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# Landscape-scale analysis of nutritional traits of New Zealand tree foliage using near-infrared spectroscopy

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ABSTRACT

Near-infrared reflectance spectroscopy (NIRS) was used to predict the nutritional quality of tree foliage from a New Zealand forest inhabited by the introduced common brushtail possum (*Trichosurus vulpecula*) as part of a study to understand the relationship between possum browsing and the nutritional quality of foliage. The traits of interest were the foliar concentrations of total nitrogen, *in vitro* dry matter digestibility, and available nitrogen (with and without incubation with polyethylene glycol to indicate the effect of tannins). A total of 1399 samples from five plant species were studied, with 650 included in a calibration set and 50 in a separate validation set. We evaluated the performance of classic (GLOBAL) and alternative (LOCAL) calibration techniques. Both were successful, although a GLOBAL equation incorporating all calibration samples was used for the final predictions. Modelling of the spectra enabled the nutritional quality of the foliage samples to be estimated with coefficients of determination ($r^2$) of 0.83–0.99 using modified partial least-squares regression. Nutritional quality varied both within and between five tree species for which possums show varied preferences. The predictive models developed in this study facilitate landscape-scale ecological investigations into the role of plant nutrients on the behaviour and demography of introduced herbivores. This provides new opportunities to test theories on invasiveness and to prioritize landscapes for conservation and possum control.



## 1. Introduction

The common brushtail possum (*Trichosurus vulpecula*) is an invasive pest in New Zealand, causing extensive damage to native forests through over-browsing (Nugent et al., 2000; Cowan, 2001). Possums exist in varying densities throughout New Zealand forests and it is likely that this is due in part to variations in the nutritional quality of the foliage. Current programs for the control of possum populations in New Zealand rely on baiting and trapping, so landscape-scale analysis of the nutritional quality of forest foods could assist possum control programs and help to identify areas at risk. Few studies have examined the nutritional quality of foliage in New Zealand on a large scale, but understanding broader patterns of demography and possum behaviour is critical to managing New Zealand forests.

Some useful measures of nutritional quality for browsing mammals include total nitrogen, dry matter digestibility, and available nitrogen (AvailN; a trait that accounts for the effects of tannins and fibre on nitrogen digestion). A further measure, AvailNP (which includes incubation with polyethylene glycol), can be used to deduce the strength of the tannin effect. These nutritional traits have previously been shown to influence possums' food choices and are thus associated with habitat suitability for possums and other browsing mammals (Moore and Foley, 2005; DeGabriel et al., 2009), with preferred foliage having a high AvailN concentration (Stalenberg et al., 2014; Jensen et al., 2015).

Measuring each of these nutritional factors in a large number of samples is expensive and time consuming. The AvailN method can take up to two weeks for only 100 samples and uses expensive reagents, but near-infrared reflectance spectroscopy (NIRS) is a viable alternative method, which would facilitate the application of these analyses more broadly. NIRS allows the chemical composition of many samples to be analysed rapidly, cheaply and repeatedly and is widely used in several industries such as the pharmaceutical industry (Roggo et al., 2007), food and agriculture (Alomar et al., 2003; Boval et al., 2004; Van Waes et al., 2005; Zornoza et al., 2008; Sánchez et al., 2012; Ren et al., 2013; de Oliveira et al., 2014), and ecology (Woolnough and Foley, 2002; Takahashi et al., 2011; Windley et al., 2013). The potential application of NIRS to ecology has been reviewed previously (Foley et al., 1998). The NIRS method relies on developing statistical models that relate the near infrared spectra of organic material to the laboratory analysis of a representative "calibration" subset of those

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samples. After developing this calibration model, the chemical composition of future samples can be estimated from the spectra alone with high precision in most cases. This would allow forest managers in New Zealand to make decisions about control measures, such as baiting and trapping, based on forest quality. For example, high nutrient quality forests may justify greater investment in control compared to low quality forests.

In ecological work, the materials of interest are usually much more heterogeneous than those investigated in industrial settings. Specifically, in studies of forest ecology, NIRS calibrations involving multiple plant species are sought whereas in agriculture, calibrations are focused more narrowly (e.g., monocultures). Such calibrations are likely to have greater errors than those using more homogenous materials. To address this issue, alternative calibration methods have been proposed.

GLOBAL calibrations (WinISI III software, Infrasoft International, Port Matilda, PA, USA) use the whole sample population but for any single sample, many of the calibration samples are so spectrally distinct as to contribute little to the precision and accuracy of the analysis. An alternative approach termed "LOCAL" calibrations includes only those samples from the calibration set that are spectrally similar to the unknown (Shenk et al., 1997). Typically the precision of prediction in analysis of pasture quality and even animal faeces has been improved by use of the LOCAL approach (Sinnaeve et al., 1995; Tran et al., 2010). The limitation of LOCAL calibrations is the need for large calibration databases whereas robust calibrations of the quality of tree foliage using the GLOBAL approach have been developed with as few as 50 samples (Ebbers et al., 2002).

In this study we aimed to (i) determine whether robust NIRS calibration equations can be established in a multi-species dataset of New Zealand trees; specifically for measuring nutritional traits that are relevant to the browsing behaviour of introduced possums, (ii) evaluate the performance of GLOBAL and LOCAL calibration techniques for this data set, and (iii) report some preliminary analysis of the nutritional quality of foliage in a New Zealand Mountain Range that highlight the spatial variability of forest quality.

## 2. Material and methods

### 2.1. Collection site and design

New Zealand's Tararua Mountain Range (40.77°S, 175.38°E) runs northeast–southwest for 80 km from near Palmerston North to the Hutt Valley. In places, the summits of the Tararua Range are in excess of 1500 m above sea level. Two 5 km transects within the Mountain Range (hereafter referred to as Line 1, and Line 2) were studied. The forest composition at Line 1 and Line 2 is typical of native forests in New Zealand, with relatively low tree diversity. The dominant tree species at the sites were *Nothofagus fusca* and *Weinmannia racemosa.* Rates of rainfall and wind run are high and there is a strong altitudinal gradient of soil quality, species diversity and temperature (Husheer, 2005). The two lines were 15 km apart running parallel, northeast, with an elevation of 300–400 m.

Sampling occurred over 10 days for each of four seasons; November 2010 (Spring), February 2011 (Summer), May 2011 (Autumn) and November 2011 (Spring). The same 297 trees were sampled each season. We collected leaf samples from five tree species on Line 2 (*W. racemosa*, *Myrsine salicina*, *Dacrydium cupressinum*, *Elaeocarpus dentatus* and *Melicytus ramiflorus*) but only *W. racemosa*, *M. salicina* and *D. cupressinum* were present at Line 1 (Table 1). These tree species were chosen based on their relative abundance at the study sites and to ensure that the samples reflected a spectrum of highly favoured to unfavoured foliage of brushtail possums in New Zealand; with *M. ramiflorus* and *M. salicina* considered to be highly favoured by possums, and to a lesser extent, the foliage of *E. dentatus* and *D. cupressinum*. Based on its local abundance and proven importance as a staple diet tree for possums in New Zealand (Nugent et al., 2000; Forsyth and Parkes, 2005; Duncan et al., 2011), *W. racemosa* was the focal species and was sampled consistently. Occasionally two samples were taken from *W. racemosa* (each from a different part of the tree) for a separate study. These samples were included in the spectral data set for calibration development but were removed from the foliage quality results presented here.

### 2.2. Plant samples

We removed branches from the canopy with a shotgun. We plucked approximately 50 g (fresh weight) of mature leaf from the fallen branch and placed it in a labelled plastic zip-lock bag. The samples were immediately frozen on a bed of solid $CO_2$ pellets and remained frozen during transport and storage before being freeze-dried. We ground the dried samples to pass a 1 mm sieve in a Tecator Cyclotec Mill (Foss, Hillerød, Denmark) and stored them in sealed containers at 5 °C in the dark before collection of NIR spectra.

### 2.3. Collection and storage of spectra

We collected spectra of the ground samples ($n = 1399$) with a Bruker MPA Fourier Transform NIR Spectrophotometer (Bruker Optik GmbH, Ettlingen, Germany). The instrument is capable of 30 sample batch measurements using a circular sample carousel. We dispensed approximately 1.5 g of dry sample into 22 mm glass vials (MGlas AG) to provide a sample thickness of 15–20 mm and ensure zero transmittance.

We recorded the spectra between 12500 $cm^{-1}$ and 4000 $cm^{-1}$ (~800–2780 nm) at a resolution of 16 $cm^{-1}$ by co-adding 64 scans using an integrating sphere. There were 1154 variables in each spectrum. We imported the spectra to the WinISI III software package (Infrasoft International, Port Matilda, PA, USA) after conversion of wavenumbers to wavelengths, interpolation and standardisation in Matlab (The Mathworks, Inc., Natick, MA, USA). Repeatability of the spectra was measured by re-collecting the spectra for a small random selection of samples after all spectra had been collected, then calculating the root mean square (RMS) error for spectra of repeated samples. The average RMS error for repeated spectra was 0.0139 ($n = 16$) and was considered to be acceptable.

### 2.4. Plant species discriminant analysis

We developed a discriminant equation using partial least squares (PLS) regression in the WinISI package to determine if different species could be grouped according to their spectra. Calibrations to discriminate the five species of tree leaves were developed by applying several mathematical transformations to the spectra. See GLOBAL calibration method (below) for a description of these mathematical transformations. Calibration models included 607 samples divided by species (Table 2). The remaining 791 samples were considered the validation set and were predicted with the final equation.

### 2.5. Calibration set and calibration equation development

We identified samples from the 1399 spectra to make up a calibration set and a validation set using the WinISI package as described by Shenk and Westerhaus (1992, 1994). Using the CENTER algorithm, samples were ranked according to their