An assessment of diffuse reflectance mid-infrared spectroscopy for measuring soil carbon, nitrogen and microbial biomass

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Abstract
Surface soils from three land-use and land-use change studies in southern Australia were used to explore mid-infrared spectroscopy (MIRS) coupled with partial least squares regression (PLSR) to measure soil total C, total N and microbial biomass carbon (MBC). The soils were from agriculture (crop and pasture), forest plantation, and native vegetation land-uses. Prediction on the validation set for total C and total N (R^2 = 0.94 and 0.86) were excellent, and that for MBC (R^2 = 0.53) was fair. The methodology has sufficient accuracy across a range of soils for application to determine the effects of land-use on these key indicator soil properties.

Key Words
Mid-infrared reflectance spectroscopy, soil, carbon, nitrogen, microbial biomass.

Introduction
Soil organic matter is heterogeneous with a wide range of functional types having variable turnover rates and nutrient release potential. Land-use and land-use change can affect the quantity and quality of soil organic matter, thus affecting soil properties and plant growth. For example, trees planted on land previously managed for agriculture (usually pastures) in southern Australia have benefited from the relatively high soil fertility arising from past fertiliser application and N-fixation by legumes. However, declines in N-availability over a rotation have been observed (e.g., O'Connell *et al.* 2003), and a challenge for plantation managers is to better understand such changes so as to maintain and build soil fertility.

Mid infrared reflectance spectroscopy (MIRS) has been demonstrated to be useful for the analysis of soils, including for total and various fractions of carbon, some nutrients, and soil texture (e.g., Janik *et al.* 2007, Viscarra Rossel *et al.* 2006). Once calibrated, it can be a cost-effective and rapid technique to assess soil fertility and health indices (e.g., Viscarra Rossel *et al.* 2008)

The work reported here draws on previous studies from a wide range of land-uses, soil textures and annual rainfall in southern Australia to assess the accuracy of MIRS to predict soil total C, total N and microbial biomass carbon (MBC).

Methods
Sites and sampling
Soils from three land-use / land-use change comparison studies were analysed:

- Pasture – *Eucalyptus globulus* plantation: Thirty one paired sites in south-western Western Australia, 0–10 cm depth, 4 replicates per site (n = 248) (O'Connell *et al.* 2003). Plantations were established on long-term pastures, and were sampled during late winter to early spring in the first rotation at 6 to 11 years of age.

- Crop – Remnant vegetation: Thirty paired sites in north-eastern, north-western and south-western Victoria, 0–10 cm depth, 6 replicates per site (n = 360) (Mele and Crowley 2008). The remnant vegetation included land under native vegetation, whereas the cropped soils had been managed conventionally for cereal, legume, vegetable, citrus and grape production.
- *Pinus radiata* plantation – Native forest (mixed *Eucalyptus* spp.): Eleven site-types across north-eastern Victoria and south-eastern NSW (3 or 4 sample plots per site-type), 0 – 5 and 5 – 10 cm depth, 3 replicates per plot (n = 204) (Kasel and Bennett 2007). The study comprised pine plantations in their first and second rotations (ex-native forest, ex-pasture), regenerated woodlands (ex-pasture, mixed eucalypt) and rehabilitated forest (ex-pine plantation, ex-pasture), and sampled during late spring and late autumn.

The three studies (n = 812) represented a wide range of soil textures (sandy to clay loam) and climate (mean annual rainfall 776 to 1400 mm). Each soil sample analysed was a composite of 6 to 9 cores.

Soil total C, total N and microbial biomass carbon
Total soil C and N were determined on finely ground (< 0.5 mm) and oven-dried (40^{o}C) subsamples using a Leco CN analyser. Microbial biomass carbon (MBC) was measured on rewetted and incubated soil subsamples (< 2 mm) by the fumigation extraction method (Sparling and West 1988), using an extraction factor (k_{EC}, 0.3 to 0.38) to convert the oxidisable organic-C flush to microbial-C, based on soil textures (Vance *et al.* 1987; Inubushi *et al.* 1991).

MIRS
Air-dried soil subsamples (< 2 mm) were finely ground in a vibrating puck mill for one minute. Mid-infrared diffuse reflectance spectra for these soils were collected using a PerkinElmer Spectrum One FT-IR spectrometer from 7800 to 450 /cm at 8 /cm resolution. Scans were co-added for one minute. A reference background spectrum was recorded at the start and after every thirty minutes or every 15 samples, whichever occurred sooner.

PLSR
Matlab (version 7.8.0.347) and PLS_Toolbox 4.2 were used to fit partial least squares regression (PLSR) calibration with leave-one-out cross-validations. Data were first transformed using a cube root (total C), square root (total N) or fourth root (MBC) to normalise the distributions. Spectra were preprocessed using multiplicative scatter correction (MSC-mean) followed by first derivative and auto scaling. One-third of the data, representing all land-uses, was randomly selected to form a validation subset and the rest used for the calibration subset.

Results and discussion
Total soil C varied from 5 to 187 g/kg, and total N from 0.25 to 7.52 g/kg (Figure 1a). Soil C to N ratio generally varied with land-use: lower (median = 16) for soils with a current or recent agricultural history (including *E. globulus* established on pasture) and higher (median = 21) for soils from forest / remnant vegetation land-uses (including *P. radiata* plantations). The overall correlation, between total N and total C was r = 0.86 (P < 0.001), and within land-uses correlations varied from 0.91 to 0.97 (P < 0.001).

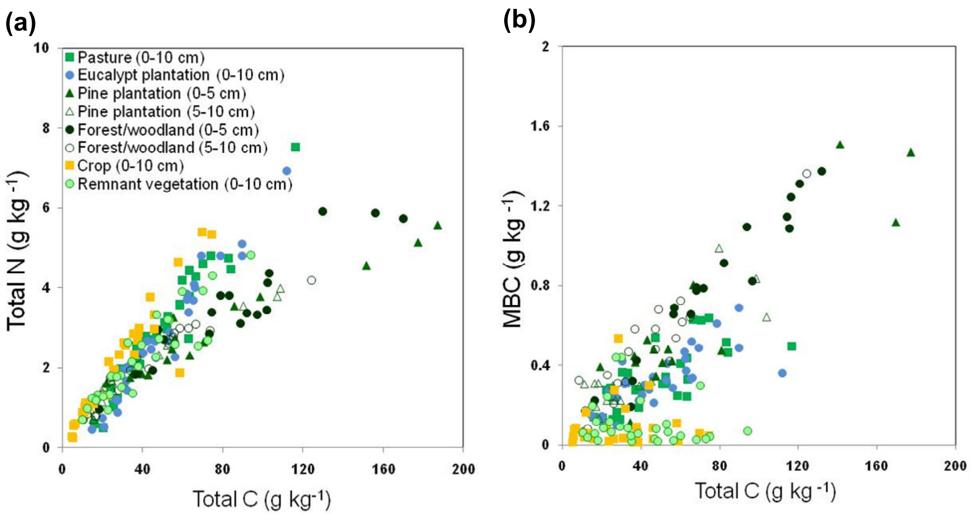

Figure 1. Scatter plots of (a) total N and total C, and (b) microbial biomass carbon (MBC) and total C. Values are means across replicate samples.

Soil MBC varied from 0.01 to 1.51 g/kg and was broadly correlated with total C (r = 0.76, P < 0.001, Figure 1b). Within land-uses, the correlation for cropped soils, generally having the lowest MBC values, was not significant, whereas correlations for other land-uses were significant (r = 0.72 to 0.96, P < 0.001).

For simple illustrative purposes here, pasture, native forest / regenerated woodlands and remnant vegetation were used as a reference land-use (RLU) to present the effects of a subject land-use (SLU, i.e. plantation or crop, Figs 2a, b, c). The present study is not concerned with analysing these effects, rather their likely magnitude in relation to the accuracy of predictions of soil variables using MIRS-PLSR. Over all data, the SLU: RLU ratios for average data from paired land-uses varied: for total C (0.14 to 1.85), total N (0.16 to 2.7) and MBC (0.14 to 3.24). Generally there was a wide scatter around the 1: 1 line, although notable was that total C and MBC were generally greater in the RLU, whereas there was little discernable difference between RLU and SLU for total N.

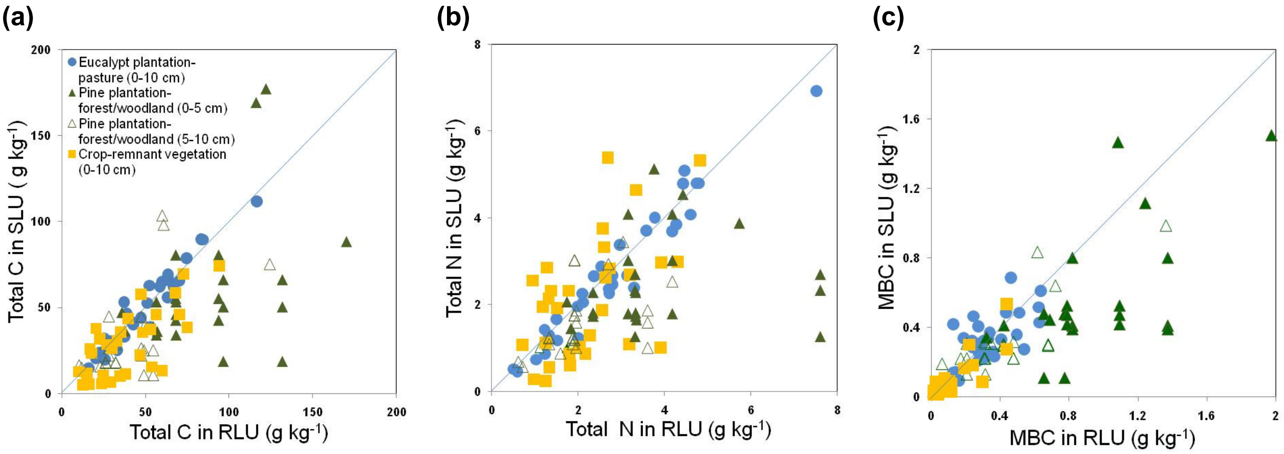

Figure 2. Scatter plots of (a) total C, (b) total N, and (c) microbial biomass carbon (MBC) in subject land-use (SLU) and that in reference land-use (RLU). SLU-RLU comparisons are: *E. globulus* plantation – pasture (WA), *Pinus radiata* plantation – native forest / regenerated woodland (Vic. / NSW), and crop – remnant vegetation (Vic.). Values are means across replicate samples.

All MIRS-PLSR calibrations (Table 1) were significant at P < 0.001. Back-transformed predictions for total C (Figure 3a) were excellent, both for calibration (R^2 = 0.96) and validation (R^2 = 0.94) data subsets. Predictions for total N (Figure 3b) were respectively very good (R^2 = 0.97) and good (R^2 = 0.86). The calibration prediction for MBC (Figure 3c) was good (R^2 = 0.73), despite MBC not being very well correlated with total C. MBC predictions for the validation subset had considerable scatter (R^2 = 0.53), although fair for microbial biomass (Stenberg and Viscarra Rossel 2008), and bias for the higher values.

Table 1. Statistics for MIRS-PLSR calibration and validation for total C, total N and microbial biomass carbon (MBC).

Variable	Total C	Total N	MBC
$n_{\text{calibration}}$	540	540	485
Transformation	Cube root	Square root	Fourth root
LV[A]	9	12	8
$R^2_{\text{calibration}}$	0.97	0.97	0.81
RMSEC[B]	0.14	0.08	0.08
RMSECV[C]	0.19	0.13	0.10
$n_{\text{validation}}$	264	264	237
$R^2_{\text{validation}}$	0.96	0.94	0.66
RMSEP[D]	0.18	0.15	0.15
Prediction Bias	-0.01	0.002	0.07

[A]Number of latent variables. [B]Root mean square error of calibration. [C]Root mean square error of cross-validation. [D]Root mean square error of prediction.

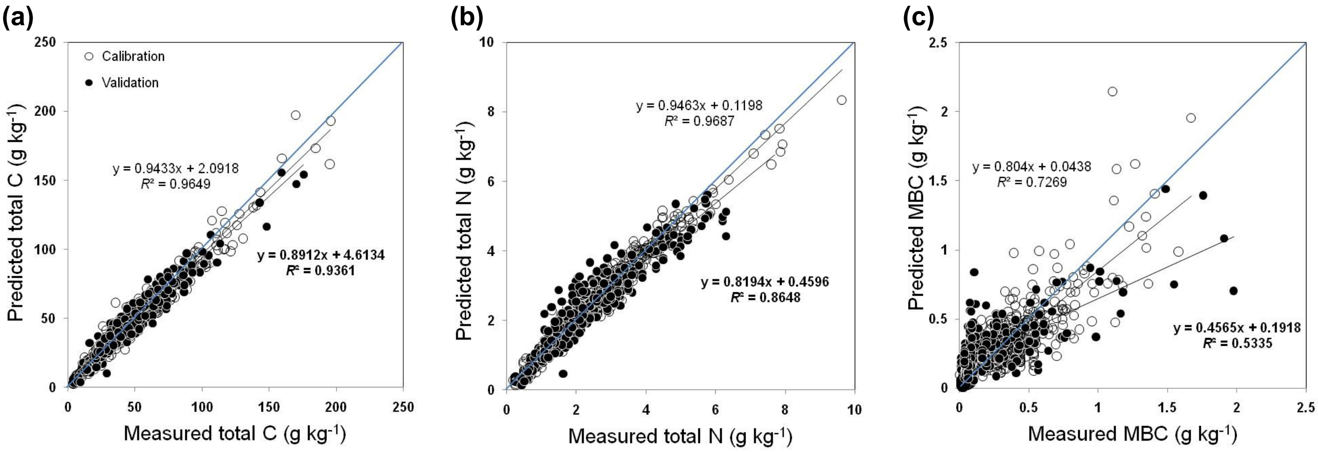

Figure 3. Relationships between the MIRS-PLSR predicted and the measured values for the calibration and validation (bold text) data subsets of (a) total C, (b) total N and (c) MBC

The present study illustrates that MIRS-PLSR predictive calibrations for total C and total N, at least, can be accurate across a wide range of soils. In the context of the magnitude of changes in these soil properties resulting from land-use and land-use change, the methodology provides rapid / cheap analysis and therefore allows for a greater application of resources to field sampling intensity or sample numbers, relative to laboratory analysis. The use of MIRS for predicting MBC requires further investigation, and may require development of local and temporal calibrations for this dynamic soil property.

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