Botanical Studies (2007) 48: 71-77.

Leaf chlorophyll content and surface spectral reflectance of tree species along a terrain gradient in Taiwan's Kenting National Park

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(Received October 21, 2005; Accepted June 27, 2006)

ABSTRACT. This study was conducted to investigate variations of leaf chlorophyll content and surface spectral reflectance of different tree species across contrasting terrain in the Nanjenshan Reserve of Kenting National Park, southern Taiwan. Tree species composition and forest types vary because of intense northeast monsoons that frequent this area. In this study, we used several remote sensing technique indices—normalized difference vegetation index (NDVI), modified normalized difference vegetation index (mNDVI), simple ratio (SR), and modified simple ratio (mSR)—to analyze the spectral reflectance data collected from portable spectroradiometers and the GER 1500 and CM1000 chlorophyll meters to estimate leaf chlorophyll content. The results showed that significant differences ($P<0.01$) arose only among the modified indices mSR 705 nm and mNDVI 705 nm. The index mNDVI705 seemed more sensitive to detecting chlorophyll content in a wide range of tree species across a terrain. Among the indices tested, the mNDVI consistently deviated from the general relationship between chlorophyll content and spectral reflection in different vegetation. The findings indicated that the modified indices were better at studying different tree species than normalized indices across terrain.

Keywords: Leaf chlorophyll; mNDVI; Portable spectroradiometers; Spectral reflectance; Vegetation index.

INTRODUCTION

In recent decades, the development of remote sensing methods to measure leaf chlorophyll content and surface spectral reflectance has received much attention, since variations in leaf chlorophyll content can provide information concerning the physiological state of a leaf or plant. Several vegetation indices estimated from remote sensing data have been considered for assessing the status of leaf chlorophyll content, plant biomass, production, and vegetation health status. Destructive methods of leaf chlorophyll content quantification include traditional methods using extraction and spectrophotometric or HPLC measurement, but they are considered time consuming and expensive. In contrast, spectral reflectance measurements are nondestructive, rapid, and can be applied across spatial scales (Gamon and Qiu, 1999). Many theoretical models have been developed for predicting leaf reflectance from leaf chlorophyll, plant water content, and vegetation structure variables (Dawson et al., 1998; Jacquemoud et al., 1996). Without such extrapolation procedures, it would be impossible to make landscale and ecosystem assessments from leaf level analysis. Most large scale research projects are now using remotely sensed data to estimate the condition of ecosystems. However, most relationships between leaf reflectance and chlorophyll contents have been derived empirically derived.

Sims and Gamon (2002) have reported that spectral indices provide relatively poor correlations with leaf chlorophyll content when applied across a wide range of species and plant functional types. They, therefore, modified some vegetation indices to demonstrate the application of spectral indices on species with widely varying leaf structure. This strategy was applied in this study and served as an impetus to further analyze leaf chlorophyll content and surface spectral reflectance in a wider range of vegetation, using modify vegetation indices mNDVI (modify normalized difference) and mSR (modify simple ratio) as test parameters. Normal

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vegetation indices are normally applied when investigating vegetative differences among similar species, whereas modified indices are specially designed to investigate different species (Sims and Gamon, 2002).

Theoretically, terrain difference affects the growth of vegetation and determines the chlorophyll content and surface spectral reflectance. Our objective in this study was to demonstrate how different leaf chlorophyll content and surface spectral reflectance characteristics are affected by terrain divergence in Kenting National Park, Taiwan.

MATERIALS AND METHODS

Study area

The experiment was conducted on site at the Nanjenshan Reserve of Kenting National Park, southern Taiwan (Figure 1). In this area, forest vegetation distribution is influenced by three major topographic positions or terrains; windward slopes, valley and leeward slopes (Hsieh and Hsieh, 1990). In the study area, the winter precipitation and the intensity of northeast winds

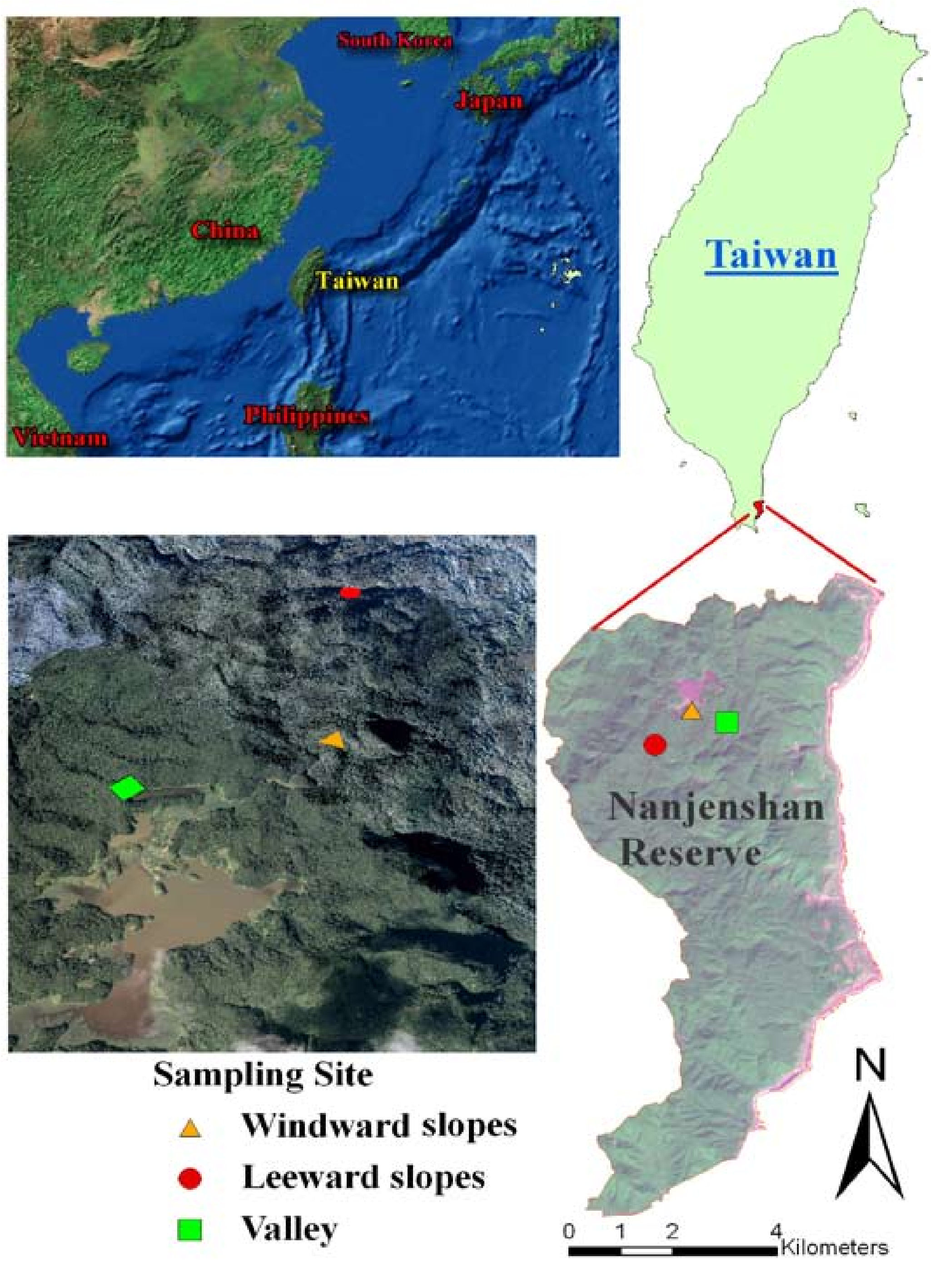

Figure 1. Location of the study sites of Nanjenshan Reserve of Kenting National Park, southern Taiwan.

were found to correlate with the differentiation of forest types. Those in the northeast district are evergreen since the wind is rather, whereas, the thorny scrubs and deciduous scrubs appear in the southwest district due to severe dry winds. The high species diversity of Kenting National Park is largely attributable to the heterogeneous environment (Su and Su, 1988).

Leaf sampling

Measurement of leaf spectral reflectance was obtained by randomly harvesting leaves from 20 tree species from the three types of terrain. From each terrain type, one sampling site from the leeward area and two sampling sites from the windward and valley areas, respectively, were selected. Fifty sample leaves taken from three to nine dominant species were randomly collected from each sampling site. Sampling was done at the top of the canopies of each species and was carried out in late April 2005. Leaf samples were stored in plastic bags and kept cool for further analysis. The leaf samples collected are listed in Table 1.

Reflectance measurements

All spectral measurements were made with a field portable spectrometer (GER-1500, SVC, Poughkeepsie, NY, USA). GER-1500 has a nominal spectral range from 350 to 1050 nm with approximately 1.5 nm nominal bandwidth. Leaf reflectance was measured with a bifurcated fiber optic cable and the spectrometer collected both leaf reflect once and reference reflect once data. Leaf samples were illuminated by sunlight. All measurements were carried out in triplicate and all tests in this study were scaled to leaf level.

Quantification of chlorophyll

Leaf chlorophyll content was estimated by using a portable chlorophyll meter (CM-1000, Spectrum Tech., Plainfield, Ill. USA). CM-1000 readings were made in situ on the plants designated for harvesting, midway along the youngest fully expanded leaf. The Minolta SPAD 502 chlorophyll meter has become recognized as a reliable substitute for total chlorophyll (Ommen et al., 1999; Daughtry et al., 2000; Bauerle et al., 2004; Van den Berg and Perkins, 2004; Netto et al., 2005). However, the CM1000 chlorophyll meter was utilized and investigated as a potential substitute for the SPAD meter in applications with small leaves and where measurements were difficult to make (i.e., turf). Four tree species (*Michelia compressa*; *Psychotria rubra*; *Daphniphyllum glaucescens*; *Gordonia axillaris*) were selected as test examples of the relationship between the CM1000 chlorophyll meter and chlorophyll content measurements, and the method for monitoring chlorophyll content followed that described in Yang et al. (1998). As the test results indicated (Figure 2), the relationship between the CM1000 chlorophyll meter and chlorophyll content is strong, in that the larger the indices of the chlorophyll meter are, the higher is the total chlorophyll content. Therefore, the CM1000 was selected as the chlorophyll meter for use in this study.

Table 1. List of all the species used in this study[a].

Species	Terrain
Aucuba chinensis Benth.	L
Bischofia javanica	L
Castanopsis carlesii	L
Champereia manillana	L
Cleyera japonica Thumb. var. *japonica*	L
Daphniphyllum glaucescens Blume subsp. var. *oldhamii* (Hemsl.) Huang	L
Dendrocnide meyeniana	L
Gordonia axillaris	V
Ilex cochinchinensis	V
Illicium dunnianum	V
Machilus kusanoi	V
Michelia formosana	W
Neolitsea buisanensis	W
Neolitsea hiiranensis	W
Reevesia formosana Sprague	W
Schefflera octophylla	W
Syzygium euphlebium	W

[a] The leaf samples measured for each species of the different terrains (L= leeward; V= valley; W = windward).

Vegetation indices

The normalized difference vegetation index (NDVI) (Eqn. 1) (Tucker, 1979) and simple ratio (SR) (Eqn. 2) were used to calculate the vegetative indices obtained from spectral reflectance measurements:

$$NDVI_{705} = \frac{R_{750} - R_{705}}{R_{750} + R_{705}} \quad (1)$$

$$SR_{705} = \frac{R_{750}}{R_{705}} \quad (2)$$

where the wavelengths for NDVI and SR were 705 and 750 nm, respectively, and are based on the chlorophyll index developed by Gitelson and Merzlyak (1994). R_{705} and R_{750} are the leaf sample spectral reflectance from the GER-1500. Based on the results of Sims and Gamon (2002), SR and NDVI indices may be affected by different species leaf surfaces. They modified these two indices to compensate for high leaf surface reflectance, which tends to increase reflectance across the whole visible spectrum of a wide range of species. Adding a constant to all reflectance values reduces both SR and NDVI even when there is no change in the absorptance of tissues below

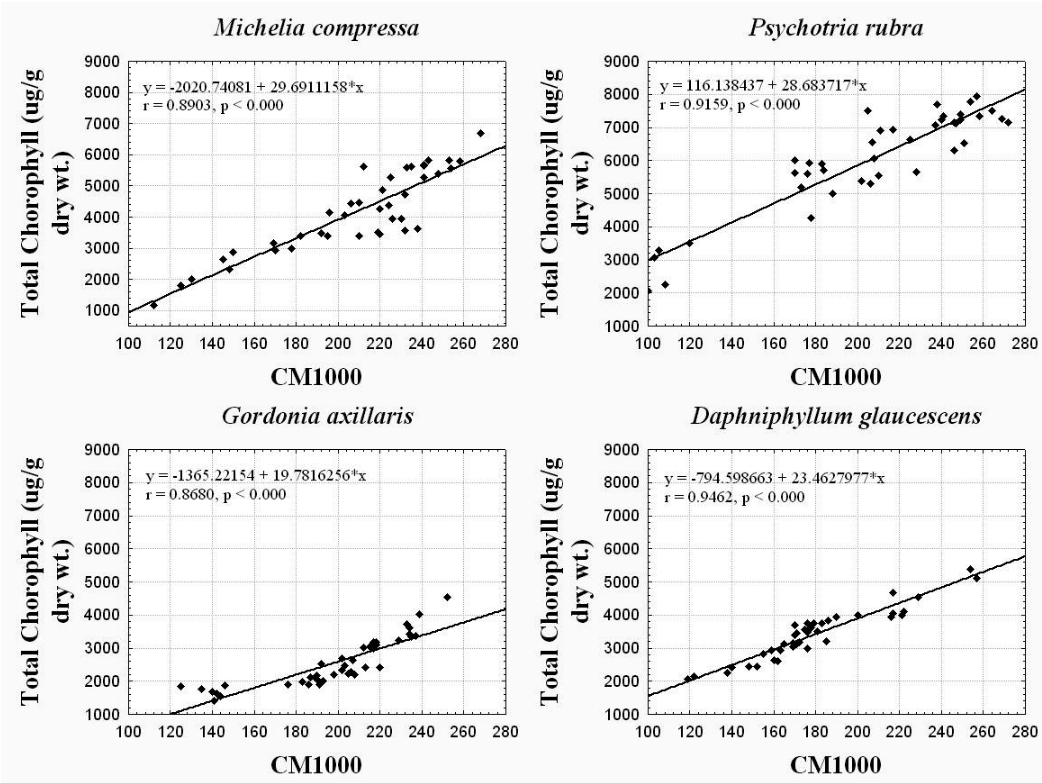

Figure 2. The relationship between leaf CM1000 meter readings and acetone extractable chlorophyll values measured from *Michelia compressa*, *Psychotria rubra*, *Gordonia axillaris* and *Daphniphyllum glaucescens*.

the epidermis. They chose R445 as a measure of surface reflectance and indicated that R_{445} is a good reference for all but the lowest chlorophyll content leaves. Besides, Le Maire et al. (2004) also pointed out that the two modified indices gave the best performance of universal broad leaf chlorophyll indices. The modified indices of NDVI and SR are as follows (Eqs. 3 and 4):

$$mND_{705} = \frac{R_{750} - R_{705}}{R_{750} + R_{705} - 2R_{445}} \qquad (3)$$

$$mSR_{705} = \frac{R_{750} - R_{445}}{R_{750} - R_{445}} \qquad (4)$$

Data analysis

The results of a Kolmogorov-Smirnov test (K-S test) for normality of the CM1000 index were matched with the normal distribution theory (Lilliefors, 1967). The indices collected from the CM1000 meter, when fitted to the normal distribution, all appeared to have the same index value of 173 for the mean, mode and median statistical parameters. Based on the above, we reclassified all the samples (N=1136) into three groups (low, middle and high) depending on the value of CM1000 indices. The three groups were classified by the percentage of the total samples 10%, 80% and 10%.

All statistical analyses were conducted using the STATISTICA statistical software (Version 6.1, StatSoft Inc. Tulsa, Oklahoma, USA, 2002). Coefficients of determination (R^2) were calculated for relationships between various chlorophyll content values from CM-1000 (independent variables) and mNDVI and mSR values from GER-1500 (dependent variable). To test and verify the relationship of chlorophyll content between mNDVI and mSR, regression analyses were used in the first data analysis step. For statistical reasons, we tried to find out the best index to indicate the differences between these four indices. The coefficient of variation (CV) was chosen. To analyze the incidence of the two modified indices, we used one way ANOVAs for each index, comparing mNDVI and mSR indices with the CM-1000 index and different terrains separately.

RESULTS AND DISCUSSION

Vegetation indices and chlorophyll content

Results for the regression analysis showed that all of the vegetation indices of this study positively correlated with the CM1000 index. The coefficients of determination (R^2) calculated for relationships between this index (independent variable) and the NDVI, SR, mNDVI and mSR indices (dependent variables) for all samples (n = 117) showed that mSR ($R^2 = 0.51$) was slightly superior to SR ($R^2 = 0.34$) in its correlation with the CM1000 index

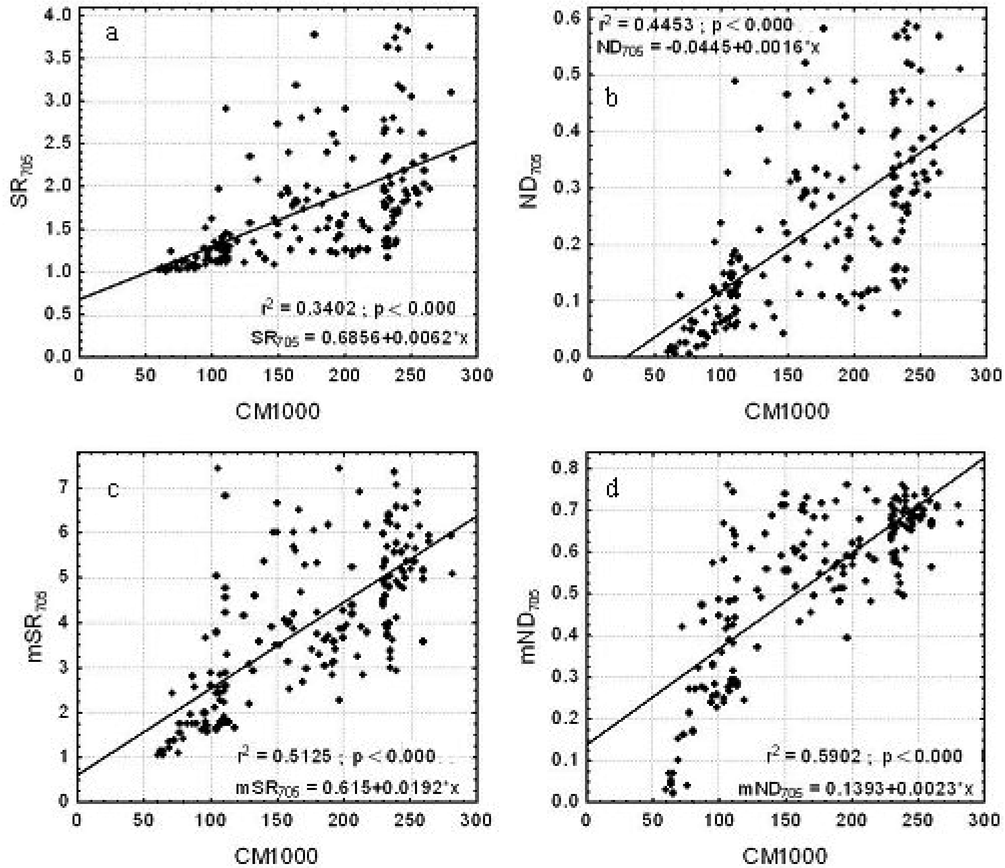

Figure 3. The normalized difference index and SR index with index wavelengths of 705 nm (a, SR_{705} and b, $NDVI_{705}$) and the modified indices (c, mSR_{705} and d, $mNDVI_{705}$) as a function of the leaf chlorophyll content. As expected from the index analyses, the (c) mSR index and (d) mNDVI were largely sensitive to chlorophyll content.

(Figure 3), and mNDVI (R^2 = 0.59) was stronger than NDVI (R^2 = 0.45). Based on the analysis, our findings were in agreement with Sims and Gamon (2002), in that the modified indices mSR_{705} and $mNDVI_{705}$ correlated better with chlorophyll content. The results of CV showed that the mNDVI had lowest CV (27%) of the four indices (Table 2).

Terrain effect

Four indices in this study were computed for the leaf samples in spectral reflectance measurement in the three terrains. Each vegetation index (SR_{705}, $NDVI_{705}$, mSR_{705} and $mNDVI_{705}$) was used in an analysis of variance (ANOVA) to determine significant effects of the three terrains (windward, valley, and leeward). The results showed significant differences (P<0.01) among the modified indices mSR_{705} and $mNDVI_{705}$, implying the advantage of using modified vegetation indices over normal indices on different terrains (Table 3).

The overall results showed that the $mNDVI_{705}$ was successfully applied in this study. The confidence intervals for leeward, windward, and valley were 0.084, 0.095, and 0.105 respectively. These results may help us to understand the variation of mND_{705} on different terrains.

Based on the discrepancies of the confidence intervals in the three terrains, the northeast wind was affected more than the other environmental factors in the study area. The degree to which terrain influenced forest composition

Table 2. The coefficient of variation of four vegetation indices[a].

	CM1000 D1		CM1000 D2		CM1000 D3		Mean
Index	Mean ± S.D.	CV %	Mean ± S.D.	CV %	Mean ± S.D.	CV %	CV %
SR_{705}	1.244 ± 0.278	22.3	2.189 ± 0.700	32.0	1.781 ± 0.571	32.1	28.8
mSR_{705}	0.099 ± 0.082	82.2	0.346 ± 0.129	37.2	0.255 ± 0.131	51.2	56.9
$NDVI_{705}$	2.317 ± 1.284	55.4	5.176 ± 1.038	20.1	4.179 ± 1.293	30.9	35.5
$mNDVI_{705}$	0.335 ± 0.179	53.5	0.666 ± 0.062	9.4	0.59 ± 0.101	17.1	26.7

[a]N=117. Data shows mean ± S.D.

Table 3. The variance of analysis of four vegetation indices between three terrains.

Index	Terrain	Mean	sd	-95%	95%	Range	F	P
	Leeward	1.781	0.078	1.628	1.935	0.307		
SR_{705}	Valley	1.669	0.097	1.478	1.860	0.382	0.412	0.663
	Windward	1.740	0.088	1.567	1.913	0.346		
	Leeward	0.245	0.018	0.209	0.280	0.071		
$NDVI_{705}$	Valley	0.231	0.022	0.187	0.275	0.088	0.395	0.675
	Windward	0.221	0.020	0.181	0.261	0.08		
	Leeward	4.346[a]	0.191	3.968	4.723	0.755		
mSR_{705}	Valley	3.311[bc]	0.238	2.842	3.780	0.938	5.907	0.003**
	Windward	3.790[ac]	0.216	3.364	4.217	0.853		
	Leeward	0.587[a]	0.021	0.545	0.629	0.084		
$mNDVI_{705}$	Valley	0.477[b]	0.026	0.425	0.530	0.105	6.304	0.002**
	Windward	0.502[b]	0.024	0.454	0.549	0.095		

**Indicates significance different among the indices, P<0.01.

was quite clear. A marked difference in forest composition existed among species in the windward and leeward forests. In the understory of the windward forest, saplings and small trees are more abundant than in the leeward forest (Hsieh et al., 2000). In the study area, winter precipitation and the intensity of the Northeast wind positively correlated with differentiation of forest types (Su and Su, 1988). Monsoon rainforests receive sufficient rains brought about by the Northeast wind during the winter, and this supports evergreen trees. Large quantities of deciduous trees occur in the leeward southwest district with semi-deciduous forest physiognomy (Su and Su, 1988). Chen (1999) used NDVI to detect seasonal variation in vegetation greenness with four SPOT XS images (Systeme Pour l'Observation de la Terre) in the same study area and reported that leeward (0.438) and windward (0.441) habitat had the highest degree of vegetation greenness from June to September. However, the NDVI between windward (0.241) and leeward (0.352) from December to March was significantly different (P>0.01). The study concluded that the northeastern monsoon would decrease vegetation greenness. Similarly, our analysis indicated that the northeast monsoon did certainly affect the vegetation physiology in the study area, and by using spectral characteristics detected the differences of terrain variance and also spectral reflection between vegetation species is possible.

CONCLUSION

We found a positive correlation between terrain and leaf chlorophyll content, indicating that modified vegetation indices, such as mNDVI, can help us to understand the relationship between a native vegetation cover and its terrain habitat. Among the indices tested, only the mNDVI consistently deviated from the general relationships between chlorophyll content and spectral reflection in different vegetation. The ecological significance of this study is based on the speed and efficiency by which modified vegetation indices can detect chlorophyll content of a complex forest vegetation. In this study, we have shown that among the indices tested, mNDVI detects the best in different terrain vegetation reflection. Nonetheless, further investigative work remains to be carried out, especially in leaf chlorophyll contents and remote sensing applications.

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墾丁國家公園地形對於不同樹種間葉綠素含量與反射光譜之影響

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本研究主要探討台灣墾丁國家公園南仁山生態保護區內，受地形影響不同樹種間之葉綠素含量與反射光譜之差異；由於本研究區域中，植群與樹種結構之組成受到地形與東北季風影響甚劇，因此以本區域作為研究區域。研究中在反射光譜與葉綠素含量資料收集上使用可攜式光譜儀 GER 1500 與葉綠素計 CM1000，應用了數個遙感探測指數作為比較之依據，包含了常態化植生指標 (NDVI)、修正後常態化植生指標 (mNDVI)、簡單比率植生指數 (SR) 與修正後簡單比率植生指數 (mSR)。研究結果顯示，在上述數個植生指數中以 mSR 705 nm 與 mNDVI 705 nm 對於不同地形之影響有顯著性之差異 ($P<0.01$)；而相較於其他指數而言，mNDVI 705 nm 更有效的呈現不同樹種間葉綠素含量與地形影響的關係。

關鍵詞：葉綠素含量；可攜式光譜儀；植生指數；反射光譜。