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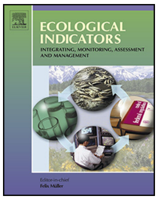

Best hyperspectral indices for tracing leaf water status as determined from leaf dehydration experiments

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ABSTRACT

Leaf water status information is highly needed for monitoring plant physiological processes and assessing drought stress. Retrieval of leaf water status based on hyperspectral indices has been shown to be easy and rapid. However, a universal index that is applicable to various plants remains a considerable challenge, primarily due to the limited range of field-measured datasets. In this study, a leaf dehydration experiment was designed to obtain a relatively comprehensive dataset with ranges that are difficult to obtain in field measurements. The relative water content (RWC) and equivalent water thickness (EWT) were chosen as the surrogates of leaf water status. Moreover, five common types of hyperspectral indices including: single reflectance (R), wavelength difference (D), simple ratio (SR), normalized ratio (ND) and double difference (DDn) were applied to determine the best indices. The results indicate that values of original reflectance, reflectance difference and reflectance sensitivity increased significantly, particularly within the 350–700 nm and 1300–2500 nm domains, with a decrease in leaf water. The identified best indices for RWC and EWT, when all the species were considered together, were the first derivative reflectance based ND type index of dND (1415, 1530) and SR type index of dSR (1530, 1895), with R^2 values of 0.95 ($p < 0.001$) and 0.97 ($p < 0.001$), respectively, better than previously published indices. Even so, different best indices for different species were identified, most probably due to the differences in leaf anatomy and physiological processes during leaf dehydration. Although more plant species and field-measured datasets are still needed in future studies, the recommend indices based on derivative spectra provide a means to monitor drought-induced plant mortality in temperate climate regions.

1. Introduction

Plant water status is closely related to forest mortality and physiological processes, which are still poorly understood (Sala et al., 2010). Studies have shown that a decreased leaf water content slows the rate of photosynthetic carbon assimilation (Lawlor and Cornic, 2002) and strongly decreases the intrinsic photochemical efficiency and electron transport rate of PS II (Augusti et al., 2001), which would result in plant carbon starvation or hydraulic failure, leading to plant death (McDowell et al., 2008, 2013; Sevanto et al., 2014). Therefore, leaf water status information is highly needed for monitoring plant physiological processes and assessing drought stress.

Traditional measurements on leaf water status are time-consuming, destructive, and point-based, which make it difficult to be up-scaled to reflect regional leaf water status (Penuelas et al., 1993). In recent decades, remote sensing has been shown to be an effective method to assess plant water status across different scales (Ceccato et al., 2001, 2002a,b; Gao, 1996; Hunt et al., 1987; Penuelas et al., 1993; Sims and Gamon, 2003; Zarco-Tejada et al., 2003). Two widespread remote sensing approaches, i.e., model inversion (Jacquemoud et al., 1996) and spectral indices (e.g., Ceccato et al., 2001), have been developed to retrieve leaf water status based on reflectance data. Compared to model inversion, the spectral indices approach, which is based on combinations of several narrow or broad spectral bands, is simple and correlates well with leaf water status. Thus, how to design a general spectral index to estimate vegetation water status by remote sensing data has consequently drawn more attention (e.g., Ceccato et al., 2002a,b; Sims and Gamon, 2003; le Maire et al., 2008).

A general approach to identify the best spectral indices is based on field measured datasets that are usually enclosed measurements of different species under different ecological conditions. Different water indices have been designed from previous

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studies such as the normalized difference water index (Gao, 1996) and water index (Penuelas et al., 1993), which generally use the typical absorption bands by water centered at around 970 nm, 1200 nm, 1450 nm, 1940 nm and 2500 nm; and strong correlations have been observed between these indices and leaf water content. However, these indices are usually calibrated based on a specific database, which means these indices may not be suitable for other databases (le Maire et al., 2008). In addition, this method is time and labor consuming, making it difficult to obtain a large dataset. Furthermore, numerous factors greatly affect the relationships, including water stress, plant species, growing conditions and phenological stages (Wang and Li, 2012b). Alternatively, simulated or a combination of simulated and measured datasets are proposed to generate universally applicable indices, e.g., Wang and Li (2012a,b) have reported two new hyperspectral indices (dSR (1510, 1560) and DDn (1530, 525)) for temperate deciduous plant water status, using both simulated and field datasets across a wide range of species. Even so, simulated and field datasets seldom include extreme cases, leading to nearly all spectral indices being unable to retrieve the leaf water content under extreme water stress conditions. This renders these indices poorly applicable to monitoring forest mortality, because all of the datasets are mainly obtained from or simulated for healthy leaves under normal natural conditions. Although, on the other hand, simulated datasets may include all extremes theoretically, biological responses to such extremes are poorly distinguished, as for most cases only physically unrealistic combinations of different model inputs are prevalent, leading to inaccuracies in applying identified indices to real applications.

The reflectance of dying leaves in different stages of water stress is quite different from that of healthy leaves, and leaf reflectance significantly increases throughout the 400–2500 nm domain during the dehydration process (Carter, 1991, 1993; Foley et al., 2006; Richardson and Berlyn, 2002; Seelig et al., 2008a). Previous studies have shown that leaf dehydration experiments can provide a dataset with a wide range of leaf water conditions and biological realities, which may be more suitable for index identification than field measurements under water stress conditions, because experiments cannot only trace the changes in leaf water status and physiological processes in a time and labor saving way, but can also obtain extreme leaf water conditions (Carter, 1991; Penuelas et al., 1993, 1997; Seelig et al., 2008a). The water indices SR (970, 900) and SR (1300, 1450) have been presented based on progressive dehydration experiments (Penuelas et al., 1993; Seelig et al., 2008a). However, these studies only focused on a single type of index (simple ratios of two wavelengths near the water absorption bands) based on a dataset with only a few plant species.

Previous studies also demonstrated that the reflectance near 700 nm and its ratio with near infrared reflectance can provide the detection of plant water stress (Carter, 1994; Carter and Knapp, 2001; Carter and Miller, 1994). However, the wavebands near 700 nm are strongly influenced by pigments, meaning they cannot directly provide plant water information. To our knowledge, there has been no study examining the relationship between leaf water status and hyperspectral indices of various types. Commonly used types of indices, including reflectance at a given wavelength (R), wavelength difference (D), simple ratios (SR), normalized differences (ND) and double differences (DDn) then need to be explicitly examined. In addition, specific conversion on leaf original reflectance can usually be performed to improve the performance of indices. For instance, the derivative spectra technique (dR) can eliminate background noise and resolve overlapping spectral features (Demetriades-Shah et al., 1990).

The objectives of the current study were: (1) to provide leaf reflectance, leaf RWC and EWT variations of different broadleaved species in the temperate climate zone during dehydration processes to compose a comprehensive dataset within biological realities; (2) to validate the performance of existing spectral indices for tracking leaf water status; and (3) to identify the best hyperspectral indices for estimating leaf water status based on different types and different treatments of reflected spectra. This study was based on leaf dehydration experiments using five common species of temperate zone ecosystems.

2. Material and methods

2.1. Leaf sampling and dehydration measurements

Leaves of four deciduous species, i.e., *Zelkova serrata, Idesia polycarpa, Liquidambar styraciflua* and *Prunus x yedoensis*, were collected around the campus of Shizuoka University, and another dominant temperate deciduous species (*Fagus crenata*) was collected from Mount Naeba, Japan. All samples were collected by the detached branch technique which is recognized to be accurate and reliable for reflectance and photosynthesis parameter measurements under non-*in situ* conditions (Koike, 1986; Foley et al., 2006; Richardson and Berlyn, 2002). The branches with target leaf samples were cut pre-dawn, and re-cut under water to avoid a loss of branch conductance. The samples were stored hydrated under dim light, high humidity and cool temperatures before measurement. In total, 24 leaf samples were collected for five plant species, including four sunlit leaves and four shaded leaves for *F. crenata* and four sunlit leaves for each of the other four species. All the samples selected were mature fully expanded leaves.

The measurements were conducted in the laboratory in the middle of September, 2013, when mean day temperature was about 25 °C and average relative humidity was around 75%. For each leaf sample, fresh leaf reflectance and weight were firstly measured, and then kept naturally under dehydrated conditions. Leaf reflectance and weights were measured synchronously at every 1 h for the first 5 h but less frequent in later during the entire leaf dehydration period (ca. 24 h) until the leaf sample was air-dried to a stable weight. Finally, the air-dried samples were oven-dried at 70 °C for 72 h and then weighed again. The total number of measurements was 224 for both leaf reflectance and leaf weight.

Leaf reflectance spectra were measured in the optical range (350–2500 nm) using a field spectroradiometer (ASD FR, USA) equipped with a leaf clip, in which a light source of a tungsten quartz halogen lamp was embedded. The spectral resolution was 3 nm at 700 nm and 30 nm at both 1400 nm and 2100 nm. The sampling interval was 1.4 nm from 350 nm to 1050 nm and 2 nm from 1000 nm to 2500 nm. White reference scan was made for the calibration before reflectance measurement, which was done in the leaf clip with matched openings for non-destructive contact measurements. Synchronously, leaf weight was measured using an electronic balance right after reflectance measurement to make sure both measurements were under similar water status as possible.

2.2. Leaf water status

Leaf relative water content (RWC) and equivalent water thickness (EWT) are commonly used as indicators for plant water status, both of which were selected for the current study. RWC refers to the ratio of the water content to the maximum water content at full turgor for one given leaf (Hunt et al., 1987). EWT is the amount of water content per unit leaf area, which is more associated with energy absorption (Jacquemoud et al., 1996). Here, we defined RWC as the ratio of the leaf water content at time T (h) to the water content of the fresh leaf, not the water content at full turgor. This ratio