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RESEARCH PAPER

Variation of SPAD values in uneven-aged leaves of different dominant species in *Castanopsis carlessi* forest in Lingshishan National Forest Park

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Abstract: The greenness (SPAD) of uneven-aged leaves of dominant species in the *Castanopsis carlessi* forest at different altitude gradients in Lingshishan National Forest Park, Fujian Province, China were measured by using portable chlorophyll meter SPAD-502. In addition, the correlation between SPAD value and the concentration of chlorophyll and foliar nitrogen was also investigated. Significant variations in SPAD values were found between the uneven-aged leaves of different dominant species and different altitude gradients. Regression analysis showed that SPAD value was significantly correlated with the concentration of chlorophyll and the content of foliar nitrogen, indicating that SPAD value could be indicators for foliar chlorophyll and nitrogen. It is suggested that SPAD meter is a useful tool for forest assessments in decision-making and operational nutrient management programs.

Keywords: *Castanopsis carlessi* forest; chlorophyll content; chlorophyll meter; dominant population; forest nutrient of nitrogen; Lingshishan; specific leaf area

Introduction

Chlorophyll plays a very important role in the trapping of solar energy for plant photosynthesis. The measurement of chlorophyll concentration is an important aspect for determine photosynthetic characteristics. Currently the methodologies of chlorophyll extraction in plant materials are mainly based upon the organic solvent extraction method of leaf tissue such as acetone and methanol. After extraction, the content of chlorophyll can be analyzed with spectrophotometer. This method is simple and accurate, but it has defect with the destructive and time consuming (Manetas et al. 1998).

Chlorophyll meter (or SPAD meter) is a simple, portable diagnostic tool for measurement of greenness or relative chlorophyll concentration of leaves. The meter makes instantaneous and non-destructive readings on a plant according to the quantification of light intensity (peak wavelength: approximately 650 nm: red LED) absorbed by the tissue sample. A second peak (peak wavelength: approximately 940 nm: infrared LED) is emitted simultaneously with red LED to compensate the leaf thickness (Netto et al. 2005). Compared with the traditional destructive methods, this equipment might provide a substantial saving in time, space and resources (Netto et al. 2005; Chang & Robison 2003).

The SPAD readings (or the leaf greenness) are positively correlated with the content of chlorophyll and they provide reliable estimate to the level of chlorophyll (Tobias et al. 1994). The variation of the chlorophyll concentration and the relationship of chlorophyll concentration with SPAD readings are important indicators of plant change with age. Chang & Robison (2003) reported that there was a close link between leaf chlorophyll concentration and foliar nitrogen (N), which makes sense because the majority of foliar N is contained in chlorophyll molecules. SPAD meters could be used as an indirect and rapid method for detecting foliar N status. Since the chlorophyll meter has the potential to detect N status, it also shows promise as a tool for improving N management (Chapman & Barreto 1997). Furthermore, as a in situ and nondestructive method, the SPAD meters can be used to carried out dynamic survey of leaf green. It has certain superiority, especially in the field experiment and is a rapid measuring way that worth spreading (HRC 2000).

The SPAD meter was initially developed in Japan to diagnose foliar N status and to determine N fertilizer (Chubachi et al. 1986). Currently it has been used for the chlorophyll or foliar N test of rice, wheat, maize, cotton, and other crops. With few exceptions, most of the publications report good utility of the SPAD meter for testing and predicting chlorophyll and foliar N concentration (Chapman et al. 1997). The applications of SPAD

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meter in measuring leaf greenness or chlorophyll concentration in crops were well reported. Also, quite a few of reports on application of SPAD meter in the field of forestry could be found (Chang & Robison 2003; Close et al. 2004; Jiang et al. 2005; Wang et al. 2006; Harrisa et al. 2008; Pinkard et al. 2006). For example, Jang et al (2005) studied the greenness (SPAD) of four broad-leaved tree species under larch (*Larix gmelinii*) plantations using SPAD-502 meter; and Wang Ting et al. (2006) explored the relation between content of nitrogen and SPAD readings in leaves of bamboo.

The relationship between SPAD readings and the concentration of chlorophyll and foliar N is affected by many factors, including species, leaf thickness, specific leaf area, leaf water content, leaf age, and geographical environment of plot for experiment (Chang & Robison 2003; Richardson et al. 2002; Sandoval-Villa et al. 2002). Research has documented that SPAD readings are positively correlated with the content of chlorophyll or foliar N in many single species. However, no available information could be found on the application of SPAD meter in population and in different aged leaves.

In the present study, we investigated the variation of uneven-aged leaf SPAD readings of different dominant species and the correlation between SPAD and the concentration of chlorophyll and foliar N of dominant populations in *Castanopsis carlessi* forest at different altitude gradients in Lingshishan National Forest Park, with the objectives to extend the application scope of SPAD meter and to discuss the utility of SPAD meter when the species and environment are not homogeneous.

Materials and methods

Site description

The study was conducted in Lingshishan National Forest Park, located at Western Fuqing (25°40′ S, 119°13′ E, a.s.l about 100 m to 900 m), Fujian Province, China. The park covers a land area of 2 275 ha and its forest coverage is 93.5%. The study area has a maritime monsoon climate, with mean monthly temperature from 7°C in January to 29°C in June and a mean annual temperature of 19.7°C. The average annual sunshine time is about 2 000 h. There is almost no frost day through the year. Precipitation is mainly concentrated from March to June, with an annual mean of 1 780 mm. The relative humidity is generally high throughout the year, with an average annual humidity of 86%. The water in the park is crystal clear and the brooks among the mountains are perennial. The dominant soil types are granite latosol with yellow-red soil above the elevation of 600 m, with deep soil and humus layers. Subtropical evergreen broadleaf forests as the main vegetation type in the park are distributed at low elevation and mountain coppice at high elevation.

Sampling methods

By using GPS location technology, we arranged nine altitude gradients within the distribution scope of *C. carlessi* forests. They were marked as A1 (157 m), A2 (200 m), A3 (242 m), A4 (332 m), A5 (442 m), A6 (531 m), A7 (632 m), A8 (762 m), and A9 (842 m). At each altitude gradient, one or two sampling plots of 20 m × 20 m were selected for dominant species and the plot was divided into 16 subplots of 5 m × 5 m. Diameter at breast height, tree height, crown size of arborous layer in each subplot were measured. We calculated the importance value for each species and selected the dominant species according to the importance values. All the dominant species from the elevation of A1 to A9 are *C. carlessi*, *M. minkweiensis*, *S. octohylla*, *S. superba*, *E. hebeclados, E. Korthals*, *C. fabri Hance*, *Rh. simisii Planch*, *P. sychotria rubra*, *A. quinquegona, P. clypearia, S. buxifolium, C. glauca, R. neriifolia*, *L. aggregata Kosterm*, *C. oleifera Abel, L. glabra, B. cochinchinensis*, *S. laurinum*, *L. megaphylla, I. elmrrilliana*, *T. dubia Ohwi*. The dominant population at each elevation gradient was not totally the same. Three standard trees were selected. Their DBH were equal to the average of the dominant species. Branches were collected from the middle and upper canopy in four orientations. One- and two-year-old leaves were identified by the leaf scar scale, and the SPAD readings were measured *in situ*. Ten measurements per leaf were immediately taken and averaged to provide a single SPAD reading. The SPAD sensor was placed randomly on leaf mesophyll tissue only, with veins avoid.

After measurement, the leaves were immediately placed into plastic bags and put into an insulated icebox full of ice. The leaves were frozen at -4°C within an hour of sampling and moved to -20°C within a day. Chlorophyll was extracted within two weeks of sample collection using the acetone method (Pinkard 2006). Chlorophyll suspensions were kept on ice in the dark between processing steps.

Leaf tissue from the study site was dried at 65°C to constant weight, and grounded in a hammer mill. Then the concentration of foliar N was measured by using the method of indophenol blue colorimetric. The specific leaf area (SLA) is the ratio of leaf dry weight and leaf area (Li et al. 2005). The concentration of chlorophyll and foliar N per unit-area was calculated by the concentration of per unit-weight and SLA.

Statistic analysis

Statistical analysis was performed using SPSS 13.0 software. Double Factorial Analysis of Variance was performed to determine significant difference for the SPAD values at $p<0.05$ level between different dominant species and between uneven-aged leaves. The variations of SPAD values between different dominant species and between different altitude gradients were analyzed by Double Factorial Analysis of Variance. Regression was used to analyze the correlation between SPAD value and the concentration of chlorophyll and foliar N.

Results and analysis

The variation of SPAD values of uneven-aged leaves

The variation of SPAD values between uneven-aged leaves and between different dominant species at each altitude gradient was analyzed. The results show that there were significant differences between different dominant species at all altitude gradients except for at altitude of A2, A3, and A6 (Table 1). Leaf age also had significant effect on the SPAD values. As indicated in Table 1, except for at altitude gradients of A3 and A6, the variation between different aged leaves reached the significant level at all altitude gradients. The variation of all 22 dominant species at nine altitude gradients indicated that there were significant differences in the variation between different dominant species and between uneven-aged leaves (Table 1).

Table 1. Variation of SPAD value between different dominant populations and between different aged leaves

Altitude	Factors	df	MS	F	*p*	Altitude	Factors	df	MS	F	*p*
A1	S	9	40.301	6.724	0.005	A2	S	5	10.199	1.675	NS
	L	1	40.146	9.625	0.005		L	1	41.070	6.744	0.048
A3	S	8	13.764	1.348	NS	A4	S	3	46.593	7.155	0.070
	L	1	50.167	4.921	NS		L	1	186.245	28.602	0.013
A5	S	10	65.162	4.307	0.015	A6	S	7	33.208	1.577	NS
	L	1	386.402	25.539	<0.001		L	1	103.023	4.892	NS
A7	S	8	133.706	33.607	<0.001	A8	S	3	145.577	17.607	0.021
	L	1	60.867	25.299	0.004		L	1	93.845	11.350	0.043
A9	S	4	120.394	111.115	<0.001	A*t*	S	21	168.894	6.705	<0.001
	L	1	22.801	21.044	0.010		L	1	788.659	31.318	<0.001

Note: A1 (157 m), A2 (200 m), A3 (242 m), A4 (332 m), A5 (442 m), A6 (531 m), A7 (632 m), A8 (762 m), and A9 (842 m); A*t* is the integration of data at all altitude gradients; S, dominant species; L, the leaf age.

High co-dominant species, *C. carlessi*, M. minkweiensis, *S. octohylla* at altitude of A2, A3, A6, and A7, were selected to make variance analysis between different altitude gradients. Variance analysis shows that significant differences in the SPAD readings existed between different altitudes (Table 2).

Table 2. Variation of SPAD value between different altitude gradients of the high co-dominant species

Leaf age	df	MS	F	*p*
one-year-old leaves	3	57.450	2.495	0.040
two-year-old leaves	3	81.796	1.953	0.045

Correlation between SPAD values and concentration of chlorophyll per unit-area (Chl_{area})

Correlative analysis showed that there were no correlation between SPAD values and the concentration of chlorophyll per unit-mass. The results are in agreement with some previous studies (Chapman & Barreto 1997; Harrisa 2008; Pinkard 2006; Wang 2006; Wu 1998; Peng 1995). Yet correlation analysis showed that Chl_{area} increased linearly with SPAD values. Regression analysis showed that the linear model best fitted the relationship between SPAD values and Chl_{area} (Table 3), which is in agreement with the results of previous studies (Chang & Robison 2003; Pinkard 2006; Close 2004). SPAD values were significantly positively correlated with Chl_{area} for one- and two-year-old leaves at all altitude gradients, except for the two-year-old leaves at the altitude of A4 and one-year-old leaves at the altitude of A6. Similar relationship was also shown in one- and two-year-old leaves of all the dominant species at the altitude of A1 to A9 (Fig. 1a). At the same altitude gradient, the different regression equations were given for uneven-aged leaves. Judging by the value of R and p, the correlation between the SPAD values and Chl_{area} in two-year-old leaves was higher than the leaves in one-year-old at altitude of A1 to A3. On the contrary, the correlation in one-year-old leaves was higher at altitude of A4 to A9. This may be caused by the leaf thickness and moisture as these factors could affect transmission of light-wave in leaves (Chang & Robison 2003; Peng 1995).

Table 3. Summary of linear regression parameters between SPAD value and chlorophyll content of different age leaves

H	One-year-old leaves				H	Two-year-old leaves			
	Equations	R	p	n		Equations	R	p	n
A1	Chl_{area}=6.9374*SPAD-262.9	0.6762	0.032	10	A1	Chl_{area}=10.409* SPAD-454.6	0.8718	0.001	10
A2	Chl_{area}=4.4285* SPAD-454.6	0.9306	0.007	6	A2	Chl_{area}=9.6635* SPAD-330.6	0.9517	0.001	6
A3	Chl_{area}=10.382* SPAD-418.3	0.8743	0.002	9	A3	Chl_{area}=8.5931* SPAD-345.8	0.8912	0.007	9
A4	Chl_{area}=3.7849* SPAD-132.0	0.946	0.050	6	A4	Chl_{area}=3.0561* SPAD-98.6	0.8793	NS	6
A5	Chl_{area}=4.3556* SPAD-97.2	0.9178	<0.001	6	A5	Chl_{area}=5.8617* SPAD-184.3	0.8326	0.001	6
A6	Chl_{area}=4.8276* SPAD-156.9	0.6267	NS	11	A6	Chl_{area}=10.71* SPAD-526.94	0.8691	0.005	11
A7	Chl_{area}=6.5638* SPAD-220.7	0.9398	<0.001	9	A7	Chl_{area}=7.2155* SPAD-257.8	0.9078	0.001	9
A8	Chl_{area}=4.9305* SPAD-139.9	0.998	<0.001	7	A8	Chl_{area}=3.4632* SPAD-78.2	0.9498	0.05	7
A9	Chl_{area}=4.4424* SPAD-125.1	0.9853	0.002	6	A9	Chl_{area}=5.4459* SPAD-166.0	0.9447	0.015	6
At	Chl_{area}=4.325* SPAD-116.7	0.7423	<0.001	70	At	Chl_{area}=4.7911* SPAD-144.5	0.6830	<0.001	70

Correlation between SPAD values and concentration of foliar nitrogen per unit-area (N_{area})

The correlation analysis (Table 4) indicated that there was significant positive correlation between SPAD values and N_{area} for one- and two-year-old leaves at all altitude gradients, except for two-year-old leaves at the altitude of A2 and A8, and one-year-old leaves at the altitude of A6 and A8. Significant positive correlation was also presented in the leaves of all the dominant species at the altitude of A1 to A9 (Fig. 1b). The correlation for one-year-old leaves was slightly higher than that for two-year-old leaves. We presumed that the correlation difference between different aged leaves might be affected by an important factor. Thus we should take care in selecting the leaves with same age for measurement.

The correlation shown in Table 3 and Table 4 between SPAD value and chlorophyll, nitrogen content per unit leaf area of all dominant species at altitude gradients of A*t* was presented in Fig. 1 to discuss whether the factor of leaf age impact the correlation.

Table 4. Summary of linear regression parameters between SPAD values and nitrogen content of different age leaves

H	One-year-old leaves				H	Two-year-old leaves			
	Equations	R	p	n		Equations	R	p	n
A1	N_{area}=0.0580*SPAD-2.09	0.7135	0.031	10	A1	N_{area} =0.0657* SPAD-2.634	0.6707	0.048	10
A2	N_{area}=0.0768* SPAD-2.27	0.4293	NS	6	A2	N_{area}=0.0832* SPAD+8.696	0.0721	NS	6
A3	N_{area}=0.1949* SPAD-8.466	0.8180	0.007	9	A3	N_{area} =0.0326* SPAD-0.906	0.7338	0.038	9
A4	N_{area}=0.0749* SPAD-2.851	0.9355	NS	6	A4	N_{area} =0.0275* SPAD-0.386	0.963	0.037	6
A5	N_{area} =0.0271* SPAD-0.418	0.6851	0.049	6	A5	N_{area} =0.0211* SPAD-0.287	0.6329	0.05	6
A6	N_{area} =0.0359* SPAD-1.124	0.8099	0.008	11	A6	N_{area} =0.0231* SPAD-0.429	05897	0.044	11
A7	N_{area} =0.0425* SPAD-1.149	0.9188	<0.001	9	A7	N_{area} =0.0256* SPAD-0.482	0.8398	0.005	9
A8	N_{area} =0.0300* SPAD-0.549	0.9052	0.035	7	A8	N_{area}=0.02795*SPAD-0.540	0.8509	NS	7
A9	N_{area} =0.0461* SPAD-1.151	0.9153	0.029	6	A9	N_{area} =0.0451* SPAD-1.052	0.9084	0.033	6
At	N_{area} =0.0301* SPAD-0.599	0.6886	<0.001	70	At	N_{area} =0.0244* SPAD-0.420	0.6670	<0.001	70

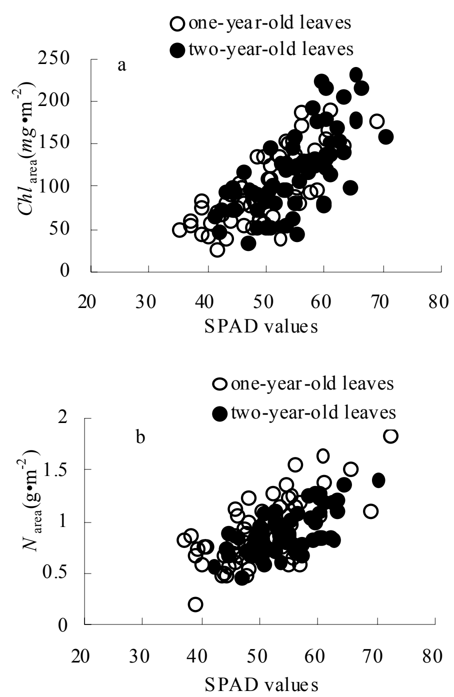

Fig. 1 The correlation between SPAD value and chlorophyll, foliar nitrogen content per unit leaf area of uneven- aged leaves. Fig. 1a was the correlation between SPAD value and the content of chlorophyll unit area. Fig. 1b was the correlation between SPAD value and content of nitrogen unit area of all dominant species at altitude gradients of A1-A9.

Conclusion and discussion

In this experiment, significant difference in SPAD values were found between different dominant species and between different aged leaves. Environmental factors are known to affect leaf morphology, which in turn affects foliar optical properties, and can be expected to affect SPAD values (Pinkard 2006). We also documented that altitude gradient, an important environmental factor, could affect SPAD values for the same dominant species (Table 1). The SPAD values were highly correlated with Chl_{area} and N_{area} for dominant population at each altitude elevation. Maybe the dominant species had formed similar ecological adaptation strategy in the same environment, despite some studies have shown that the correlation was related with gene of species (Chang & Robison 2003; Pinkard 2006; Sandoval-Villa 2002; Thompson 1996; Kim 2002). Thus we concluded that the SPAD meters provided good estimates of chlorophyll and foliar N status for the dominant population in this experiment. It is not just suitable for a single species but a population.

The relationships between SPAD values and Chl_{area}, N_{area} showed that SLA was expected to affect absorbance by the leaf of two wavelengths of light (660 nm and 940 nm), which could account for the SPAD values. SLA, moisture content, and leaf optical properties are likely to be related to differences in leaf age; thus there was difference for uneven-aged leaves in the relationship. Chang et al. (2003) concluded that incorporating moisture content could improve the correlation. Therefore calibrations of the SPAD meter may be needed to help account for site variation, tree vigor, and the range of N concentration.

The relationship between the SPAD values and Chl_{area} shows that the SPAD values can reflect the status of chlorophyll concentration and the SPAD meter is superior to the conventional spectrophotometry in the field investigation and research. This method allows measurement of chlorophyll on the same leaf over time. Furthermore, the research related to photosynthesis should be carried out together.

SPAD meters could assist to diagnose the status of foliar N in field and provide basic data for analysis of N nutrition. From the SPAD values, we could also judge the abundance or scarcity of N nutrition. For example, we can conclude that less than 40 of SPAD values denotes the shortage of nitrogen nutrition, and more than 70, the nitrogen nutrition is in abundance. As the SPAD values are between 40 and 70, the supply of nitrogen nutrition is in normal (Fig. 1b). However accurate prediction of N concentration by the SPAD meter may be difficult; perhaps more complicated equations are needed. After calibrated, the SPAD meters can be used for the estimation of N status over time or the species in the similar stand. What important is, the SPAD meters have efficient application for field nutrient assessments and forest management for hardwood species (Chang & Robison 2003; Pinkard 2006). The correlation of SPAD values and foliar N is related with the range of nitrogen content. For example, foliar N concentration of *P. clypearia* at some altitude gradient was more than 30 $mg \cdot g^{-1}$ per unit mass, and the content unit area ranged from 1.5 $g \cdot m^{-2}$ to 2 $g \cdot m^{-2}$, leading to no significant correlation. Therefore, when analyzed, we eliminated some data of *P. clypearia* foliar N. Pinkard (2006) found that foliar N content was not highly correlated with SPAD values in *E. nitens* and *E. globulus* because *Eucalyptus* often stores N at levels greater than the physiological requirements for photosynthesis (Niinemets 2003), which is in agreement with our results.,

SPAD meter is a potential tool for the time-consuming diagnosis of nutrient and more detailed forest management. It is somewhat limited by the design as the area of the chamber measuring light transmittance on the meter is only 2 mm × 3 mm. The meter is sensitive to many factors such as leaf veins, leaf thickness and moisture content, thus multiple measurements for the same leaf is needed. Modifications such as increasing the area of the chamber of the SPAD meter may be needed to make the instrument more applicable for hardwood species. Another restriction is that SPAD meter is not suitable for the application in conifers yet.

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