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Connecting infrared spectra with plant traits to identify species

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ABSTRACT

Plant traits are used to define species, but also to evaluate the health status of forests, plantations and crops. Conventional methods of measuring plant traits (e.g. wet chemistry), although accurate, are inefficient and costly when applied over large areas or with intensive sampling. Spectroscopic methods, as used in the food industry and mineralogy, are nowadays applied to identify plant traits, however, most studies analysed visible to near infrared, while infrared spectra of longer wavelengths have been little used for identifying the spectral differences between plant species. This study measured the infrared spectra (1.4–16.0 μm) on individual, fresh leaves of 19 species (from herbaceous to woody species), as well as 14 leaf traits for each leaf. The results describe at which wavelengths in the infrared the leaves' spectra can differentiate most effectively between these plant species. A Quadratic Discrimination Analysis (QDA) shows that using five bands in the SWIR or the LWIR is enough to accurately differentiate these species (Kappa: 0.93, 0.94 respectively), while the MWIR has a lower classification accuracy (Kappa: 0.84). This study also shows that in the infrared spectra of fresh leaves, the identified species-specific features are correlated with leaf traits as well as changes in their values. Spectral features in the SWIR (1.66, 1.89 and 2.00 μm) are common to all species and match the main features of pure cellulose and lignin spectra. The depth of these features varies with changes of cellulose and leaf water content and can be used to differentiate species in this region. In the MWIR and LWIR, the absorption spectra of leaves are formed by key species-specific traits including lignin, cellulose, water, nitrogen and leaf thickness. The connection found in this study between leaf traits, features and spectral signatures are novel tools to assist when identifying plant species by spectroscopy and remote sensing.

1. Introduction

The global biological diversity of all plants exceeds >350,000 species ranging from grasses to woody plants (Plant List, 2013). Every year, new plant species are discovered, especially in remote areas where diversity has not been previously assessed. A combination of plant traits make each species unique and reflect the outcome of evolutionary and community assembly processes responding to abiotic and biotic environmental constraints (Valladares et al., 2007). These traits determine how plants respond to environmental factors and how they can influence ecosystem function (Kattge et al., 2011). Making quantitative observations of traits can be used to classify plants into species as well as determine a plant's health and its potential to provide ecosystem services (Lavorel and Garnier, 2002). The correct estimation of leaf traits is essential for the conservation of natural ecosystems since efforts towards the conservation of plant biodiversity rely on the identification of traits for the accurate detection of species (e.g. Chiarucci et al., 2011; Nagendra, 2001; Pereira et al., 2013; Skidmore and Pettorelli, 2015).

Airborne and satellite spectral sensors can improve the efficiency of plant species identification compared with conventional field identification (Adam et al., 2010; Nagendra, 2001). Remote sensing studies to identify species have focused on the use of absorption features to classify species by identifying changes in the visible (VIS) and near-infrared (NIR) spectra (0.7–1.4 μm) (e.g. Clark et al., 2005; Cochrane, 2000; Martin et al., 1998; Schmidt and Skidmore, 2001; Vaiphasa et al., 2007). The bands in this part of the spectrum, governed by the strong absorption of radiation by leaf pigments, are often used to quantify photosynthetic absorption, from which vegetation productivity and species identity can

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be retrieved (Kerr and Ostrovsky, 2003; Turner et al., 2003). The strong absorption of light in the blue and red and the reflected energy in the green have a masking effect of additional features for species differentiation. In this VIS-NIR region, most studies have focused on classifying functional groups, vegetation types, or ecosystems based on their productivity (Ustin and Gamon, 2010).

The strong influence of pigment absorption decreases in the NIR section of the electromagnetic spectrum and beyond. As a consequence, signals in the short-wave infrared (SWIR, 1.4–2.5 μm), mid-wave infrared (MWIR, 2.5–6.0 μm) and long-wave infrared (LWIR, 6–20.0 μm) are probably more sensitive to (other) leaf compounds such as water, lignin and cellulose, which are essential to the functioning and structure of the leaf (Elvidge, 1988; Ribeiro da Luz and Crowley, 2007). Studies have found that within the infrared spectral region, structural traits and leaf compounds can be detected, including leaf and canopy water content (e.g. Fabre et al., 2011; Gerber et al., 2011; Harrison et al., 2018; Neinavaz et al., 2016; Ullah et al., 2014), nitrogen (Serbin et al., 2011), lignin and cellulose (e.g. Curran, 1989; Elvidge, 1988; Martin and Aber, 1997; Meerdink et al., 2016), epidermis thickness and cuticle compounds such as fatty acid esters, waxes, cutin and cutan (e.g. Ribeiro da Luz, 2006; Stewart et al., 1997) as well as carbohydrates and proteins (e.g. Curran, 1989; Elvidge, 1988). Some researchers have suggested that these leaf traits, which are species dependent, can be connected to specific features in the MWIR and LWIR (2.5–20.0 μm) and that these features can be used to differentiate species (Elvidge, 1988; Harrison et al., 2018; Ribeiro da Luz and Crowley, 2007). For example, Ullah et al. (2012) found that 13 species show significant spectral differences in the wavelength range from 1.4–6.0 μm and 8.0–14.0 μm. However, a possible mechanistic explanation for these differences in the spectra of plant species was not explored. In a more recent experiment, Harrison et al. (2018) explored the differentiation of group of species and connected spectral differences to the main leaf constituents, however, the differentiation of single species was not tackled. These knowledge gaps are addressed here. Therefore, this study determines the wavelengths (within the SWIR, MWIR and LWIR) that most effectively differentiate nineteen plant species. This study investigates the spectral features that differentiate species, and that can be explained to a large extent by the structure and composition of their leaves, identifying the most likely leaf traits that cause the spectral differences for these 19 species.

2. Methods and data

This study was conducted at the University of Twente, The Netherlands, during July and September 2015. Fresh leaves were selected from 19 broadleaf herbaceous and woody species of different taxonomic families occurring from the tropics to temperate regions (Table 1). All leaves of woody species were collected from adult trees at the university gardens or surroundings. Herbaceous plants were collected from mature plants from the university gardens or from plants coming from a local plant nursery. For each species, nine different individuals were selected that appeared to be of good health and physiological condition. From these individuals, leaves attached to twigs were clipped, placed in moist cotton to avoid desiccation and brought to the laboratory. Spectral measurements were made within five minutes after the clipping, followed by the microstructural measurements which were made on the same location (middle of the leaf). Chemical analyses were made using the same entire leaf.

2.1. Spectral measurements

The infrared spectra of each selected fresh leaf was measured by locating the middle of the leaf in the sample port (3cm diameter) of the external integrating sphere of the Bruker Vertex 70 FTIR spectrometer (Hecker et al., 2011). The reflectance measured with the spectrometer was converted to emissivity using Kirchhoff's law (*emissivity + reflectance = 1*), assuming that leaves behave as opaque objects (Fabre et al., 2011; Gerber et al., 2011). For consistency, emissivity was calculated for SWIR, MWIR and LWIR, even though in the SWIR and MWIR it is more common to use reflectance data. The calibration with the spectrometer is described by Hecker et al. (2011), and consisted of measuring the reflectance of a reference (infragold plate) before each leaf sample measurement. The ratio between the reference and the leaf sample measurements is calculated to convert the spectrum to reflectance percentages. Each sample leaf was placed against the sample port of the integrating sphere, with a diameter of 3 cm (filling the entire sample port, and two additional leaves of the same species were layered and taped behind the selected leaf to reduce possible loss of energy due to transmittance in the MWIR (Gerber et al., 2011). The spectrum was measured in the range 7000–600 cm^{-1} (1.4–16.6 μm) with a resolution of 4 cm^{-1} (0.0008–0.110 μm). Per leaf, eight measurements of 512 scans on the same spot were averaged in order to increase the signal-to-noise ratio. Although the spectra were measured in the wavenumber domain, the data was presented in wavelengths (in μm – micrometres) as this is customary in the remote sensing community. Infrared spectra of liquid demineralised water and powders of lignin (Sigma-Aldrich code: 471003) and cellulose fibres (Sigma-Aldrich code: S6790) were also measured, following the same procedure with the Bruker Vertex 70 FTIR. These spectra were used to compare with leaf spectra.

2.2. Anatomical and biochemical measurements

For each leaf, microstructural leaf traits (Fig. 1, Table 2) were measured using an optical microscope (Leitz Wetzlar model) with an amplification of 40 times for bigger structures (i.e. leaf thickness) and 630 times for smaller structures (i.e. cuticle thickness). For the same leaves used in the spectroscopic measurements, a thin sample from the transverse section at the middle length of the leaf (Fig. 1a and b) was dissected with a cutting edge. A digital picture was taken covering all structures at their most suitable magnification (40–630 times). The average of three measurements of the upper cuticle, epidermis and leaf thickness (in μm) was calculated. The main vein thickness (in μm) was measured a single time at the same location. Similarly, a digital picture of the tangential section of the adaxial side of the leaf was taken with an amplification of 630X (Fig. 1c). For each image, at least five stomatal structures were measured (including guard cells) to calculate the average stomatal size (in μm^2), and the number of stomata in the picture area were counted to calculate the stomatal density (number of stomata/mm^2).

Individual leaf area was measured with the LI3100c area meter (in cm^2). Leaf water content (in %) was gravimetrically calculated by drying the leaves and recording fresh and dry weights (in grammes). Lignin and cellulose percentages were calculated with the ANKOM Acid Detergent Fibers method (Ankom, 2011), and then lignin/cellulose ratios were calculated. Carbon (C) and nitrogen (N) content (in %) were measured with a Perkin-Elmer Elemental analyser (Culmo and Shelton, 2013), and from these measurements, the carbon/nitrogen ratio was calculated. The instrument measurement errors were 0.29 μm for the cuticle, epidermis, vein and leaf thickness, 0.57 μm^2 for bundle area, 1 mm^2 for leaf area, 0.01 gr for LWC, 1% for lignin and cellulose percentages and 0.01% for carbon and nitrogen percentages.

2.3. Statistical methods

The statistical analyses used in this paper can be summarised as follows: