502* readings.

	Castanopsis sieboldii		*Cinnamomum tenuifolium*		*Eurya japonica*		*Machilus thunbergii*		*Neolitsea sericea*	
b_0	41.710	(3.024, 80.312)	0.063	(–10.281, 10.031)			4.525	(–4.504, 13.682)	–3.097	(–10.100, 4.002)
b_1	–3.074	(–5.768, –0.352)	0.073	(–0.408, 0.575)			–0.623	(–1.584, 0.373)	0.275	(–0.115, 0.666)
b_2	0.0910	(0.0306, 0.1512)	0.0202	(0.0141, 0.0260)			0.0530	(0.0227, 0.0831)	0.0189	(0.0134, 0.0243)
b_3	–0.000571	(–0.001000, –0.000134)					–0.000415	(–0.000698, –0.000123)		
α_0					6.798	(–1.046, 14.710)				
β_0					9.885	(–3.851, 23.362)				
γ_0					7.1271	(–3.8882, 17.9805)				
α_1					–0.0341	(–0.3668, 0.2928)				
β_1					–0.476	(–1.029, 0.078)				
γ_1					–0.351	(–0.784, 0.093)				
α_2					0.0146	(0.0115, 0.0178)				
β_2					0.005781	(0.000366, 0.011190)				
γ_2					0.001959	(–0.002194, 0.005980)				
σ	4.828	(4.249, 5.530)	6.236	(5.470, 7.118)	5.675	(4.992, 6.485)	4.505	(3.946, 5.144)	4.064	(3.546, 4.637)
σ_{re}			3.081	(0.581, 7.084)					1.942	(0.280, 4.429)

Table 2. Posterior means and 95% credible intervals (in brackets) for the parameters of models that best estimated leaf nitrogen content from *Agriexpert PPW-3000* readings.

	Castanopsis sieboldii		*Cinnamomum tenuifolium*		*Eurya japonica*		*Machilus thunbergii*		*Neolitsea sericea*	
b_0	102.7	(–29.8, 236.3)	–54.0	(–107.2, –0.3)	137.7	(–56.1, 339.7)	9.1	(–39.6, 59.6)	172.2	(–80.1, 450.5)
b_1	51.0	(–1,082.0; 1,203.0)	–189.4	(–1,460.3; 1,146.1)	386.5	(–886.6; 1,653.1)	886.3	(–339.3, 2136)	369.4	(–810.0; 1,587.1)
b_2	–154.4	(–282.6, –20.6)	106.2	(63.4, 151.6)	–161.7	(–248.4, –78.4)	90.5	(42.7, 136.2)	–240.5	(–546.7, 49.2)
b_3	–1,456.0	(–2,580.2; –335.5)	–59.9	(–1,358.1; 1,175.1)	–936.1	(–2,131.1; 216.1)	–670.8	(–1,868.1; 508.1)	–649.0	(–1,785.1; 506.5)
b_4	1,483.9	(569.3; 2,350.0)	249.9	(–475.0, 899.3)	627.5	(–354.4; 1,631.1)	–198.5	(–789.6, 392.1)	455.9	(–481.4; 1,396.0)
b_5	1,192.2	(–150.3; 2,443.1)			497.1	(–986.2; 1,914.1)			371.8	(–1,034.2; 1,714.0)
b_6	48.4	(11.9, 83.7)			53.7	(33.0, 75.2)			106.6	(15.6, 202.0)
b_7	–126.8	(–1,345.0; 1,112.0)			–277.7	(–1,710.2; 1,126.1)			–333.6	(–1,630.0; 1,022.0)
b_8	–831.8	(–1,590.1; –31.6)			–256.2	(–1,399.0; 852.3)			–135.3	(–1,100.1; 834.2)
σ	14.5	(12.3, 17.0)	24.1	(20.4, 28.9)	16.6	(14.0, 19.8)	19.1	(16.1, 22.7)	16.4	(14.0, 19.3)
σ_{re}			12.3	(0.0, 30.6)	2.2	(0.0, 11.3)	19.1	(7.5, 47.3)	12.8	(5.3, 27.5)

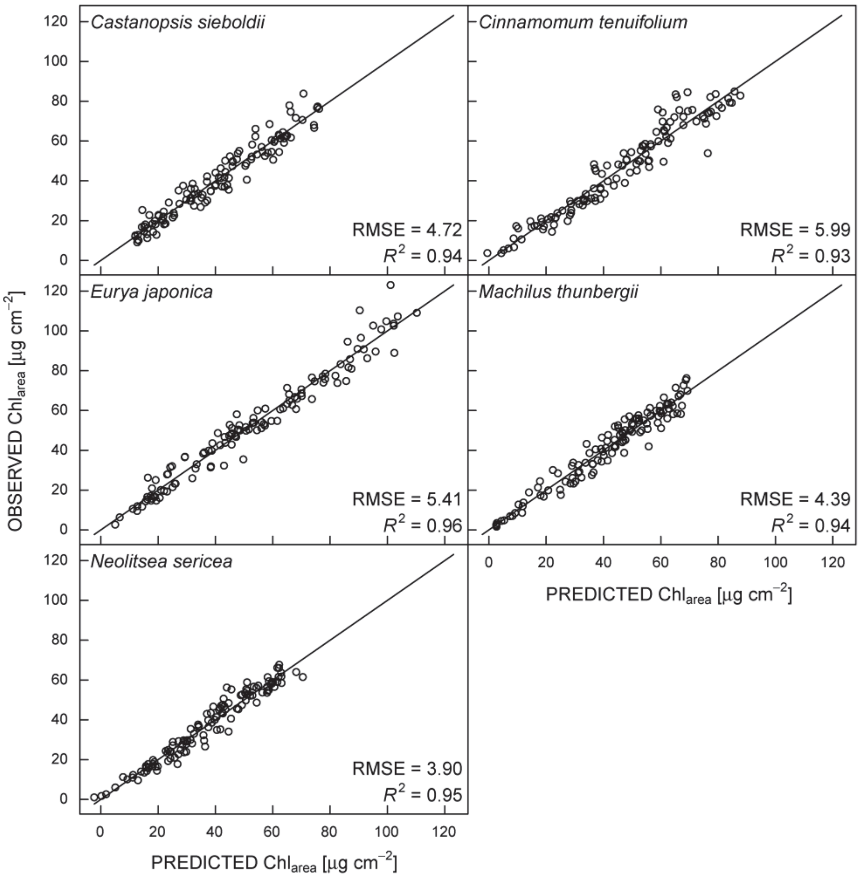

Fig. 2. Relationships between observed and predicted leaf chlorophyll content per unit area (Chl_{area}). The predicted values were calculated using the best models with the parameter values presented in Table 1. The *solid line* represents a 1:1 relationship. R^2 – coefficient of determination; RMSE – root mean squared error.

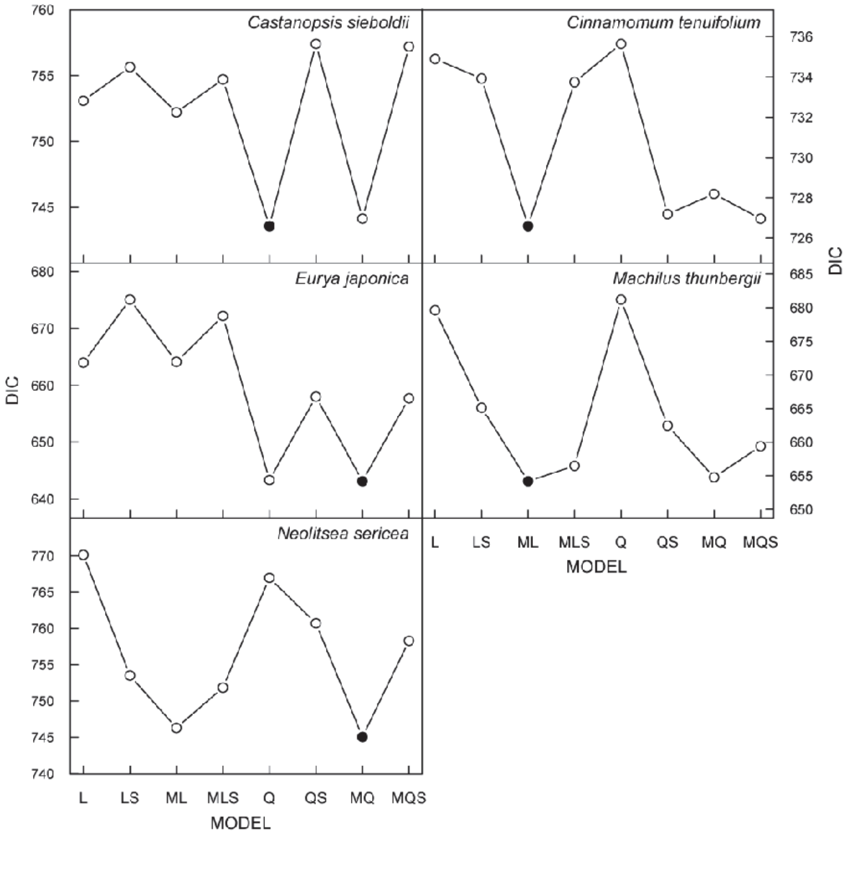

Fig. 3. Deviance information criterion (DIC) values for the models used to estimate leaf nitrogen content per unit area (N_{area}) using Agriexpert readings. L – linear model, LS – linear model with seasonal effects, ML – mixed-effects linear model, MLS – mixed-effects linear model with seasonal effects, Q – quadratic model, QS – quadratic model with seasonal effects, MQ – mixed-effects quadratic model, MQS – mixed-effects quadratic model with seasonal effects.

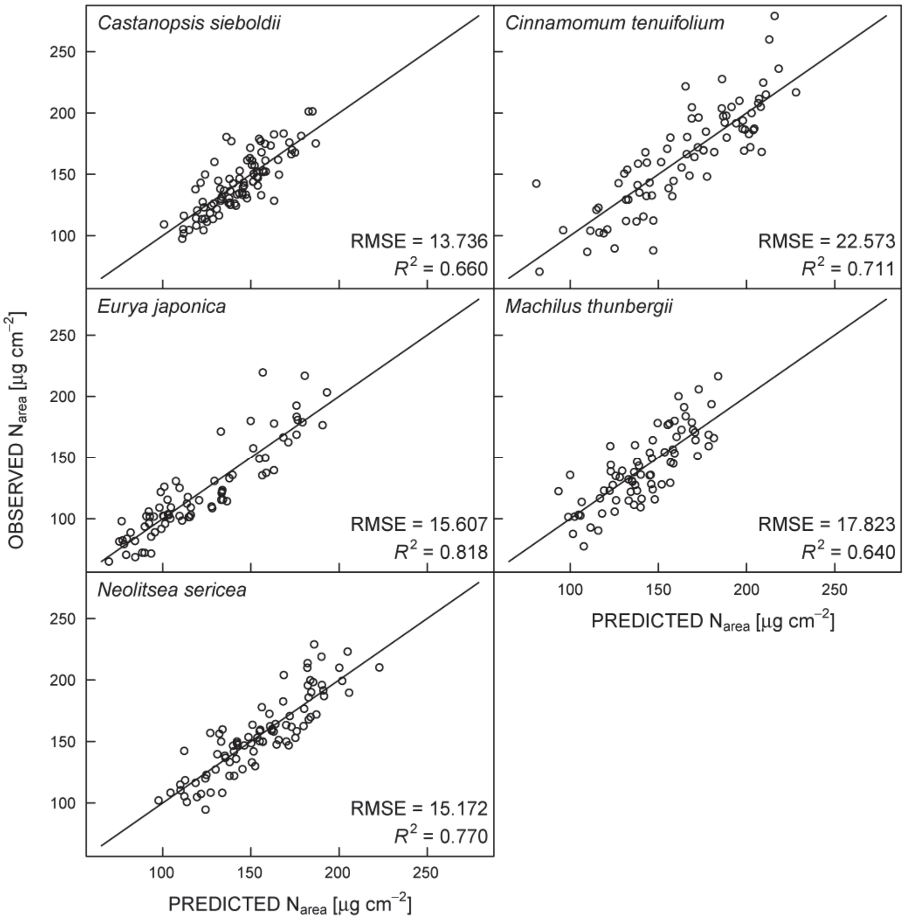

Fig. 4. Relationships between observed and predicted values of leaf nitrogen content per unit area (N_{area}). The predicted values were calculated using the best models with the parameter values presented in Table 2. The *solid line* represents a 1:1 relationship. R^2 – coefficient of determination; RMSE – root mean squared error.

selected model formulation, significances of seasonal adjustments (discussed below), DIC patterns among models, and parameter values. Previous studies have also shown that relationships between Chl_{area} or N_{area} and optical meter readings are species- or cultivar-specific (Neilsen *et al.* 1995, Pinkard *et al.* 2006, Uddling *et al.* 2007, Marenco *et al.* 2009, Coste *et al.* 2010). Thus, the evolvement of species-specific models is a prerequisite in order to assess Chl_{area} or N_{area} using optical sensors.

In most cases, the selected models did not include terms for seasonal adjustments, whereas the relationship between Chl_{area} and SPAD values for EuJ clearly showed a seasonal change. This indicated that the extent of seasonal change in the relationship was also species-specific. Similarly, previous studies of deciduous species did not produce consistent results regarding seasonal changes for the relationships between Chl_{area} or N_{area} and optical meter readings (Neilsen 1995, Chang and Robison 2003, Ichie *et al.* 2006). The mechanism underlying the seasonal change for the relationship between SPAD and Chl_{area} in EuJ was not clear. To date, many factors, including irradiance, leaf water status, leaf thickness, time of measurement, growth conditions, and movement of chloroplast have been reported to affect this relationship (Campbell *et al.* 1990, Martínez and Guiamet 2004, Jifon *et al.* 2005, Schlemmer *et al.* 2005, Marenco *et al.* 2009, Nauš *et al.* 2010). Among these factors, leaf water content might have caused the change in the relationship in EuJ, because seasonal water availability fluctuates at the study site and the relationship between SPAD and Chl_{area} could be affected by leaf dehydration/rehydration even after leaf maturation (Martínez and Guiamet 2004).

In conclusion, species-specific estimation models were generated for Chl_{area} and N_{area} in five evergreen, broad-leaved tree species. The models were species-specific at multiple levels, including the selected model formulation, significances of seasonal adjustments, patterns in DIC values among different models, and parameter values. Multiple-species estimation models should be developed in the future.

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Appendix 1

The details of four classes of models used in the present study are presented below.

Basic models: Basic models related SPAD values and Chl_{area} using quadratic or cubic equations, and Agriexpert readings and N_{area} using linear or quadratic equations. Quadratic and cubic models were used to relate SPAD values and Chl_{area}, because their relationship is often curvilinear (Markwell *et al.* 1995, Jifon *et al.* 2005, Uddling *et al.* 2007, Coste *et al.* 2010, Nauš *et al.* 2010), as follows:

$$\mathrm{Chl_{area}} = b_0 + b_1\,\mathrm{SPAD} + b_2\,\mathrm{SPAD}^2, \quad \text{and}$$
$$\mathrm{Chl_{area}} = b_0 + b_1\,\mathrm{SPAD} + b_2\,\mathrm{SPAD}^2 + b_3\,\mathrm{SPAD}^3,$$

where Chl_{area} is Chl content on a unit leaf area basis [μg cm^{-2}], SPAD is the *SPAD-502* reading, and b_0–b_3 are model parameters that need to be determined.

The linear and quadratic models used for N_{area} were:

$$\mathrm{N_{area}} = b_0 + \sum_{i=1}^{4}(b_i\ \mathrm{LED_i}) \quad \text{and}$$
$$\mathrm{N_{area}} = b_0 + \sum_{i=1}^{4}(b_i\ \mathrm{LED}_i) + \sum_{i=5}^{8}(b_i\ \mathrm{LED_{(i-4)}}^2),$$

where N_{area} is the nitrogen content on a unit leaf area basis (μg cm^{-2}), LED_i is the ith reading with an *Agriexpert PPW-3000*, and b_0–b_8 are model parameters.

Models with seasonal effects: To detect possible seasonal changes in the relationships between optical meter readings and Chl_{area} or N_{area}, it is assumed that model parameters in the basic models changed seasonally:

$$b_i = \alpha_i \sin\left(\frac{2\pi}{365}\,\mathrm{DOY}\right) + \beta_i \cos\left(\frac{2\pi}{365}\,\mathrm{DOY}\right) + \gamma_i \quad (i = 1\text{–}8), \tag{1}$$

where DOY is day of year and α_i, β_i, and γ_i are model parameters. Eq. 1 can be transformed as

$$b_i = B_i\left\{\cos\left(\frac{2\pi}{365}\mathrm{DOY} - \theta_i\right) + \gamma_i'\right\}, \tag{2}$$

where B_i is the amplitude, θ_i is the phase shift, and γ_i' is a constant. They are given by

$$B_i = \sqrt{\alpha_i^2 + \beta_i^2}, \tag{3}$$
$$\gamma_i' = \gamma_i / B_i, \tag{4}$$
$$\text{and}\ \ \theta_i = \begin{cases} \arctan|\beta_i/\alpha_i| & (\alpha_i \ge 0, \beta_i \ge 0) \\ -\arctan|\beta_i/\alpha_i| & (\alpha_i \ge 0, \beta_i < 0) \\ \pi - \arctan|\beta_i/\alpha_i| & (\alpha_i < 0, \beta_i \ge 0) \\ \pi + \arctan|\beta_i/\alpha_i| & (\alpha_i < 0, \beta_i \ge 0) \end{cases} \tag{5}$$

Eqs. 2–5 indicate that the b_i parameter changes seasonally following a cosine function with a fixed cycle (1 year). The amplitude (B_i), phase shift (θ_i), and intercept (γ_i) were determined by the data through model fitting. Hereafter, this class of model was referred to as "models with seasonal effects" (*e.g.*, a quadratic model with seasonal effects). The extent of seasonality in relationships between optical meter readings and Chl_{area} or N_{area} can be assessed by comparing the model fit between a basic model and a corresponding model with seasonal effects (details of the comparison are given in the Bayesian inference subsection).

Mixed models with random effects: Because data were collected 6 times within a year period, they included random errors caused by unknown factors associated with each measurement event. These random errors may distort the relationships between optical meter readings and Chl_{area} or N_{area}. To capture random effects, which were associated with the measurement time, a term for random effects was added to the models. Hereafter, this class of models was referred to as "mixed-effects models" (*e.g.*, mixed-effects linear model). Although models with a random-effect term were considered to be different from corresponding models without a random-effect term, they were essentially similar because the relationships between Chl_{area} or N_{area} and optical meter readings were the same. A hierarchical Bayesian approach was used to cope with the random effects.

Mixed models with seasonal effects and random effects: The last class of models considered both seasonal changes in the relationships between optical meter readings and Chl_{area} or N_{area}, and random errors associated with each measurement event. Mathematically, they were expressed by adding a term for random effects (described in the subsection above) to models with seasonal effects (described above). Hereafter, this class of model was referred to as "mixed-effects models with seasonal effects" (*e.g.*, a mixed-effects quadratic model with seasonal effects).

Appendix 2

The details of Bayesian inference used in this study were as follows. Observations of Chl_{area} and N_{area} were assumed to follow normal distributions with means provided by the models described in Appendix 1. The precision (*i.e.*, inverse of the variances) of the normal distribution was assumed to have a noninformative prior (gamma distribution with shape parameter 1.0×10^{-4} and rate parameter 1.0×10^{-4}). It was also assumed that all of the model parameters had non-informative priors (normal distributions with means of zero and precisions of 1×10^{-6}). For hierarchical models with a random-effect term, the random effects were assumed to follow a normal prior with mean zero and a precision that followed a noninformative hyperprior (gamma distribution with shape parameter 1.0×10^{-4} and rate parameter 1.0×10^{-4}).

The model that related the best SPAD readings to Chl_{area} was selected among 8 for each species (2 basic models *vs.* [quadratic or cubic models] *vs.* [with or without seasonal effects] *vs.* [with or without random effects]), The model that was related the best Agriexpert readings to N_{area} was selected among 8 models [2 basic models (linear or quadratic models) *vs.* (with or without seasonal effects) *vs.* (with or without random effects)] for each species. During model selection, the model with the lowest DIC value was considered the best. For all of the models selected, R^2 was calculated to express the goodness of fit in a relative sense and root mean squared error (RMSE) was used to express the accuracy of model prediction in an absolute sense.

Sampling from the posterior distributions of all parameters was performed using a Markov chain Monte Carlo methods (MCMC) with the software *WinBUGS* (Spiegelhalter *et al.* 2003). For most of the models, three independent MCMC chains were run and 20,000 samples were recorded after a burn-in of 50,000. The chains were thinned every 20 runs, yielding independent samples from a posterior of size 3,000. For some N_{area} models (the mixed-effects linear model with seasonal effects and the mixed-effects quadratic model with seasonal effects), 100,000 samples were recorded for each chain after a burn-in of 20,000, and the chains were thinned every 100 runs because the MCMC samplings did not converge, when the previous sampling method was used. Convergence of the MCMC chains was checked using $\hat{R}$ (Gelman *et al.* 2004) for each parameter. The mean and 95% Bayesian credible intervals for each parameter were evaluated based on their posterior samples.