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Field sampling protocol for foliage chemistry assessment. Applicability over varied forest sites in Australia.

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Abstract

Photosynthetic rate is an indicator of vegetation performance as a carbon sequestration element on earth. At the same time, primary productivity is also function of photosynthetic rate and canopy cover. The photosynthetic capacity of an ecosystem is limited by the foliage pigment content due to the active participation of pigmentation in photon capture. Hence, the quantitative assessment of foliage pigment content is of high importance in order to monitor forest primary productivity and ecosystem performance in carbon sequestration.

Pigment content estimation over large areas can be assessed using remote sensing data using empirical algorithms or by inverting radiative transfer models. Both techniques need accurate leaf pigment measurement for parameterisation and validation. Leaf sampling field protocols are then needed in order to collect and store leaf tissue without altering leaf pigment composition before its analysis in the laboratory.

The Terrestrial Ecosystem Research Network (TERN) is a platform for researchers and land managers in Australia to work together on terrestrial ecosystem inventory and monitoring. Leaf sampling for pigment content estimation in Australian forests is then part of TERN field sites activities.

This paper presents a leaf sampling field protocol used for TERN field activities during 2012. Two set of leaf samples were collected from sun exposed branches from each individual stand. The first set of samples was frozen immediately in dry ice and kept at -70 degrees Celsius for subsequent pigment quantification in the laboratory. The second set of leaves was kept under 10 degrees Celsius in humid conditions. This second set was later used for leaf spectroscopy measurement and to quantify water and dry matter content per unit area. Additionally, pertinent metadata was collected to characterise each sampled stand. The field protocol presented here has been used for leaf sampling on a broad range of study areas varying in species composition, canopy height and foliage density.

Keywords: pigment estimation, leaf sampling, protocol.

Dr Lola Suárez has a background in Agriculture Engineering, GIS and remote sensing. She did her Ph.D. research at IAS, Spanish Council of Scientific Research, Cordoba. Then, she worked as a postdoctoral fellow in the Remote Sensing Laboratories (University of Zurich) and INRA-EMMAH in Avignon (France). At the moment she works in the School of Mathematical and Geospatial Science at RMIT University (Melbourne, Australia). Her research interests include vegetation biophysical properties estimation and up-scaling methodologies to different spatial resolutions using hyper/multispectral remote sensing, and radiative transfer modeling. Simon Jones is a Professor of remote sensing in the School of Mathematical and Geospatial Science at RMIT University. Dr M. Soto-Berelov is a post doctoral research fellow in remote sensing at RMIT, specializing in land-use change science (LUCC), vegetation mapping/ modelling, geographic information science, and remote sensing. P. Wilkes is a PhD candidate at RMIT University investigating the use of LiDAR for assessment of forest structure. W. Woodgate is a PhD candidate at RMIT University investigating Leaf Area Index of forests at different scales from remote sensing data. Andrew Mellor and Dr. Andrew Haywood are with the forest monitoring and reporting section of the Department of Sustainability and Environment, Victoria.

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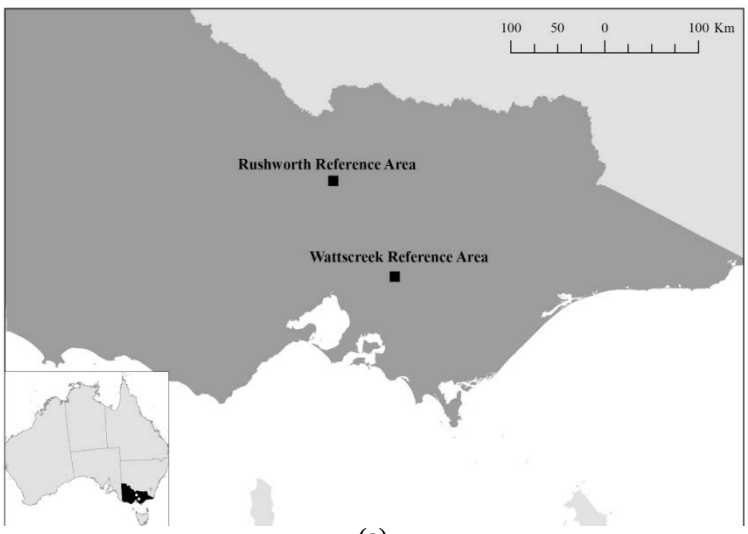
Introduction

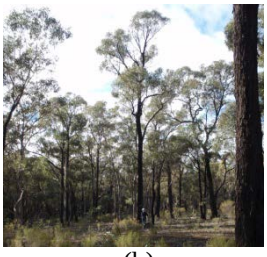
Foliage chemistry refers to components existing in the leaf tissue of a canopy. It is represented as the mean concentration in the leaf tissue of a crown when the assessment is at the stand level or the mean concentration in the leaf tissues of all the crowns over a specific unit area when the assessment is done at a larger scale. The foliage composition of a canopy has been found to be correlated with canopy health and biodiversity in Australian forest (Stone and Simpson, 2006; Asner *et al*., 2009). Moreover, it can be used as input for models to predict net ecosystem productivity (Martin and Aber, 1997; Smith *et al.*, 2002). The interaction between vegetation and herbivores within the ecosystem has also been studied as function of the chemical composition of foliage and soils. According to Robertson (1991), leaf chemical composition is driving herbivore preferences towards specific tree species. The levels of soluble tannins and carbon/nitrogen ratio determine the leaf palatability for insects. Herbivores feed from plants and are the proximal cause of mortality in eucalypt dieback, but as a feedback they play an important role as seed dispersal agents. All the above-mentioned factors demonstrate how critical the foliage chemistry is for the assessment of the ecosystem services (Martin and Aber, 1997). Standard field assessment is based on an estimation of the percentage of discoloured leaves made by a given operator, and in consequence, can be subjective. Alternatively, remote sensing of foliar chemistry has been recognised as an important element in producing large-scale, spatially explicit estimates of forest ecosystem function (Mooney *et al*., 1987; Steudler *et al*., 1989; Wofsy *et al*., 1993). Nevertheless, remote sensing techniques require accurate ground truth for parameterisation/calibration and validation of models. This paper presents a detailed protocol that has been successfully used for leaf sample and corresponding tree metadata collection for chemical analysis. Each of the steps in the protocol has been extensively used by different research groups. The existing protocols are not always well adapted for every forest type; the leaf sampling protocol presented here have been tested in typical sclerophyll forest sites located in Victoria, Australia.

Study sites

The two study sites are located in Victoria, Australia. Figure 1a shows the location of the study sites within Victoria. They consist of reference areas, meaning they are relatively undisturbed, sufficiently large to ensure the viability of ecosystems, and that the area contributes to a network of Reference Areas representative of the Victorian land systems. Reference areas are tracts of public land containing viable samples of one or more land types that are relatively undisturbed and that are proclaimed under the Reference Areas Act 1978. The first study site is the Rushworth forest reference area (36.749535S, 144.967344E), located close to Nagambie. The area is a medium eucalypt woody forest (National Vegetation Information System, NLWRA, 2001) populated with red iron bark (Eucalyptus tricarpa), red stringybark (Eucalyptus macrorhyncha), red box (Eucalyptus polyanthemos), long leaf box (Eucalyptus goniocalyx) and grey box (Eucalyptus microcarpa). Figure 1b presents the typical vegetation structure present in Rushworth forest. The second study site is a rainforest located in Watts Creek reference area 15 km east of Healsville (37.69S, 145.68E). It is representative of the plateau and slopes of the upper watershed areas south of the Great Dividing Range and comprises a mature tall eucalypt open forest of mountain ash on soils derived from Devonian volcanics. The area largely comprises a mature open forest of Mountain Ash (*Eucalyptus regnans*). Regrowth and older mature stands of Mountain Ash, Shining Gum (*Eucalyptus nitens*) and Alpine Ash (*Eucalyptus delegatensis*) occur at higher elevations and there is a small area of scrub. The vegetation in Watts Creek reference area is much denser and the vertical structure predominantly consists of more than 2 canopy layers (Figure 1b).



(a)



(b)

(c)

Figure 1. Location of the study sites used in this study within Australia and Victoria (1a). Overview of the vegetation type and density in Rushworth (b) and Watts Creek reference areas (c)

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Field protocol

A dedicated field protocol was designed for stand selection and leaf sampling based on the previous experience of other research groups (e.g. Global Ecology department, Carnegie Institution for Science). From each study site a set of crowns representing the main species present was selected. All the selected stands had a large crown diameter (over 6-7 metres), and an emergent crown having a dominant participation on the spectral signal of the projected area. There was a minimum distance of 50 m between the sampled stands of the same species in order to ensure genetic individuality.

The pigment pool and the spectral characteristics of a leaf depend on the illumination conditions and consequently of the location of the leaf within the crown. The leaves were collected from branches located in the upper-most third part of the crown; in this way, they were representative of the spectra extracted from the imagery. In many cases, tree height was above 30 metres, especially in Watts Creek. In these cases, a rifle was needed in order to reach the branches at the top of the tree. For the other samples, a shot gun was used. The average number of bullets needed per sampled branch was 4. After the branch was brought down, only mature, full-open leaves were taken, avoiding those that were partly-eaten or damaged. The purpose was to acquire a representative sample set of leaves, young/developing leaves are presenting different pigment pool resulting in high spectral differences in the visible and near infrared (Stone *et al.*, 2001). At the same time, if the percentage of damaged leaves in the crown is below 5%, those leaves are not representative of the overall pigment content. Three leaf sample sets were collected. Two sets of 50 g were stored in zip-bags labeled with the study site name, the date and the stand identifier. A third bag was collected in case expert assistance was required for species identification. The first set of 50 g was frozen in dry ice immediately and kept at -70 degrees Celsius until it was analysed in a laboratory. The second set was stored with a humid tissue in fresh temperature (at 5-10 degrees) and processed within a day of sampling (see post-field processing). From each sampled tree, specific metadata was collected including species, height, diameter at breast height, location and estimated canopy cover (Fist 8 columns of Table 1). Moreover, for better documentation and future identification of every stand, pictures were taken using a digital camera. The trees were flagged and tagged with their corresponding identifier. The location was recorded using a handheld GPS device (Juno SP, Trimble) and the tree height was measured using a rangefinder (Tru Pulse, Laser Technology Inc.). Table 1 shows the data sheet populated with the data collected in the field. The last five columns (i.e. Wet weight, Dry weight, Leaf area, SLA and Leaf water content) were populated during the post-field processing.

Table 1. Data sheet used for leaf sampling where: Tree ID: tree ID specified in the tree label, bags and files obtained through the processing procedure, Spp: tree species, DBH: Tree trunk diameter at chest height (cm), Tree height: Height of the tree in metres (m), Approx crown Ø: Approximation of tree crown diameter (m), E/C/I: crown position in the field with respect to the surrounding tree crowns, % cover: estimated percentage of the leaf fractional cover in the crown, Comments: every comment added in the field or during the laboratory processing, Date: day the leaves were collected (format yymmdd), Wet weight: weight measure the same day the leaves are collected from the tree (g), Dry weight: weight of the same leaves measured after drying them in the oven (g), Leaf area: Area corresponding to the same leaves computed from the scanned image (cm^2), SLA: Specific leaf area, calculated as (Wet weight/Leaf area) in g/cm^2.

Date:			Site:									
Tree ID	Spp	DBH (cm)	Height	Approx crown diameter	E=emergent C= canopy I= isolated	% cover	Comments	Wet weight	Dry weight	Leaf area	SLA	Leaf water content

Post-field processing protocol

Back in the laboratory, the same day the samples were collected, spectroscopy and leaf specific area measurements were completed. From the zip-bag that has been kept at 5-10 degrees, three leaves were taken to measure their reflectance and transmittance. Spectroscopy measurements were carried out using a portable spectrometer attached to an integrating sphere (ASD Inc, Boulder, CO). From the suggested manufacturer protocols, the one that corrects for the lamp misalignment was used. Then, straylight was quantified as part of every measurement and reflectance and transmittance spectra were corrected for lamp misalignment errors. As a result, every leaf reflectance and transmittance measurement requires 8 different configurations of the elements on the integrating sphere ports. To improve the efficiency of this process, two integrating spheres can be positioned in parallel, measuring reflectance with one of them and transmittance with the other. An experiment carried out with the same operators demonstrated the time to measure reflectance and transmittance of one leaf decreased from 4.2 to 2.88 when measuring with two integrating spheres measuring in parallel instead of using only one integrating sphere. The spectral files were later loaded into a spectral database where the reflectance and transmittance calculation was made automatically (Hueni *et al.*, 2012).

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From the same bag, enough leaves to cover an A4 scanner surface were taken. The leaves were placed on the scanner so as not to overlap or touch and with the petiole removed. The resulting scanned image was later processed to estimate the total leaf area. The same leaves were then weighted using a 0.01 g precision scale; and kept in paper bags labelled with the stand identification name, study site and day. The leaves inside the paper bags were dried in an oven at 60 degrees for 24 hours and weighted again. To ensure all dry leaf tissue was weighed, the bags with the content were weighed, then properly emptied and weighed again. The water content can then be calculated depending on the procedure as in [1] or [2]. Specific leaf area was calculated as the ratio of total leaf area to the corresponding dry mass [3].

$$LWC = \frac{(WetMass - DryMass)}{LeafArea} \quad [1]$$

$$LWC = \frac{(WetMass - DryMassW/bag - BagMass)}{LeafArea} \quad [2]$$

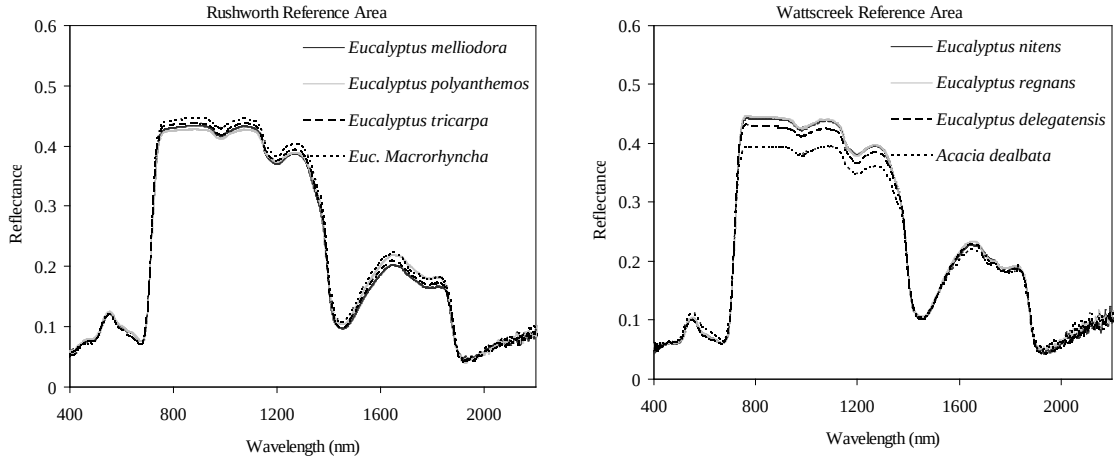
$$SLA = \frac{LeafArea}{DryMass} \quad [3]$$

Finally, the samples that immediately frozen in the field were kept at -70º Celsius until the different chemical components could be extracted in the laboratory. The chemical analysis was conducted in the Research School of Biology, Department of Ecology, Evolution and Genetics at the Australian National University (ACT, Australia).

Results and discussion

A total of 96 stands were sampled in Rushworth reference area representing the four main species present (i.e. *Eucalyptus macrocarpa*, *Eucalyptus macrorhyncha*, *Eucalyptus tricarpa* and *Eucalyptus polyanthemos*). In Watts Creek reference area, 49 individuals from the main three eucalypt species present (*Eucalyptus regnans*, *Eucalyptus delegatensis* and *Eucalyptus nitens*), myrtle beech and silver wattle were sampled. A summary of the stand characteristics can be found in Table 2. Mean values of tree height, canopy cover and DBH show the structural differences between the vegetation in both study sites. While Rushworth reference area has smaller trees with a lower canopy cover, Watts Creek reference area has much bigger and dense canopy. At the same time, standard deviation values demonstrate Watts Creek rainforest bears a higher biodiversity.

The differences found between the spectra of eucalypt leaves were very small while the silver wattle presented lower absorption in the visible and higher absorption in the near- and shortwave- infrared (Figure 2). Those differences may be due to higher wax content in eucalypt leaves (Barry et al., 2009), or due to the thickness of the spongy mesophyll cells (Gausmann, 1977). In a study focused on Australian forests, Asner et al (2009) demonstrated that the vegetation spectral variability was mainly driven by biodiversity; our results may reaffirm these findings as in this case there is no relationship between leaf spectra and leaf pigment content or structure.



(a) (b)

Figure 2. Average reflectance spectra for the main four species present in Rushworth reference area (2a) and in Watts Creek reference area (2b).

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Table 2. Statistical overview of the structural, water content and specific leaf area values of the stands sampled in the two study sites

Study site	Spp	Tree height (m)	Canopy cover (%)	Diameter at breast height (cm)	Water content (g/cm^2)	Specific leaf area (g/cm^2)
Rushworth Reference Area n= 96	*Eucalyptus macrocarpa* *Eucalyptus macrorhyncha* *Eucalyptus tricarpa* *Eucalyptus polyanthemos*	Avg:25.69 Min: 6.2 Max:65.9 Stdev:14.73	Avg:27.18 Min:15 Max:45 Stdev:5.89	Avg:41.07 Min:14.3 Max:115.4 Stdev:21.88	Avg:0.021 Min: 0.014 Max: 0.028 Stdev:0.0023	Avg:37.64 Min:30.31 Max:73.70 Stdev:5.16
Watts Creek Reference Area n= 49	*Eucalyptus regnans* *Eucalyptus delegatensis* *Eucalyptus nitens* *Nothofagus cunninghamii* *Acacia dealbata*	Avg:43.35 Min:11.90 Max:70.0 Stdev:14.09	Avg:34.75 Min:20 Max:80 Stdev:14.28	Avg:103.97 Min:37.2 Max:227.0 Stdev:41.59	Avg:0.021 Min:0.012 Max:0.024 Stdev:0.0027	Avg:57.74 Min:43.64 Max:85.68 Stdev:8.52

Conclusions

This paper presents a leaf sampling protocol used in two study sites representative of Victorian forests. The aim of leaf collection was to quantify leaf pigment and nutrient content for each of the selected stands. Additional stand structural information was collected for a full characterization of every sampled tree. Moreover, leaf spectroscopy, specific leaf area and dry matter and water content were measured. The protocol presented could be used for leaf sampling in similar Australian study sites. Results are consistent, and show small spectral differences between leaves collected from eucalypt species; and spectra collected from other species such as *Acacia dealbata*. These results reinforce the notion of utilising spectral differences to characterise species richness and biodiversity.

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