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Disentangling long- and short-term changes in perennial organ functions in seasonal environments: A model of foliar chlorophyll and nitrogen in saplings of four evergreen broad-leaved trees

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Abstract

Perennial organ functions of trees living in seasonal environments exhibit temporal changes that can be classified as long-term interannual changes and seasonal fluctuations within single years. However, few studies have separately quantified these changes from longitudinal measurement data or analyzed the relationships between them. We developed a hierarchical Bayesian statistical model consisting of three parts: a long-term interannual change expressed by consecutive annual linear trends, seasonal fluctuations with 26 values for two-week periods in a year, and a random effect for repeated measurements. The model can extract long-term interannual changes and seasonal fluctuations from longitudinal repeated measure data. The pattern of seasonal fluctuation, the amount of seasonal fluctuation, and the net annual change are expressed by the estimated model parameters. We applied our model to foliar chlorophyll (Chl) and nitrogen (N) content measured repeatedly on more than 1-year-old leaves of saplings in four evergreen broad-leaved tree species using nondestructive optical methods. The model successfully explained large variations in the Chl and N content. In general, seasonal fluctuations corresponded to the phenology of current-year leaves; Chl and N tended to decrease from the opening to maturation of new leaves and increased during the rest period. The magnitude of the decrease in the Chl and N content in the growth period of current-year leaves ($\Delta\gamma$) did not decrease noticeably as leaves aged. For the Chl content, $\Delta\gamma$ was positively correlated with the maximum value before leaf opening across species. For the N content, $\Delta\gamma$ and the maximum value before leaf opening were not clearly correlated across species, but were positively correlated within some species. A model parameter for annual linear trends in Chl and N varied from positive (indicating increasing trends) to negative values (indicating decrease) depending on species and leaf age in years.

Additional key words: Agriexpert; *Castanopsis*; *Cinnamomum*; leaf aging; *Machilus*; *Neolitsea*; retranslocation; SPAD-502.

Introduction

The perennial organ functions of trees living in seasonal environments exhibit temporal changes that can be divided into two categories: long-term interannual changes and seasonal fluctuations within single years. For example, the foliar nutrient content of evergreen tree species undergoes long-term changes associated with increasing leaf age and short-term seasonal changes (*e.g.*, Chapin and Kedrowski 1983, Silla and Escudero 2003, Milla *et al.* 2005). Long-term trends and slow aging appear through repeated seasonal fluctuations (Senser *et al.* 1975). Therefore, these two kinds of temporal change are thought to be related. However, long-term interannual and short-term seasonal changes in tree functions often occur through different mechanisms, and are subject to different influences. Therefore, it is important to discriminate between them and examine their relationship.

However, extracting temporal changes at different time scales from longitudinal data is challenging because these changes are combined within single measurement values. Moreover, such data are often obtained by measurements repeated on fixed subjects, and are structurally complex. To date, few studies have analyzed or modeled both

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Abbreviations: CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; intrinsic CAR – intrinsic Gaussian conditional autoregressive model; MaT – *Machilus thunbergii*; MCMC – Markov chain Monte Carlo; NeS – *Neolitsea sericea*; R – correlation coefficient; R^2 – coefficient of determination; $\hat{R}$ – Gelman-Rubin's scale reduction factor.

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long-term interannual and short-term seasonal changes in tree functions and size (growth) (*e.g.*, Kimberley and Richardson 2004, Ye *et al.* 2014). These studies often used fixed mathematical functions to represent long-term interannual changes (*e.g.*, logistic function) and short-term seasonal changes (*e.g.*, sine function); thus, they generally lacked flexibility and failed to account for potentially complex data structure. Therefore, the primary objectives of this study were (*1*) to propose a statistical model based on a hierarchical Bayesian approach using the intrinsic Gaussian conditional autoregressive model (CAR) (Besag *et al.* 1991) for extracting long-term interannual and short-term seasonal changes from longitudinal data obtained through repeated measurements, and (*2*) to apply this model to the content of foliar nutrients (specifically, N and Chl) of evergreen broad-leaved tree species in Japan as an example of the model application. We presented the detailed features of temporal changes in foliar N and Chl content based on the results of the analysis. This was the secondary objective of this paper.

Foliar nutrient content is influenced by many factors, including leaf shedding pattern (del Arco *et al.* 1991, Orgeas *et al.* 2002, Milla *et al.* 2005, Katahata *et al.* 2007), branch position within the crown (Niinemets 1997), light intensity (Hikosaka *et al.* 1994), supply from soil (Silla and Escudero 2003), and changes temporally depending on season and leaf age (*e.g.*, Chapin and Kedrowski 1983, Silla and Escudero 2003, Milla *et al.* 2005). Comprehensive knowledge of temporal changes in foliar nutrient content leads to a better understanding of the strategies for absorption, storage, and use of nutrients in trees growing in an ever-changing environment (Nambiar and Fife 1991, Saur *et al.* 2000, Silla and Escudero 2003, Muller *et al.* 2011). Moreover, it improves estimations of functions over the life span of single leaves (Kikuzawa and Lechowicz 2006, Selaya and Anten 2010, Umeki *et al.* 2010) and integrates the functions of individual plants or canopies (Koyama and Kikuzawa 2009) because many leaf functions depend on their nutrient content.

Because the foliar N content is closely related to important leaf functional parameters, 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), its temporal change has been intensively studied (*e.g.*, Chapin and Kedrowski 1983, Silla and Escudero 2003, Milla *et al.* 2005). These studies have shown that temporal patterns in the foliar N content of evergreen trees differ from those of deciduous trees (Cherbuy *et al.* 2001). The pattern in evergreens can be summarized as follows: the N content of existing leaves increases before bud burst, decreases from the spring to summer as new shoots develop, and increases again after current-year shoots finish their growing (Fife and Nambiar 1982, Millard and Proe 1992, Wendler *et al.* 1995, Silla and Escudero 2003). These changes result from the retranslocation of N from existing older leaves to new growing shoots, and its replenishment into the older leaves after shoot maturation. Older leaves have a N-storage function; N is retranslocated repeatedly during their life span, not just before abscission (Fife and Nambiar 1984, Wendler *et al.* 1995).

The amount of N retranslocated is affected by multiple factors, including the source-sink balance and leaf aging. For example, it is positively correlated with the N content just prior to retranslocation (Millard and Proe 1993, Saur *et al.* 2000, Milla *et al.* 2005, Fife *et al.* 2008). It is also positively correlated with the rate of shoot growth, which should be a strong sink of N (Nambiar and Fife 1991, Miyazawa *et al.* 2004). Nutrients are remobilized primarily from leaves in the youngest age classes (Fife and Nambiar 1984, Greenway *et al.* 1992, Millard 1996, Cherbuy *et al.* 2001).

While seasonal fluctuation in evergreen tree foliar N contents has been studied extensively, less attention has been paid to long-term trends. Moreover, the few studies on long-term trends in foliar nitrogen content have shown inconsistent patterns. N in relatively young needles of *Pinus radiata* was replenished in the winter to a level similar to that before retranslocation, but N in relatively old needles did not increase from the level lowered by retranslocation (Fife and Nambiar 1984, Nambiar and Fife 1991). In this case, the needle N content decreased through repeated fluctuations as leaves aged. However, N contents (concentration per area) did not decrease with leaf age of other species (Escudero and Mediavilla 2003, Ethier *et al.* 2006, Han *et al.* 2008).

Chl is the main pigment involved in photosynthesis; a foliage Chl content determines leaf absorptance and thus the fraction of light absorbed (Niinemets 2010). The foliar Chl content per unit of leaf dry mass is known to increase with decreasing light availability across and within individual canopies (Niinemets 2010). However, few studies have dealt with temporal changes in the Chl content of evergreen trees. Moreover, most of these studies reported on changes caused by various stresses such as low temperature (Senser *et al.* 1975, Ottander *et al.* 1995, Öquist and Huner 2003) or drought (Gratani *et al.* 1998, Munnè-Bosch and Alegre 2000). A few studies observed temporal changes during periods without extreme stress, but they found no significant seasonal or long-term changes (Fife and Nambiar 1982, Wendler *et al.* 1995, Muller *et al.* 2011).

We used parameters estimated for the model of foliar Chl and N contents to answer the following questions: (*1*) Do seasonal fluctuations coincide with the development of new leaves? (*2*) Is the extent of seasonal fluctuation correlated with the leaf age or maximum nutrient content? (*3*) Is the annual linear trend correlated with leaf age or seasonal fluctuations? (*4*) How great are the seasonal fluctuations and annual linear trends relative to the maximum content?

Materials and methods

Model for temporal change in perennial organ functions: We developed a statistical model for temporal changes in sizes, traits, or functions of perennial organs that included long-term interannual change comprising annual linear changes, seasonal fluctuations, and a random effect for repeated measurements. Although the model is generally applicable to various perennial organ functions, it is explained below with the terminology for the N content of evergreen leaves.

In the model (Eq. 1), the N content for a leaf of a particular age (in years) during a year is expressed as the sum of a linear function expressing an annual linear trend, an autocorrelated random effect term expressing seasonal fluctuation, and a leaf-specific random effect term. It describes the temporal change in the N content in one year; the interannual change can be expressed by a simple sequence of the model for single years. We used two measures of leaf age in the model: age in years and age in days. The age in years was a discrete integer variable defined as the difference between the year when the leaf emerged and the year when it was measured. This variable is referred to hereafter as "leaf age class". The leaf age class appears as a suffix of variables and parameters. Age in days was a continuous variable expressing the length of time (in days) from leaf emergence to measurement (hereafter "leaf age"). The leaf age was used as an explanatory variable.

The model is expressed as:

$$y_{\mathrm{ijk}} = \alpha_{\mathrm{i}} + \beta_{\mathrm{i}} \cdot A_{\mathrm{ijk}} + \gamma_{\mathrm{il}} + R_{\mathrm{leaf\,j}} \qquad (1)$$

where y_{ijk} is the N content for the k^{th} measurement on the j^{th} leaf in the i^{th} age class, A_{ijk} is the leaf age in days, and α_{i} and β_{i} are model parameters specific to each leaf age class. The first and second terms of the model $(\alpha_{\mathrm{i}} + \beta_{\mathrm{i}} \cdot A_{\mathrm{ijk}})$ together express a linear trend (either increasing or decreasing) in the N content within a year. γ_{il} is a temporally autocorrelated random effect representing seasonal fluctuation (explained in detail below). $R_{\mathrm{leaf\,j}}$ is a random effect for the j^{th} leaf to capture leaf-specific variance in the data caused by repeated measurement of the same leaves.

To express seasonal fluctuation in the N content, we divided a year into 26 2-week periods and assigned an autocorrelated random effect (γ_{il}) that followed the intrinsic CAR (Besag *et al.* 1991) to each period. The conditional probability distribution of γ_{il} is expressed as:

$$\gamma_{\mathrm{il}} |\, \gamma_{\mathrm{iL}} \sim N\left(\frac{\sum_{L\in\delta_l} \gamma_{iL}}{2}, \frac{\sigma^2_{\gamma\, i}}{2}\right) \qquad (2)$$

where δ_{l} is a set consisting of two neighboring periods (one just before and the other just after the target period) and L is a member of δ_{l} . $\sigma_{\gamma\mathrm{i}}{}^2$ is the age-class-specific conditional variance. γ_{il} follows a normal distribution (N) with the expected value being the average of the values for the two neighboring periods. Note that interdependency among γ_{il} is circular: the first period in a year is a neighbor of the last period, and *vice versa*. Intrinsic CAR has been often used to represent spatially autocorrelated random effects but its application to temporal data has been rather rare (*e.g.*, Shaddick and Wakefield 2002). One interesting feature of the intrinsic CAR model is that we do not need data in all divisions (2-week periods, for instance) to estimate the expected value for divisions (however, data in all divisions does improve accuracy). Details on Bayesian inference for the model, including priors for parameters, and on Markov chain Monte Carlo (MCMC) sampling are given in Appendix 1.

To quantify the magnitude of seasonal fluctuations, we calculated the difference ($\Delta\gamma$) between the maximum γ_{il} in the period from January to August and the minimum γ_{il} in the period from May to December. These periods were based on typical seasonal fluctuations of the N content (maximum in the spring and minimum in early autumn) that correspond to the retranslocation of nutrients from the spring to early autumn followed by replenishment (detailed patterns are described in the Results). The 26 γ_{il} values had a circular structure such that they return to the same value at the end of a year and do not contribute to the net change in y_{ijk}. We also calculated the ratio of $\Delta\gamma$ to the maximum value before the summer (hereafter relative $\Delta\gamma$).

The net change in y_{ijk} in a year was expressed as $365\beta_{\mathrm{i}}$. We calculated the relative $365\beta_{\mathrm{i}}$, the ratio of $365\beta_{\mathrm{i}}$ to the maximum value before the summer. These relative values enabled us to compare the extents of annual fluctuations and net changes between species with different absolute contents of nutrients, as well as temporal changes in various nutrients (*e.g.*, N *vs.* P content).

Application of the model: We applied the model to data on the N and Chl content measured in more than 1-year-old leaves from saplings of four evergreen broad-leaved tree species in Japan. Measurements were taken using the nondestructive optical methods described below.

Species and study site: We studied four evergreen broad-leaved tree species occurring in Japan: *Castanopsis sieboldii* (Makino) Hatus. ex T. Yamaz. *et* Mashiba (CaS), *Cinnamomum tenuifolium* (Makino) Sugim. ex H. Hara (CiT), *Machilus thunbergii* Siebold *et* Zucc. (MaT), and *Neolitsea sericea* (Blume) Koizumi (NeS). All are common species in Japanese evergreen broad-leaved forests. CaS is a dominant canopy species in the warm-temperate forests of central Japan (Nitta and Ohsawa 1997). MaT is another canopy species in these forests; it often dominates the canopy layer in coastal stands. CiT and NeS are subcanopy species that often codominate in these temperate forests. Saplings of the four species frequently occur in the understories of our study site. Therefore, all four species are considered shade-tolerant. Leaves of these species emerge in a single short flush in the spring, usually once a year. Leaf fall has one peak in the spring (CaS), coincident with leaf emergence, or two peaks with the first peak in the spring (coincident with leaf emergence) and the second in the summer or autumn (CiT, MaT, and NeS) (Nitta and Ohsawa 1997).

Our study site was on the Matsudo Campus of Chiba University, Japan (35°46'34"N, 139°54'0"E). The mean temperature at the nearest meteorological station (15.2 km from the research site) over a 12-year period (1999–2010) was 15.3°C, with a maximum value (26.5°C) in August and a minimum value (4.9°C) in January. The mean annual precipitation was 1,463 mm.

Measurements: In April 2006, we selected 9–10 saplings of each target species at the study site. The saplings had not been artificially planted. Some of the selected saplings were under the canopy of deciduous trees and others were under the canopy of evergreens. Tree heights ranged from 0.2 to 3 m.

We measured all leaves on the main stem of the selected saplings for their Chl and N content, except very old leaves in the most proximal positions. About one-fourth of leaves (318 leaves out of 1,227 for the Chl measurement, and 379 leaves out of 1,343 for the N measurement) were shed during the research period. We used *SPAD-502* (*Konica-Minolta*, Osaka, Japan; hereafter SPAD) and *Agriexpert PPW-3000* (*Satake Corp*., Hiroshima, Japan; hereafter Agriexpert) optical meters for estimating the Chl and N content, respectively. SPAD measures light absorption rates of 650 and 950 nm and calculate SPAD value, and Agriexpert measures absorption rates of four wavelengths (560, 660, 900, and 950 nm). The measurements were repeated on all target leaves once or twice a month. Foliar Chl and N content per unit of leaf area were estimated with equations developed for these species (Mizusaki *et al.* 2013). These equations relate readings of SPAD and Agriexpert to the Chl and N content, respectively, using species-specific parameters. Because they were developed with data obtained from leaves of various ages (up to five years old) and colors, they can be used for the estimation of foliar Chl and N content in this study. Measurements of the Chl content were performed from April 2006 to March 2007, and those for the N content were carried out from April 2007 to December 2008.

Leaf age determination: The year when a leaf emerged was determined using bud scars on the main trunk. The years of leaf emergence were used to determine the leaf age class. The estimated leaf emergence years ranged from 2000 to 2008. Because we excluded the current-year leaves from the analyses, the smallest leaf age class was one. The number of leaf age classes used in the analyses was four for CaS and NeS and six for CiT and MaT.

Leaf emergence dates were used to calculate leaf age in days. For leaves that emerged in 2006, 2007, and 2008, we used the dates when leaf emergence was actually observed, but for leaves that emerged before 2006, we used the sapling-specific average leaf emergence dates calculated from data from 2006, 2007, and 2008 as the estimated leaf emergence date. The average leaf emergence dates ranged from late April to early June (23 April for CaS, 12 May for CiT, 5 June for MaT, and 25 April for NeS).

Maturing phenology of current-year leaves: We focused on the temporal change in the Chl and N content of leaves more than 1-year-old and used data for current-year leaves as references against which the temporal changes in older leaves were compared. Specifically, we determined the development period of current-year leaves (from the average leaf emergence date to the average leaf maturation date) for each species, and examined how the Chl and N content of older leaves changed both during and outside this period.

To determine the leaf maturation date, we fitted the following model, which has an asymptotic maximum, to data on the Chl or N content of the current-year leaves:

$$y = a - b \cdot e^{-c \cdot A} \qquad (3)$$

In this function, y is the Chl or N content, A is leaf age in days, a is a model parameter for an asymptotic maximum value, and b and c are coefficients. Details of model fitting are given in Appendix 2.

We calculated the date when the Chl or N content reached 90% of the asymptotic maximum value as the leaf maturation date. We calculated leaf maturation dates for the Chl and N content and used them for the analyses of the Chl and N content, respectively. The leaf maturation dates for the Chl content were 31 July for CaS, 11 September for CiT, 2 September for MaT, and 2 September for NeS. The leaf maturation dates for N were 10 August for CaS, 3 August for CiT, 19 July for MaT, and 30 August for NeS.

Software: We used *WinBUGS* software (Spiegelhalter *et al.* 2003) to sample parameter values from posterior distributions and *R software* (*R Core Team* 2012) for other statistical procedures and data manipulation.

Results

Variation in nutrient content: Scatterplots for the N and Chl content *vs*. leaf age (> 1 year) showed long-term trends and seasonal fluctuations in the N and Chl content (Fig. 1). They also showed large variations in observed values at each measurement date. These variations were due to considerable differences among leaves.

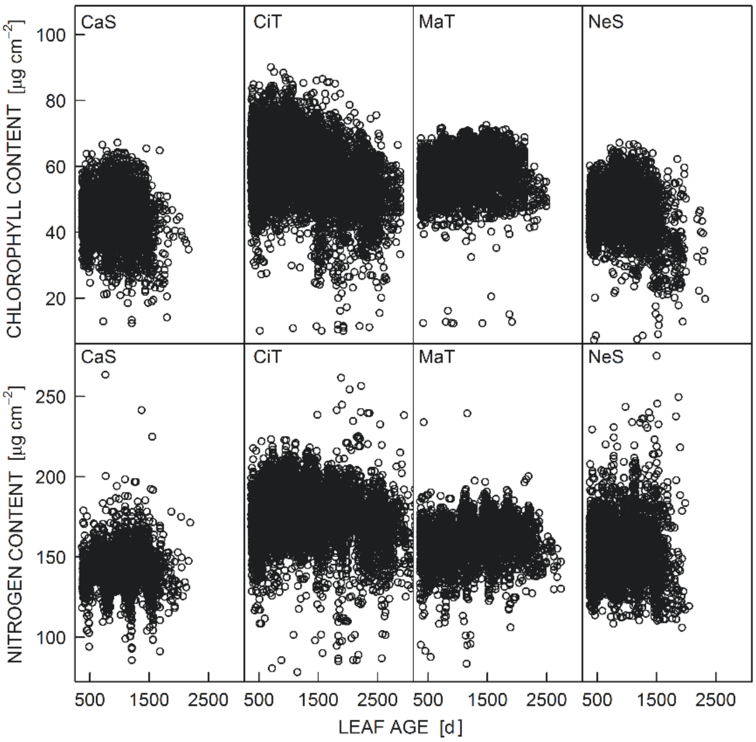

Fig. 1. Relationships between leaf age and chlorophyll content estimated from SPAD-502 and leaf age and nitrogen content estimated from Agriexpert of leaves older than current-year leaves. CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; MaT – *Machilus thunbergii*; NeS – *Neolitsea sericea*.

Model fitting: In all model fittings (two variables × four species), the $\hat{R}$ (Gelman-Rubin's scale reduction factor) values were lesser than 1.1 for all parameters in Eq. 1, indicating that three chains of MCMC samples converged. The R^2 values for the model of Chl content were relatively high, ranging from 0.717 for CaS to 0.751 for MaT. The R^2 values for the model of the N content were smaller than those for the Chl model, ranging from 0.458 for CaS to 0.684 for CiT. These relatively high R^2 values indicated that a major portion of the variation in the Chl and N content was explained by the three components of the model.

Seasonal fluctuations: We observed a marked common pattern in the seasonal fluctuations of the Chl content (Fig. 2); it peaked around leaf opening (late April to early June), decreased from this peak to a minimum value at leaf maturation (mid-July to early September), and increased or remained stable over the rest of the period. This pattern was found in leaves of all age classes for CiT and MaT and in leaves of most age classes for CaS. We found similar fluctuations for NeS where the Chl content decreased from the spring until the end of summer and then increased toward the winter; however, the range in fluctuations was small and the period, when Chl content decreased, did not clearly accord with the maturation phenology of the current-year leaves.

We also observed a clear common pattern in the seasonal fluctuation of N (Fig. 2). The N content peaked around leaf opening (late April to early June), decreased from this peak to minimum values around leaf maturation (mid-July to early September), and increased or remained stable over the rest of the period. This pattern was found in leaves of all age classes for CaS, CiT, and MaT, although the decrease began in January for leaves in some age classes in CaS. The seasonal fluctuation in the N content for NeS was somewhat similar to the seasonal fluctuation in the Chl content for this species. The N content decreased from the spring to the end of summer and then increased toward the winter, but the period, when the N content decreased, did not clearly match the maturation phenology of the current-year leaves.

Relationship between Δγ and age: In general, Δγ for the Chl content did not change markedly as leaves aged (Fig. 3*A*), although it decreased sharply from the age class 3 to 4 for CaS, and slightly over the whole leaf–life span for MaT. Δγ for the N content decreased clearly for CaS and slightly for CiT as leaves aged (Fig. 3*B*). For MaT and NeS, it remained stable for leaves in all age classes with few exceptions.

Relationship between Δγ and maximum value: We detected a positive interspecific correlation between Δγ and the maximum value for the Chl content, but no clear intraspecific relationships (Fig. 4*A*). No clear interspecific correlations were detected between Δγ and the maximum value for the N content. However, Δγ and the maximum N content were positively correlated within each of three species (CaS, CiT, and NeS). The relative Δγ for the Chl content ranged from 1.4% for CaS to 10.7% for MaT (Table 1). The relative Δγ for the N content ranged from 6.1% for CiT to 16.6% for CaS.

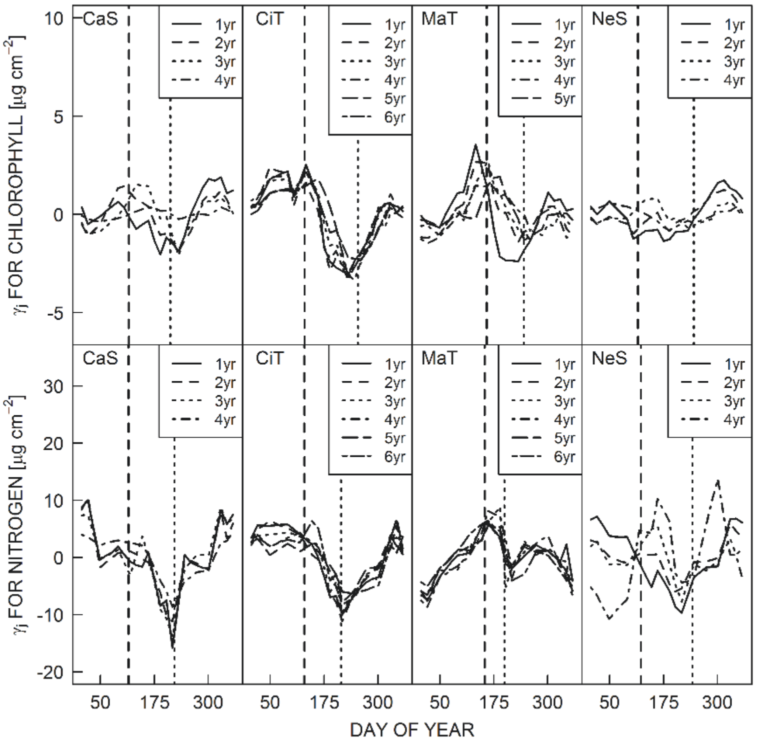

Fig. 2. Values of the seasonal fluctuation term (γ) in Eq. 1 for chlorophyll content and nitrogen content. *Vertical dashed lines* show average leaf opening dates and *vertical dotted lines* show average leaf maturation dates. CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; MaT – *Machilus thunbergii*; NeS – *Neolitsea sericea*.

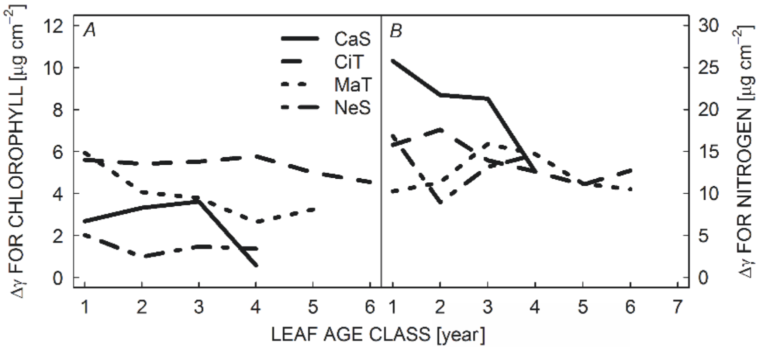

Fig. 3. Relationships between Δγ and leaf age class for chlorophyll (*A*) and nitrogen (*B*). Each line represents different species. Δγ is the difference between the maximum γ from January to August and the minimum γ from May to December. CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; MaT – *Machilus thunbergii*; NeS – *Neolitsea sericea*.

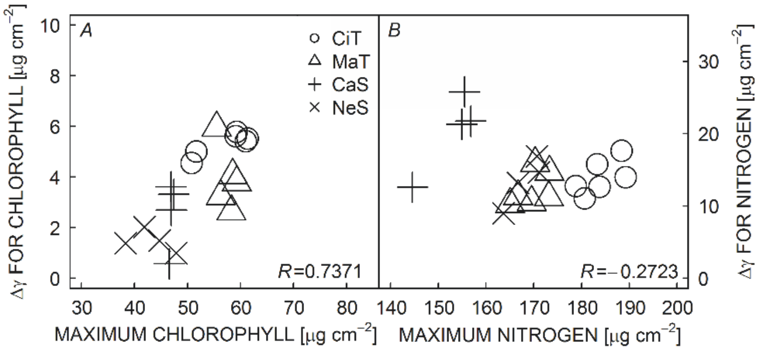

Fig. 4. Relationship between the maximum content before summer and Δγ for chlorophyll (*A*) and for nitrogen (*B*). *R* is the correlation coefficient calculated from all data points.). CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; MaT – *Machilus thunbergii*; NeS – *Neolitsea sericea*.

Relationship between β and age: With few exceptions, β for the Chl content decreased as leaves aged (Fig. 5*A*). Except for NeS, β was positive when the leaf age class was one, indicating that the Chl content increased with the increasing leaf age during the second year after leaf emergence. β became negative with the increasing leaf age class for CaS and CiT, indicating that the Chl content began to decrease with leaf aging, whereas it stayed positive for MaT.

For MaT and CaS, β for the N content was positive and decreased with the increasing leaf age class (Fig. 5*B*), indicating that the N content increased as leaves aged, at

Table 1. Amount of seasonal fluctuation ($\Delta\gamma$) and annual net change (365β) in chlorophyll and nitrogen content. Values in parentheses are relative values (the ratios of $\Delta\gamma$ or 365β to the maximum value before summer; referred to as relative $\Delta\gamma$ and relative 365β, respectively, in the text). CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; MaT – *Machilus thunbergii*; NeS – *Neolitsea sericea*.

Species	Age	Chlorophyll		Nitrogen	
		Δγ [μγ χμ^{-2}]	365β [μγ χμ^{-2}]	Δγ [μγ χμ^{-2}]	365β [μγ χμ^{-2}]
CaS	1	3.94 (5.73)	0.73 (1.55)	25.8 (16.6)	5.64 (3.62)
	2	3.32 (7.00)	0.12 (0.26)	21.7 (13.9)	4.93 (3.14)
	3	3.61 (7.71)	–0.48 (–1.02)	21.3 (13.7)	3.27 (2.11)
	4	0.721 (1.24)	–1.39 (–2.97)	12.6 (8.72)	1.65 (1.14)
CiT	1	5.61 (9.49)	1.52 (2.57)	15.8 (8.61)	5.68 (3.10)
	2	5.41 (8.87)	–2.43 (–3.98)	17.6 (9.35)	–1.51 (–0.80)
	3	5.52 (8.99)	–3.04 (–4.95)	14.0 (7.37)	0.84 (0.44)
	4	5.78 (9.75)	–3.99 (–6.74)	12.6 (6.87)	0.60 (0.32)
	5	5.01 (9.69)	–3.32 (–6.42)	11.1 (6.11)	–0.54 (–0.30)
	6	4.55 (8.96)	–4.17 (–8.21)	12.7 (7.12)	–1.53 (–0.86)
MaT	1	5.94 (10.7)	0.61 (1.09)	10.3 (6.22)	8.70 (5.27)
	2	4.08 (6.97)	0.79 (1.35)	11.3 (6.79)	7.46 (4.47)
	3	3.79 (6.39)	0.24 (0.41)	15.9 (9.34)	6.00 (3.53)
	4	2.93 (4.53)	0.28 (0.48)	14.7 (8.48)	4.78(2.76)
	5	3.25 (5.77)	–0.44 (–0.77)	11.2 (6.45)	4.21 (2.43)
	6			10.5 (6.21)	3.80 (2.24)
NeS	1	3.09 (4.85)	–0.37 (–0.88)	16.9 (9.88)	–1.64 (–0.96)
	2	2.23 (2.08)	–2.79 (–5.83)	8.97 (5.48)	0.87 (0.53)
	3	1.49 (3.32)	–4.07 (–9.09)	13.2 (7.90)	0.23 (0.14)
	4	1.38 (3.60)	–2.01 (–5.25)	14.7 (8.57)	–1.15 (–0.67)

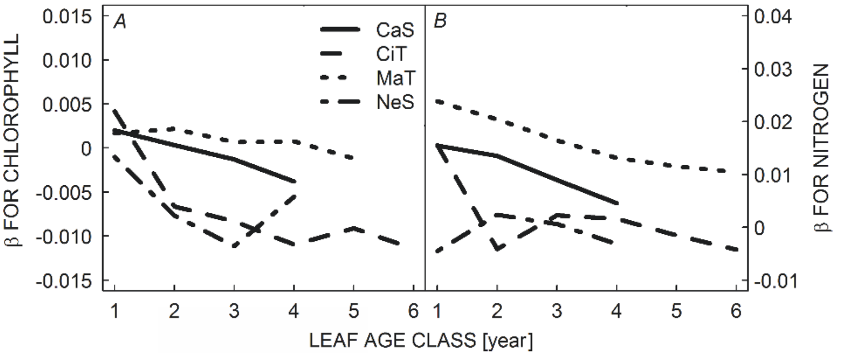

Fig. 5. Relationship between β and leaf age class for chlorophyll (*A*) and nitrogen (*B*) contents. CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; MaT – *Machilus thunbergii*; NeS – *Neolitsea sericea*.

rates that decreased with the leaf age class. β was positive but decreased rapidly to values near 0 for CiT, indicating that the N content increased only during the second year after leaf emergence. For NeS, β stayed near 0, indicating that the N content did not change perceptibly.

The relative 365β for the Chl content ranged from –9.09% for NeS to 2.57% for CiT. The relative 365β for the N content ranged from –0.96% for NeS to 5.27% for MaT (Table 1).

Relationship between Δγ and β: We noted no clear intra- or interspecific relationships between Δγ and β for the Chl content (Fig. 6*A*). The variations in both Δγ and β were larger among species than within them; points for each species were located within a narrow, distinct region of the scatterplot. No clear interspecific relationships were observed between Δγ and β for the N content (Fig. 6*B*). The variation in β for the N content was larger among species than within them. When Δγ and the absolute value of 365β were compared for the Chl or N content for each species (Table 1), the former was generally larger than the latter, indicating that the amount of fluctuation was larger than the annual net change.

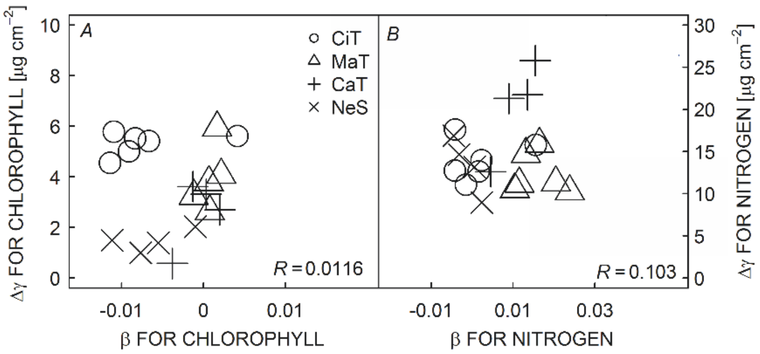

Fig. 6. Relationship between Δγ and β for chlorophyll (*A*) and nitrogen (*B*) contents. *R* is the correlation coefficient calculated from all data points. CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; MaT – *Machilus thunbergii*; NeS – *Neolitsea sericea*.

Discussion

Extraction of long-term interannual changes and seasonal fluctuations: Large variations in the Chl and N content were successfully explained by our model, which included three components: annual linear trends, seasonal fluctuations, and a leaf-specific random effect. The pattern of fluctuations, the amount of seasonal fluctuation, and the net annual change are respectively expressed by γ, Δγ, and 365β. Therefore, this model enabled us to understand patterns of the temporal change in the Chl and N content and to compare patterns quantitatively within and among species. The temporal patterns in long-term interannual changes and seasonal fluctuations within single years can be complicated as described above for the N and Chl content. Therefore, it is insufficient to assume fixed mathematical functions (*e.g.*, logistic and sine functions) to model them. In previous studies, some attempts (*e.g.*, selection of appropriate functions from multiple candidates or Fourier series approximations) have been made to make modeling flexible (Kimberley and Richardson 2004, Ye *et al.* 2014). Our model was flexible enough to represent interannual changes in the Chl and N content containing both increasing and decreasing phases and variously patterned seasonal fluctuations. Including a leaf-specific random effect was also effective for extracting linear annual trends and seasonal fluctuations from "noisy" data. This modeling method is general enough to be used in a wide range of analyses of temporal patterns in perennial plant organ functions in seasonal environments, and the example of the application of the model (*i.e.*, the analysis of foliar Chl and N contents) clearly showed the applicability of the model.

Seasonal fluctuations and their possible causes: We found seasonal fluctuations in the N content in the leaves of the evergreen tree species we studied, similarly to many previous studies (Nambiar and Fife 1991, Millard and Proe 1992, Cherbuy *et al.* 2001, Miyazawa *et al.* 2004, Yasumura *et al.* 2011). In contrast to several previous studies (Fife and Nambiar 1982, Wendler *et al.* 1995, Muller *et al.* 2011), we found seasonal fluctuations in Chl (Gond *et al.* 1999). The temporal pattern of variation in the Chl content may depend on the species or environment.

Despite differences in leaf opening and maturation dates among species, the periods of decreasing Chl and N content in older leaves coincided with the developmental period of the current-year leaves. This finding strongly suggests that the decreases were caused not by leaching or acclimation to changing environments (Muller *et al.* 2011), but by retranslocation of nutrients from older to current-year leaves (Fife and Nambiar 1984, Nambiar and Fife 1987, 1991; Helmisaari 1992). Current-year leaves should be strong sinks during their development, influencing the nutrient dynamics in previously formed organs; they can absorb many nutrients from older leaves. When the current-year leaves finished their development, the decrease in the Chl and N content in the older leaves also stopped in the absence of strong sinks. Chl is a large compound and is unlikely to be transported as it is, but can be decomposed and transported to sinks (hereafter, this process is referred to as retranslocation of Chl for simplicity).

After the Chl and N content of the older leaves stopped decreasing, they began to increase again. Previous studies also found similar increasing phases (Nambier and Fife 1987, 1991), termed replenishment. Nitrogen absorbed by roots may contribute to the increased N content in the older leaves, and Chl might be assimilated in the older leaves using N allocated to them. Although the increasing phase was found in all species studied just after leaf maturation, the patterns of fluctuation in the winter differed among species. For example, the N content in the winter was stable in CiT, but decreased in CaS. These differences may reflect possible differences in the sensitivity of roots to low temperatures and in the activity of other organs.

The range of seasonal fluctuation was the smallest in NeS, and the period, when the N and Chl content decreased, did not clearly match the maturing phenology of the current-year leaves for this species. This may be due to the leaf maturation phenology of this species, which was the longest of the species studied (*e.g.*, for the N content, 106 d in CaS, 85 d in CiT, 45 d in MaT, and 119 d in NeS). This prolonged period of leaf maturation reduced nutrient absorbtion rates, weakening the sink activity of new shoots.

Amount of seasonal fluctuation: The relative $\Delta\gamma$ we observed for the N content (5–17%) was smaller than the values previously reported in evergreen conifers (*e.g.*, 42% for *Pinus strobus*, 32% for *Picea glauca*, and 25.8–42.5% for *Pinus radiata*) (Nambiar and Fife 1991, Munson *et al.* 1995). These differences may be attributable to the species studied. We also found considerable differences in $\Delta\gamma$ among species, although they all had the same leaf habit (*i.e.*, evergreen broad-leaved trees growing in a seasonal environment) and grew in the same habitat. Different environmental factors can also affect the amount of retranslocation. Yasumura *et al.* (2011) showed in *Quercus myrsinaefolia* that N retranslocation was larger in the outer crown than in the inner one. This result may indicate that more N was exported from unshaded than from shaded leaves. Trees usually grow slowly under shaded conditions as did our target saplings. In this situation, sinks (new shoots) are smaller and less active, resulting in relatively low nutrient demands. The low relative $\Delta\gamma$ we observed for the N content was also attributable to the limited growth under shaded conditions.

Relationship of $\Delta\gamma$ with leaf age and maximum nutrient content: Although Millard (1996) reported that retranslocation of nutrients, irrespective of senescence, was larger in younger than in older leaves, our results showed that $\Delta\gamma$ for both Chl and N content did not decrease greatly as leaves aged ($\Delta\gamma$ for he N content in CaS and CiT were exceptions). Older leaves of the study species also had a relatively important role as nutrient suppliers. Fife and Nambiar (1984) reported that the proportion of N retranslocated from 4–6-month-old needles was only half that from 12–16-month-old needles. Therefore, the relationship between the amount of retranslocated nutrients and the leaf age seems variable. More detailed physiological studies may explain this variation.

We found a positive interspecific correlation between the maximum value before the summer and $\Delta\gamma$ for the Chl content. Previous studies reported that the amount of retranslocated N is positively correlated with the maximum N content within a single species, *P. radiata* (Nambiar and Fife 1991), and across multiple species of evergreen broad-leaved trees (Fife *et al.* 2008). Our result for the Chl content indicates that the nutrient content in leaves is one determinant of the amount of retranslocation. The total amount of nutrients before retranslocation may primarily reflect the amount of nutrients stored for retranslocation.

Consistent with a previous study (Nambiar and Fife 1991), $\Delta\gamma$ for N was positively correlated with the maximum content within each of three species (CaS, CiT, and NeS). However, unlike for Chl, we could not find an interspecific correlation for N. The lack of an interspecific correlation could be explained by among-species differences in the proportion of various forms of N in leaves. Among the various forms of N present in leaves, N in soluble proteins is more easily retranslocated than that of structural proteins. The ratio of soluble to structural proteins is known to differ among species (Takashima *et al.* 2004). If the ratio differed considerably among the study species, $\Delta\gamma$ and the maximum content of N would not be correlated across species even if they were correlated within species. In contrast, Chl does not have various forms that could lead to a clear positive interspecific correlation between $\Delta\gamma$ and the maximum value.

Temporal patterns in β: A typical pattern in the net annual change in the Chl and N content expressed by an annual linear trend (β) was that the Chl and N content increased with leaf age when leaves were young and decreased with leaf age when leaves were old. This pattern indicates that leaves were sinks for Chl and N, when they were young, and became sources as they aged. Although the current-year leaves have been considered strong sinks, and this was the case in this study, the 1-year-old leaves still acted as sinks for Chl and N after the current-year leaves finished maturing. The negative annual net changes ($365\beta < 0$) in the older leaves were consistent with previous studies for Chl content (Šesták and Čatský 1962, Ciganda *et al.* 2008) and N content (Field 1983, Hikosaka 1994). However, β for the leaf age class 1 was relatively large or the decreasing rates of β were relatively small for some species so that β was still positive for the oldest leaf age class in Chl and N content, indicating that the Chl and N content increased over the life span. The long-term trends in the Chl and N content differed among species of evergreen broad-leaved trees.

Relationship between $\Delta\gamma$ and β: We found no clear intra- or inter-specific relationships between $\Delta\gamma$ and β for either Chl or N content. The lack of clear relationships between $\Delta\gamma$ and β may indicate that the amounts of seasonal fluctuation and the net annual change were independent. Larger retranslocation did not necessarily result in a larger net reduction in a year, which indicates that replenishment was also an important factor in determining the annual net change of nutrients in this study.

Future research: First, future research can use the model and the results of the analysis presented in this paper to make inter- and intra- specific comparisons of the temporal changes in foliar Chl and N contents. As previous studies showed, patterns in temporal changes in N and Chl contents are various depending on species and growth conditions (Cherbuy *et al.* 2001, Fife and Nambiar 1984, Nambiar and Fife 1991, Escudero and Mediavilla 2003). Second, future research should test if the estimation of photosynthetic gains can be improved by considering the detailed temporal changes of N and Chl contents as was quantified in this study. Third, future research should reveal N dynamics (*i.e.*, absorption, allocation, utilization, and discharge) at the individual level. The detailed analysis of the foliar N content as described in this paper will be an indispensable part of this research and our model can be used in N dynamics of other perennial plant organs because they also have the long-term interannual trends and seasonal fluctuations.

Conclusion: We developed the statistical model to analyze general temporal changes in the functions of perennial organs of trees in seasonal environments. The model was flexible enough to capture various types of temporal changes at two different time scales (*i.e.*, interannual and seasonal). Random effects associated with repeated measurements helped extract temporal patterns common to all measurement subjects from "noisy" longitudinal repeated measures data. We applied our model to the foliar nitrogen and chlorophyll content of evergreen tree species. The parameters estimated by the model quantified some important physiological processes (*e.g.*, retranslocation of nitrogen) and enabled us to examine the dependence of long-term and short-term (seasonal) changes on various factors, such as the leaf age class.

References

Besag J., York J., Mollié A.: Bayesian image restoration, with two applications in spatial statistics. – Ann. I. Stat. Math. **43**: 1-20, 1991.

Ciganda V., Gitelson A.A., Schepers J.S.: Vertical profile and temporal variation of chlorophyll in maize canopy: quantitative "crop vigor" indicator by means of reflectance-based techniques. – Agron. J. **100**: 1409-1417, 2008.

del Arco J.M., Escudero A., Garrido M.V.: Effects of site characteristics on nitrogen retranslocation from senescing leaves. – Ecology **72**: 701-708, 1991.

Ellsworth D.S., Reich P.B.: Leaf mass per area, nitrogen content and photosynthetic carbon gain in *Acer saccharum* seedlings in contrasting forest light environments. – Funct. Ecol. **6**: 423-435, 1992.

Escudero A., Mediavilla S.: Decline in photosynthetic nitrogen use efficiency with leaf age and nitrogen resorption as determinants of leaf life span. – J. Ecol. **91**: 880-889, 2003.

Ethier G.J., Livingston N.J., Harrison D.L. *et al.*: Low stomatal and internal conductance to CO_2 versus Rubisco deactivation as determinants of the photosynthetic decline of ageing evergreen leaves. – Plant Cell Environ. **29**: 2168-2184, 2006.

Field C., Mooney H.A.: The photosynthesis-nitrogen relationship in wild plants. – In: Givnish T.J. (ed.): On the Economy of Plant Form and Function. Pp. 25-55. Cambridge University Press, Cambridge 1986.

Field C.: Allocating leaf nitrogen for the maximization of carbon gain: Leaf age as a control on the allocation program. – Oecologia **56**: 341-347, 1983.

Fife D.N., Nambiar E.K.S.: Accumulation and retranslocation of mineral nutrients in developing needles in relation to seasonal growth of young radiata pine tree. – Ann. Bot.-London **50**: 817-829, 1982.

Fife D.N., Nambiar E.K.S.: Movement of nutrients in radiata pine needles in relation to the growth of shoots. – Ann. Bot.-London **54**: 303-314, 1984.

Fife D.N., Nambiar E.K.S., Saur E.: Retranslocation of foliar nutrients in evergreen tree species planted in a Mediterranean environment. – Tree Physiol. **28**: 187-196, 2008.

Gond V., de Pury D.G.G, Veroustraete F. *et al.*: Seasonal variations in leaf area index, leaf chlorophyll, and water content; scaling-up to estimate fAPAR and carbon balance in a multilayer, multispecies temperate forest. – Tree Physiol. **19**: 673-679, 1999.

Gratani L., Pesoli P., Crescente M.F.: Relationship between photosynthetic activity and chlorophyll content in an isolated *Quercus ilex* L. tree during the year. – Photosynthetica **35**: 445-451, 1998.

Greenway K.J., MacDonald S.E., Lieffers V.J.: Is long-lived foliage in *Picea mariana* an adaptation to nutrient-poor conditions? – Oecologia **91**: 184-191, 1992.

Han Q., Kwasaki T., Nakano T. *et al.*: Leaf-age effects on seasonal variability in photosynthetic parameters and its relationships with leaf mass per area and leaf nitrogen concentration within a *Pinus densiflora* crown. – Tree Physiol. **28**: 551-558, 2008.

Helmisaari H.-S.: Nutrient retranslocation within the foliage of *Pinus sylvestris*. – Tree Physiol. **10**: 45-58, 1992.

Hikosaka K., Terashima I., Katoh S.: Effects of leaf age, nitrogen nutrition and photon flux density on the distribution of nitrogen among leaves of a vine (*Ipomoea tricolor* Cav.) grown horizontally to avoid mutual shading of leaves. – Oecologia **97**: 451-457, 1994.

Chapin F.S. III, Kedrowski R.A.: Seasonal changes in nitrogen and phosphorous fractions and autumn retranslocation in evergreen and deciduous taiga trees. – Ecology **64**: 376-391, 1983.

Cherbuy B., Joffre R., Gillon D. *et al.*: Internal remobilization of carbohydrates, lipids, nitrogen and phosphorus in the Mediterranean evergreen oak *Quercus ilex*. – Tree Physiol. **21**: 9-17, 2001.

Katahata S., Naramoto M., Kakubari Y. *et al.*: Seasonal changes in photosynthesis and nitrogen allocation in leaves of different ages in evergreen understory shrub *Daphniphyllum humile*. – Trees **21**: 619-629, 2007.

Kikuzawa K., Lechowicz M.J.: Toward synthesis of relationships among leaf longevity, instantaneous photosynthetic rate, lifetime leaf carbon gain, and the gross primary production of forests. – Am. Nat. **168**: 373-383, 2006.

Kimberley M.O., Richardson B.: Importance of seasonal growth patterns in modelling interactions between radiata pine and some common weed species. – Can. J. Forest Res. **34**: 184-194, 2004.

Koyama K., Kikuzawa K.: Is whole-plant photosynthetic rate proportional to leaf area? A test of scalings and a logistic equation by leaf demography census. – Am. Nat. **173**: 640-649, 2009.

Lusk C.H., Reich P.B.: Relationships of leaf dark respiration with light environment and tissue nitrogen content in juveniles of 11 cold-temperate tree species. – Oecologia **123**: 318-329, 2000.

Milla R., Castro-Díez P., Maestro-Martínez M. *et al.*: Relationships between phenology and the remobilization of nitrogen, phosphorus and potassium in branches of eight Mediterranean evergreens. – New Phytol. **168**: 167-178, 2005.

Millard P.: Ecophysiology of the internal cycling of nitrogen for tree growth. – J. Plant. Nutr. Soil Sc. **159**: 1-10, 1996.

Millard P., Proe M.F.: Nitrogen uptake, partitioning and internal cycling in *Picea sitchensis* (Bong.) Carr. as influenced by nitrogen supply. – New Phytol. **125**: 113-119, 1993.

Millard P., Proe M.F.: Storage and internal cycling of nitrogen in relation to seasonal growth of Sitka spruce. – Tree Physiol. **10**: 33-43, 1992.

Miyazawa S.-I., Suzuki A.A., Sone K. *et al.*: Relationships between light, leaf nitrogen and nitrogen remobilization in the crowns of mature evergreen *Quercus glauca* trees. – Tree Physiol. **24**: 1157-1164, 2004.

Mizusaki D., Umeki K., Honjo T.: Development of models for estimating leaf chlorophyll and nitrogen contents in tree species with respect to seasonal changes. – Photosynthetica **51**: 531-540, 2013.

Muller O., Hirose T., Werger M.J.A. *et al.*: Optimal use of leaf nitrogen explains seasonal changes in leaf nitrogen content of an understorey evergreen shrub. – Ann. Bot.-London **108**: 529-536, 2011.

Munnè-Bosch S., Alegre L.: Changes in carotenoids, tocopherols and diterpens during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. – Planta **210**: 925-931, 2000.

Munson A.D., Margolis H.A., Brand D.G.: Seasonal nutrient dynamics in white pine and white spruce in response to environmental manipulation. – Tree Physiol. **15**: 141-149, 1995.

Nambiar E.K.S., Fife D.N.: Growth and nutrients retranslocation in needles of Radiata pine in relation to nitrogen supply. – Ann. Bot. **60**: 147-156, 1987.

Nambiar E.K.S., Fife D.N.: Nutrient retranslocation in temperate conifers. – Tree Physiol. **9**: 185-207, 1991.

Niinemets Ü.: A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. – Ecol. Res. **25**: 693-714, 2010.

Niinemets Ü.: Distribution patterns of foliar carbon and nitrogen as affected by tree dimensions and relative light conditions in the canopy of *Picea abies*. – Trees **11**: 144-154, 1997.

Nitta I., Ohsawa M.: Leaf dynamics and shoot phenology of eleven warm-temperate evergreen broad-leaves tree near their northern limit in central Japan. – Plant Ecol. **130**: 71-88, 1997.

Öquist G., Huner N.P.A.: Photosynthesis of overwintering evergreen plants. – Annu. Rev. Plant Biol. **54**: 329-55, 2003.

Orgeas J., Ourcival J.-M., Bonin G.: Seasonal and spatial patterns of foliar nutrients in cork oak (*Quercus suber* L.) growing on siliceous soils in Provence (France). – Plant Ecol. **164**: 201-211, 2002.

Ottander C., Campbell D., Öquist G.: Seasonal changes in photosystem II organization and pigment composition in *Pinus sylvestris*. – Planta **197**: 176-183, 1995.

R Development Core Team: R: A language and environment for statistical computing. Pp. 3411. R Foundation for Statistical Computing, Vienna 2012.

Reich P.B., Walters M.B., Ellsworth D.S.: From tropics to tundra: Global convergence in plant functioning. – P. Natl. Acad. Sci. USA **94**: 13730-13734, 1997.

Saur E., Nambiar E.K.S., Fife D.N.: Foliar retranslocation in *Eucalyptus globulus*. – Tree Physiol. **20**: 1105-1112, 2000.

Selaya N.G., Anten N.P.K.: Leaves of pioneer and later-successional trees have similar lifetime carbon gain in tropical secondary forest. – Ecology **91**: 1102-1113, 2010.

Senser M., Schötz F., Beck E.: Seasonal changes in structure and function of spruce chloroplasts. – Planta **126**: 1-10, 1975.

Shaddick G., Wakefield J.: Modelling daily multivariate pollutant data at multiple sites. – J. Roy. Stat. Soc. C-App.: **51**: 351-372, 2002.

Silla F., Escudero A.: Uptake, demand and internal cycling of nitrogen in saplings of Mediterranean *Quercus* species. – Oecologia **136**: 28-36, 2003.

Spiegelhalter D., Thomas A., Best N. *et al.*: WinBUGS User Manual. Version 1.4. Pp. 60. MRC Biostatistics Unit, Cambridge 2003.

Šesták Z., Čatský J.: Intensity of photosynthesis and chlorophyll content as related to leaf age in *Nicotiana sanderae* hort. – Biol. Plantarum **4**: 131-140, 1962.

Takashima T., Hikosaka K., Hirose T.: Photosynthesis or persistence: nitrogen allocation in leaves of evergreen and deciduous *Quercus* species. – Plant Cell Environ. **27**: 1047-1054, 2004.

Umeki K., Kikuzawa K., Sterck F.J.: Influence of foliar phenology and shoot inclination on annual photosynthetic gain in individual beech saplings: A functional-structural modeling approach. – Forest Ecol. Manag. **259**: 2141-2150, 2010.

Wendler R., Carvalho P.O., Pereira J.S. *et al.*: Role of nitrogen remobilization from old leaves for new *Eucalyptus globulus* seedlings. – Tree Physiol. **15**: 679-683, 1995.

Wright I.J., Reich P.B., Westoby M. *et al.*: The worldwide leaf economics spectrum. – Nature **428**: 821-827, 2004.

Yasumura Y., Ishida A.: Temporal variation in leaf nitrogen partitioning of a broad-leaved evergreen tree, *Quercus myrsinaefolia*. – J. Plant Res. **124**: 115-123, 2011.

Ye M., Jiang L., Mao K. *et al.*: Functional mapping of seasonal transition in perennial plants. – Brief. Bioinform. doi:10.1093/bib/bbu025, 2014.

Appendix 1. Details of Bayesian inference for the model of temporal change in foliar nitrogen content

The expected value of nitrogen content (y_{ijk}) for the k^{th} measurement on the j^{th} leaf in the i^{th} age class was assumed to be a function including annual linear trends, seasonal fluctuations, and a leaf-specific random effect,

$$y_{\mathrm{ijk}} = \alpha_{\mathrm{i}} + \beta_{\mathrm{i}} \cdot A_{\mathrm{ijk}} + \gamma_{\mathrm{il}} + R_{\mathrm{leaf\,j}} \qquad \text{(A1)}$$

where A_{ijk} is leaf age in days, γ_{ij} is a temporally autocorrelated random effect representing seasonal fluctuation, and $R_{\mathrm{leaf\,j}}$ is a random effect for the j^{th} leaf. We assumed the observed values of foliar nitrogen content (Y_{ijk}) followed a normal distribution as $Y_{\mathrm{ijk}} \sim \mathrm{N}(y_{\mathrm{ij}k}, \sigma_{\mathrm{y}}^2)$, where σ_{y}^2 is variance. We assumed that the prior of the precision of the normal distribution

($1/\sigma_y^2$) was noninformative as $1/\sigma_y^2 \sim \text{Gamma}(10^{-4}, 10^{-4})$, where $\text{Gamma}(a_g, b_g)$ represents a gamma distribution with shape parameter a_g and rate parameter b_g. We assumed that the model parameters α_i and β_i had hierarchical priors as $x_i \sim N(\mu_x, \sigma_x^2)$, where x_i represents α_i or β_i, and μ_x and $\sigma_x^2$are hyperparameters. We assumed that the priors for μ_x and $1/\sigma_x^2$ were noninformative as $\mu_x \sim N(0, 10^6)$ and $1/\sigma_x^2 \sim \text{Gamma}(10^{-4}, 10^{-4})$.

Similarly, we assumed that the random effect $R_{\text{leaf j}}$ had a hierarchical prior as $R_{\text{leaf j}} \sim N(\mu_{\text{leaf}}, \sigma_{\text{leaf}}^2)$, $\mu_{\text{leaf}} \sim N(0, 10^6)$, and $1/\sigma_{\text{leaf}}^2 \sim \text{Gamma}(10^{-4}, 10^{-4})$.We assumed that the reciprocals of hyperparameters ($1/\sigma^2_{\gamma\,i}$; $\sigma^2_{\gamma\,i}$ are age-class-specific conditional variances for a temporally autocorrelated random effect, γ_{il}) for the temporally autocorrelated random effects (γ_{il}) had noninformative priors: $1/\sigma^2_{\gamma\,i} \sim \text{Gamma}(10^{-4}, 10^{-4})$.

Sampling from the posterior distribution of all parameters was performed using Markov chain Monte Carlo (MCMC) simulations with *WinBUGS* software (Spiegelhalter *et al.* 2003). Three independent MCMC chains were run and 50,000 samples were recorded after a burn-in of 1,000. The chains were thinned every 147 runs, yielding independent samples from a posterior of size 1,000. For each model parameter, a mean value was calculated from the sampled values and used as the estimated values for the parameter.

Appendix 2. Determination of leaf maturation date

To determine the leaf maturation date, we modeled the temporal change in chlorophyll or nitrogen contents in the current-year leaves (Fig. A1) with a Bayesian approach and calculated the date when chlorophyll or nitrogen contents reached 90% of the asymptotic maximum value.

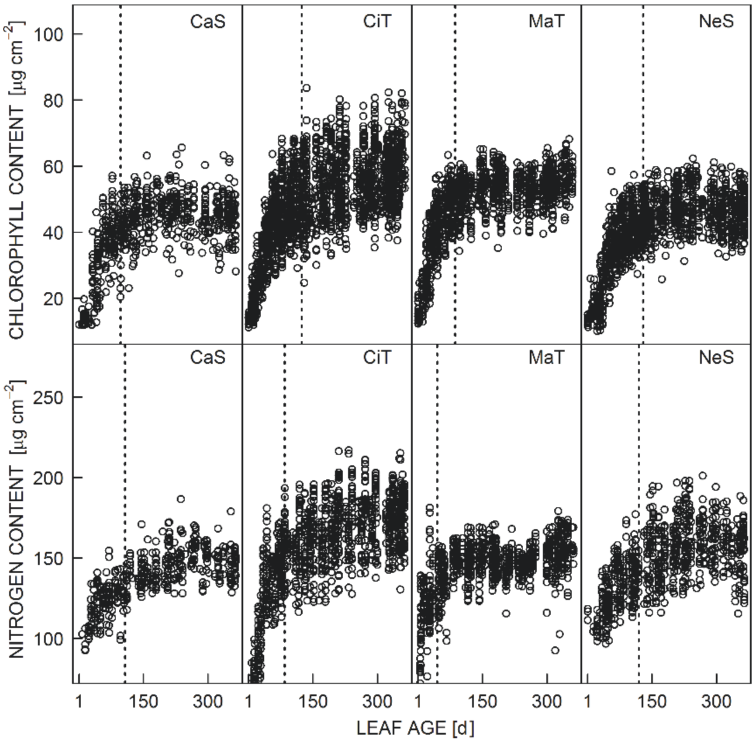

Fig. A1. Relationships between leaf age and chlorophyll and nitrogen contents of current-year leaves. *Vertical dotted lines* show average leaf maturation ages. CaS – *Castanopsis sieboldii*; CiT – *Cinnamomum tenuifolium*; MaT – *Machilus thunbergii*; NeS – *Neolitsea sericea.*

The expected value of the chlorophyll or nitrogen content (y) was assumed to be a function of leaf age in days with an asymptotic maximum,

$$y = a - b \cdot e^{-c \cdot A} \tag{A2}$$

where A is leaf age in days, a is a model parameter for an asymptotic maximum value, and b and c are coefficients. The observed values for the chlorophyll or nitrogen content (Y) were assumed to follow a normal distribution as $Y \sim N(y, \sigma^2)$, where σ^2 is a variance. We assumed that the prior for the precision of the normal distribution ($1/\sigma^2$) was noninformative as $1/\sigma^2 \sim \text{Gamma}(10^{-4}, 10^{-4})$. We also assumed that the priors for the model parameters a, b, and c were noninformative as $x \sim N(0, 10^6)$, where x represents a, b, or c.

Sampling from the posterior distribution of all parameters was performed using Markov chain Monte Carlo (MCMC) simulations with the *WinBUGS* software (Spiegelhalter *et al.* 2003). Three independent MCMC chains were run and 10,000 samples were recorded after a burn-in of 1,000. The chains were thinned every 27 runs, yielding independent samples from a posterior of size 1,000. For each model parameter, a mean value was calculated from the sampled values and used as the estimated values for the parameter.

The above Bayesian inference was conducted for each combination of four species and two nutrients (*i.e*., chlorophyll and nitrogen). We then calculated leaf age and a corresponding date that gave 90% of the asymptotic maximum value of the chlorophyll or nitrogen content and used that as the date of leaf maturation.