

Near infrared spectroscopy for rapid assessment of foliar nitrogen and phosphorus



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INTRODUCTION

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Site preparation and early fertiliser application is essential when planting tree seedlings in commercial forest plantations.¹ Among the seventeen essential nutrients required by plants, nitrogen (N) and phosphorus (P) are key macro-elements for growth. During planting it is common to add both nitrogen and phosphorus in fertiliser along with other macro- and micro-nutrients depending on the local soil conditions. To monitor the response to fertiliser application, foliar samples are often collected from trees in designated fertiliser response trials to provide feedback on the availability of the nutrients. Foliar analysis is traditionally conducted using chemical methods of analysis, which are costly and labour intensive, and while high-throughput elemental analysers are used, the sample throughput is limited. The nature of destructive sampling also precludes the ability to monitor individual leaves over time, which limits the ability to undertake longitudinal studies. Instead of monitoring an individual leaf over time, “matched pairs” or bulked samples are used, which introduces sampling errors associated with sample representation and inhomogeneity.² Research into fertiliser response of plantation tree species, such as *Acacia mangium* and *Eucalyptus pellita*, would greatly benefit from the ability to monitor the foliar response over time to correlate nutrient status with tree growth. However, due to destructive sampling, poor sample representation, low throughput and high cost, there is great difficulty in designing an experiment to separate the site and species response. To provide detailed data in longitudinal studies it is therefore desirable to implement a technology that is non-destructive to foliage and could be used for repeated measurements of individual leaves of a tree. Near infrared spectroscopy is already used in the forestry and wood products sector for rapidly and non-destructively predicting wood quality and wood properties. In recent years, NIR spectroscopy of tree foliage has been used to discriminate between species and hybrids^{3,4} and to monitor leaf physiology.⁵ It is therefore proposed that NIR spectroscopy would be well suited to non-destructively determine the foliar nutrient status of individual leaves in trees, before, and at regular intervals after, fertiliser application. This would enable the flush of individual nutrients into and out of leaves to be monitored without the confounding effect of destructive sampling introducing sampling error. The true benefit of rapid, large-scale foliar analysis comes in conjunction with NIR calibrations for soil nutrients, offering the ability to monitor the soil and foliage macronutrients in the field in real time in a non-destructive manner. This could provide a step-change in the ability of foresters and agronomists to evaluate controlled fertiliser trials and optimize the application of fertilizer to operational areas by assessing the availability and demand for nutrients at a local scale.

METHODOLOGY

Table 1. Fertiliser treatments for the nursery seedlings and the field trial plot at establishment.

Treat.ID	Rate (kg m ⁻³)	Fertiliser (N:P:K)	Comments
Nursery			
N-T1	0	Control	
N-T2	6	Osmocote 14:14:14	SOP
N-T3	3	Osmocote 14:14:14	SOP-50% application rate
N-T4	9	Osmocote 14:14:14	SOP+50% application rate
Field	(g tree ⁻¹)		
F-T1	0	Control	
F-T2	50	(NH ₄) ₂ HPO ₄ 18:46:0	Fertilised at 15 cm radius from stem, SOP- 50%
F-T3	100	(NH ₄) ₂ HPO ₄ 18:46:0	Fertilised at 15 cm radius from stem, SOP
F-T4	150	(NH ₄) ₂ HPO ₄ 18:46:0	Fertilised at 15 cm radius from stem, SOP+50%

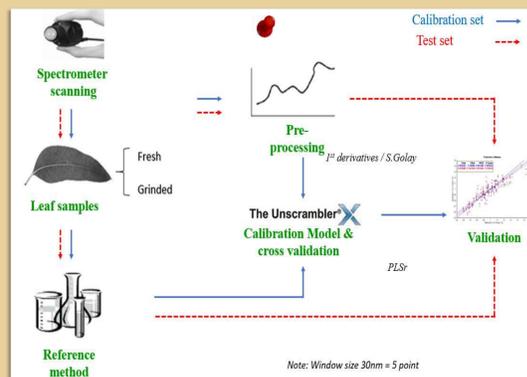


Figure 1. left. Process flow of calibration development and right is foliar sampling at nursery, field stage and sample scanning using a microNIR 1700 (Viavi Solutions, Milpitas, CA, USA)

NIR spectra were acquired both fresh on the tree and again after air-drying and grinding (Figure 1, right). Foliar nitrogen and phosphorus were determined on a subsample of foliage in an ISO laboratory following the methods of Kjeldahl and Bray-2 respectively. Partial least squares regression analysis of the NIR spectra and the nutrient values (Figure 1, left) was performed using The Unscrambler v10.4 (Camo Analytics, Oslo, Norway).

RESULT

Like all fresh biological samples, the NIR spectrum of foliage is dominated by the water absorbance bands near 1160 and 1452 nm (Figure 2). The dominance of these bands frequently degrades the overall performance of calibrations developed using NIR spectra from fresh samples, so that drying and preparing samples often improves calibration performance. Nonetheless, calibrations of foliar nitrogen and maybe phosphorus are possible using fresh NIR spectra of the foliage, acquired in situ on the tree. Calibrations developed using combined samples from the 10-week-old seedlings in the nursery and from trees in the field at 3-months and 6-months post planting, were used to predict the foliar nutrition using spectra acquired on the same trees at 9-months of age (Table 2). For foliar nitrogen it was possible to develop working calibrations using the raw spectra and any of the pretreatment algorithms, whereas for phosphorus using spectra acquired on the fresh foliage it was only possible to develop a calibration after orthogonal signal correction was applied. The calibrations for total foliar nitrogen are suitable for use in a screening process while the phosphorus calibration should be used with care and is suitable only for a qualitative, high/low classification.

Table 2. PLS regression statistics for calibrations developed from *E. pellita* dry foliage using nursery (10-week), 3-month, and 6-month samples, and the prediction of the 9-month samples as an independent test set.

Element	Pretreatment	LV	R ² _C	RMSEC (g 100g ⁻¹)	r ² _{CV}	RMSECV (g 100g ⁻¹)	RPD
Nitrogen	1SG7+SNV	3	0.90	0.19	0.88	0.21	3.0
Phosphorus	1SG7+SNV	4	0.77	0.019	0.71	0.021	1.9

1SG7 = Savitzky-Golay first derivative with 7-point window
 RMSEC, RMSECV = root-mean-square error of calibration, cross-validation
 RPD = ratio of performance to deviation

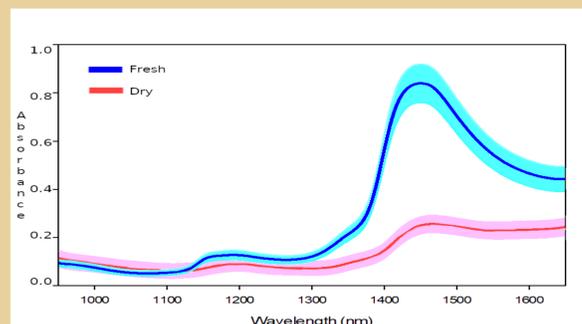


Figure 2. Mean spectra with standard deviation for NIR spectra acquired from *E. pellita* fresh foliage (blue) and dry, milled foliage (red).

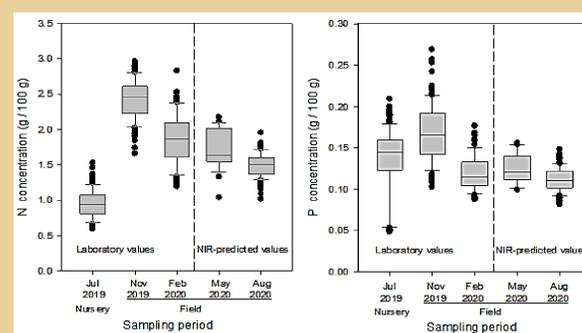


Figure 3. Box-whisker plots showing the distribution of nitrogen and phosphorus concentrations collected from *E. pellita* leaves at each stage of sampling in the nursery and at 3, 6, 9 and 12-months post planting.

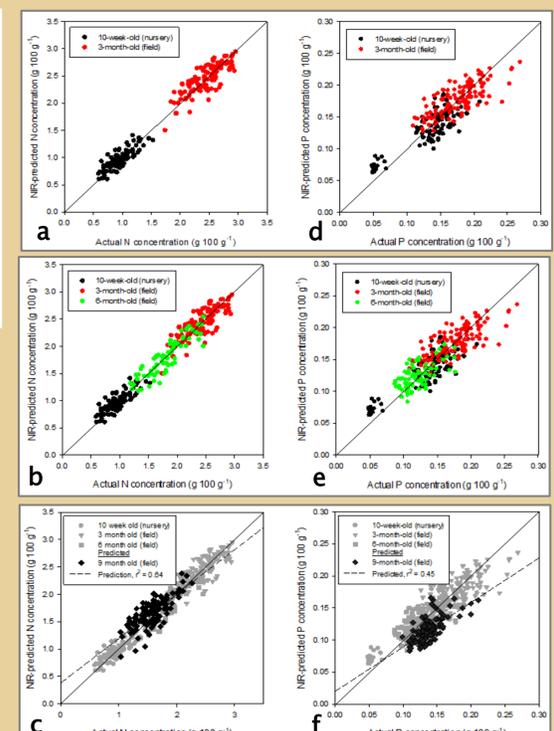


Figure 4. NIR-predicted vs measured calibration plots of dry *E. pellita* foliar nitrogen (a-c) and phosphorus (d-f) for: (a,d) nursery-stage and 3-month-old data, (b,e) nursery-stage, 3-month-old and 6-month-old data, and (c,f) predicted foliar nitrogen in 9-month-old samples (black-dot) superimposed on the calibration. N: raw spectra, P: spectra with 1st derivative + SNV transformation. The diagonal line represents the line of unity Y=X

CONCLUSION

This study has shown the development and validation of PLS regression calibrations for foliar nitrogen and foliar phosphorus in the foliage of *Eucalyptus pellita* from nursery to 9 months of age in the field. Calibration of foliar N and P on fresh leaves was successful for N and problematic for P, which is consistent with NIR foliar analysis in other crops. Calibrations developed using spectra acquired on dry, milled foliage were improved with fewer latent variables required, lower RMS errors and larger values for R²_c and r²_p.

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