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Spectroscopic determination of leaf traits using infrared spectra

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ABSTRACT

Leaf traits characterise and differentiate single species but can also be used for monitoring vegetation structure and function. Conventional methods to measure leaf traits, especially at the molecular level (e.g. water, lignin and cellulose content), are expensive and time-consuming. Spectroscopic methods to estimate leaf traits can provide an alternative approach. In this study, we investigated high spectral resolution (6612 bands) emissivity measurements from the short to the long wave infrared (1.4-16.0 μm) of leaves from 19 different plant species ranging from herbaceous to woody, and from temperate to tropical types. At the same time, we measured 14 leaf traits to characterise a leaf, including chemical (e.g., leaf water content, nitrogen, cellulose) and physical features (e.g., leaf area and leaf thickness). We fitted partial least squares regression (PLSR) models across the SWIR, MWIR and LWIR for each leaf trait. Then, reduced models ($PLSR_{red}$) were derived by iteratively reducing the number of bands in the model (using a modified Jackknife resampling method with a Martens and Martens uncertainty test) down to a few bands (4–10 bands) that contribute the most to the variation of the trait. Most leaf traits could be determined from infrared data with a moderate accuracy ($65 < R^2_{cv} < 77\%$ for observed versus predicted plots) based on $PLSR_{red}$ models, while the accuracy using the whole infrared range (6612 bands) presented higher accuracies, $74 < R^2_{cv} < 90\%$. Using the full SWIR range (1.4–2.5 μm) shows similarly high accuracies compared to the whole infrared. Leaf thickness, leaf water content, cellulose, lignin and stomata density are the traits that could be estimated most accurately from infrared data (with R^2_{cv} above 0.80 for the full range models). Leaf thickness, cellulose and lignin were predicted with reasonable accuracy from a combination of single infrared bands. Nevertheless, for all leaf traits, a combination of a few bands yields moderate to accurate estimations.

1. Introduction

Plant traits consist of morphological, chemical and anatomical features that occur at scales of individual cells to entire plants (Violle et al., 2007). The high diversity of plant traits is expressed in the biochemistry (e.g., water, fibres and proteins content), the morphology (e.g., height, diameter, leaf area) and the physiology (e.g., photosynthesis, respiration, carbon storage) of each species, but is also expressed in individuals under specific growing conditions. Traits from leaves, which are the bulk of the canopy measurements, can contain relevant, measurable information to characterise a plant species but also to determine the health status of an individual plant.

Plant traits show strong variations between species but also vary within species, and this can be a proxy for growing conditions. Morphological leaf traits such as leaf area, leaf thickness, trichomes, and stomata, are commonly used for species differentiation by recognising the traits that are an expression of the genetic differences between species, while the smaller variations within single species can be used to differentiate plant status, growing conditions and health (Rhodes and Nadolska-Orczyk, 2001). These intraspecific differences can carry valuable information about the exposure of a plant to different environments (McGill et al., 2006). Plants under optimal conditions tend to express different leaf traits than individuals under suboptimal conditions, even when belonging to the same species and varieties (e.g., Buitrago et al., 2016)

The quantification of leaf traits such as water, lignin, cellulose and nitrogen content is used for the assessment of plant status (e.g., plant stress, productivity), but also in global ecological modelling as proxies of ecosystem processes. The estimation of leaf traits is needed for large areal extents to build global models that help in understanding the role of terrestrial ecosystems in the exchange of energy and material between soil, water, vegetation and atmosphere (e.g. Cash and Moser 2000; Randin et al., 2009). For example, leaf water content has been related to plant stress (e.g. Buitrago Acevedo et al., 2017; Levitt 1980;

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Morgan 1984), nitrogen to plant productivity (e.g. Martin and Aber 1997; Tilman et al., 1996), and lignin and cellulose have been related to plant biomass and wood quality (e.g. Boyd 1972; Parton et al., 1993). Similarly, in ecological modelling, leaf biochemicals are used as local inputs to calibrate models of ecosystems processes such as carbon storage, nutrient- and water availability, primary net production and carbon decomposition (e.g. Kattge et al., 2009; Kucharik et al., 2000; Lloyd et al., 1995).

Conventionally, the most accurate methods to identify and quantify leaf traits consist of laboratory measurements, such as wet chemistry methods to calculate lignin or cellulose (Curran 1989). Most of these methods are restricted to pure chemicals and are based on destructive methods (Allison et al., 2009), which are expensive and time-consuming. The development of high-resolution spectrometers has provided tools for the identification of chemical components of organic materials in a non-destructive way, based on the vibrational bonds of individual simple or complex molecules (e.g. water and lignin molecules respectively), tracking the fingerprints in their spectra of the vibrational bonds of most organic materials at the molecular level (Lin et al., 2009; Subramanian and Rodriguez-Saona 2009). The development of such new techniques to estimate the biochemistry of fresh leaves is needed, for example, for the non-destructive assessment of plant health and performance at the level of individual plants and plant populations (Curran 1989; Serbin et al., 2014). Spectroscopy, which forms the basis of this technique, can in some cases also be scaled up to the level of remote sensing.

The identification of leaf traits with spectroscopy and remote sensing has been performed mainly in the visible to near-infrared (VIS-NIR, 0.7–1.4 μm) wavelength range. This part of the electromagnetic spectrum is governed by the strong absorption of leaf pigments and often used to differentiate the photosynthetic absorption of electromagnetic radiation by vegetation (Knipling 1970). Since the strong influence of pigment absorption decreases in the NIR and further in the electromagnetic spectrum, signals in the short-wave infrared (SWIR, 1.4–2.5 μm), mid-wave infrared (MWIR, 2.5–6 μm) and long-wave infrared (LWIR, 6–20 μm) are known to be sensitive to other leaf compounds such as water, lignin and cellulose, which are essential to the functioning and structure of the leaf (e.g. Elvidge 1988; Ribeiro da Luz 2006; Salisbury and Milton 1988).

Recent studies have found that spectral differences in the infrared regions can be caused by leaf traits associated with the leaf́s composition, such as water content (e.g., Fabre et al., 2011; Gerber et al., 2011; Ullah et al., 2014), lignin and cellulose content (e.g., Curran 1989; Elvidge 1988; Martin and Aber 1997), epidermis thickness and cuticle composition such as fatty acid esters, waxes, cutin and cutan content (e.g., Ribeiro da Luz 2006; Stewart et al., 1997) as well as carbohydrate and protein content (e.g. Curran 1989; Elvidge 1988). Other researchers have suggested that additional structural and microstructural leaf traits can leave fingerprints in infrared spectra, such as epidermis thickness and cell wall composition (e.g. Fabre et al., 2011; Ribeiro da Luz 2006; Salisbury and Milton 1988). All these studies looked at a limited number of leaf traits for a few plant species for different sections of the infrared spectrum. This needs to be better integrated, and hence this study considers a wide range of leaf traits (14 in total) and species (20 in total) at the same time, over a large part of the infrared spectrum. We expect that features in infrared spectra of leaves contain information about their composition and structure. Therefore, we estimate both biochemical and structural leaf traits from hyperspectral spectroscopic data and to identify which parts of the spectra are more sensitive to changes in leaf traits. For structural traits, we measured some variables that are not directly related to spectral features (e.g., leaf thickness or area), but that are well-known indicators of plant status and might be related to other leaf traits that are less easy to determine, but which are detectable through spectroscopy. This study aims to link leaf traits to the most sensitive infrared bands.

Table 1
Plant species.

Code	Species	Family	Type	Climate	Foliage
Am	Aglaonema spp.	Araceae	Herbaceous	Tropical	Evergreen
An	Asplenium nidus	Aspleniaceae	Herbaceous	Tropical	Evergreen
Ap	Acer platanoides	Aceraceae	Woody	Temperate	Deciduous
Cr	Calanthea rufibarba	Marantaceae	Herbaceous	Tropical	Evergreen
Df	Dieffenbachia spp.	Araceae	Herbaceous	Tropical	Evergreen
Fs	Fagus sylvatica	Fagaceae	Woody	Temperate	Deciduous
Fv	Fittonia verschaffeltii	Acanthaceae	Herbaceous	Tropical	Evergreen
Gb	Ginkgo biloba	Ginkgoaceae	Woody	Temperate	Deciduous
Gm	Geranium macrorrhizum	Geraniaceae	Herbaceous	Temperate	Evergreen
Hh	Hedera helix	Araliaceae	Woody	Temperate	Evergreen
Io	Ilex opaca	Aquifoliaceae	Woody	Temperate	Evergreen
Ls	Liquidambar styraciflua	Altingiaceae	Woody	Temperate	Deciduous
Pa	Persicaria amplexicaulis	Polygonaceae	Herbaceous	Temperate	Deciduous
Pl	Prunus laurocerasus	Rosaceae	Woody	Temperate	Evergreen
Po	Platanus orientalis	Platanaceae	Woody	Temperate	Deciduous
Qu	Quercus robur	Fagaceae	Woody	Temperate	Deciduous
Rc	Rhododendron caucasicum	Ericaceae	Woody	Temperate	Evergreen
Rh	Rhododendron cf. catawbiense	Ericaceae	Woody	Temperate	Evergreen
St	Spathiphyllum cochlearispathum	Arecidae	Herbaceous	Tropical	Evergreen
Tp	Tilia platyphyllos	Tiliaceae	Herbaceous	Temperate	Deciduous

2. Methods

This study was conducted in Enschede, The Netherlands, between July and September 2015. Nine leaves were collected from different individuals from 20 different plant species (Table 1). Nine herbaceous species which are mainly small plants from indoor and outdoor gardens, and eleven woody species, which are mainly shrubs and trees from the gardens of the University of Twente and surroundings. The species were selected from herbaceous to woody plants, as well as from tropical and temperate climates, to guarantee a large variance in leaf traits.

2.1. Spectral measurements

We harvested fresh leaves shortly before making spectral measurements. The spectra of each leaf were measured with a Bruker Vertex 70 FTIR spectrometer adapted with an external integrating sphere for hemispherical reflectance (Hecker et al., 2011). This reflectance was converted to emissivity using Kirchhoff's law (*emissivity + reflectance = 1*), assuming the leaves behave as opaque objects (Fabre et al., 2011; Gerber et al., 2011). For consistency, emissivity was calculated for both MWIR and LWIR, even though in MWIR it is more common to use reflectance data.

We placed each sampled leaf against the sample port of the integrating sphere, with a diameter of 3 cm, and we taped two additional leaves of the same species to the measured 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 1 cm^{-1} (0.008–0.110 μm). Per leaf eight measurements of 512 scans on the same spot were averaged to increase the signal-to-noise ratio. Although the spectra were measured in the wavenumber domain, the data is presented in wavelengths (in μm – micrometres) as this is customary in the remote sensing community.

We also measured the spectra, of liquid demineralised water and powders of lignin (Sigma-Aldrich code: 471003) and cellulose fibres (Sigma-Aldrich code: S6790), following the same procedure with the Bruker Vertex 70 FTIR.

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