Defining soil sample preparation requirements for MIR spectroscopic analysis using principal components

Jeff Baldock[A] and Bruce Hawke[B]

[A]CSIRO Sustainable Agriculture Flagship/CSIRO Land and Water, Urrbrae, SA 5051, Australia, Email jeff.baldock@csiro.au
[B]CSIRO Sustainable Agriculture Flagship/CSIRO Land and Water, Urrbrae, SA 5051, Australia, Email bruce.hawke@csiro.au

Abstract
Diffuse reflectance MIR (mid-infrared) spectroscopy when combined with PLS (partial lest squares) statistical analyses can be used to quantify various soil chemical properties. Of particular interest is the application of MIR/PLS analysis to provide a rapid and cost-effective methodology for predicting soil organic carbon content and its allocation to component fractions for use in soil carbon scenario modelling. The technique applies a beam of approximately 3.14 mm^2 (2 mm diameter circle) to the surface of a powdered soil sample to define chemical composition. Given the small area sampled, soil sample homogeneity and particle size become important issues to examine when interpreting the spectral results obtained. MIR analysis was completed on three different soils with variable texture that had been ground for durations ranging from 0 – 180s. Four aliquots of four replicate samples prepared for each soil by grinding time combination were analysed. Principal components analysis (PCA) was applied to the acquired MIR spectra to define to spectral variability and PLS analysis of the spectra was used to quantify the variability in predicted values for soil carbon and carbon fraction contents. PCA provided an excellent means of assessing the impact of grinding on the spectral homogeneity for each of the soils examined. The sandy soil required significantly more grinding time to reduce the variability of acquired MIR spectra. However the variability in predicted organic carbon and carbon fraction contents stabilised at shorter grinding times.

Key Words
Soil carbon, mid-infrared spectroscopy, principal components analysis, partial least squares analysis.

Introduction
Soil carbon accounts for a significant component of terrestrial carbon. Much interest exists in defining agricultural management practices that have the potential to capture and retain soil organic carbon (SOC) and thus mitigate anthropogenic greenhouse gas emissions. Management effects on SOC can be quantified through repeated temporal measurements; however, changes in SOC are slow and often require >10 years to become statistically significant. In the absence of archived soil samples, estimates of the impact of management on SOC can obtained through the application of calibrated computer simulation models.
In Australia, a variant of the RothC model has been calibrated for use in predicting management impacts on SOC (Skjemstad *et al.* 2004). In addition to calibrating the dynamics of total SOC, Skjemstad *et al.* (2004) also showed that measurable fractions of SOC could be substituted for the conceptual carbon pools originally defined within the RothC model. As a result estimates of management impacts can be obtained by measuring the current SOC content and its allocation to a set of measurable fractions. Determining the allocation of SOC to these measurable fractions is time consuming and expensive. Development of an accurate, rapid and cost-effective methodology to estimate the allocation of SOC to these fractions is required. Diffuse reflectance mid-infrared spectroscopy (MIR) coupled with a partial least squares (PLS) statistical analysis has been shown to offer such potential (Janik *et al.* 2007). The successful application of this MIR/PLS technique to predict carbon fractions requires the use of homogenised fine powdered samples given that only a 3.14 mm^2 area (2 mm diameter circle) of the surface of sample is analysed. Large soil particles (>0.5 mm) and in homogeneity can result in acquisition of results that are not representative of the entire sample, but instead are biased towards specific components. In this study, the influence of grinding time during sample preparation on the variability of MIR spectra acquired for a sand, loam and clay and subsequent predictions of the contents of SOC and carbon fractions was quantified.

Methods
Three soils (a sand from WA, a fine sandy loam from SA and a clay from SA) were dried overnight at 60°C (approximately 16 hours). The dried samples were ground in a Restch MM400 grinding mill set at 24 Hz. Approximately 10 g of soil was placed into a 35 ml milling cup lined with zirconium oxide and containing a 15 mm zirconium oxide milling ball. Four replicate samples of each soil were ground for durations ranging

from 0 s through to 180 s. For each of the four replicates, four separate MIR spectra were collected using a different aliquot of the ground sample giving. As a result, a total of 16 MIR spectra were collected for each combination of soil and grinding duration. All MIR spectra were acquired using a Nicolet 6700 FTIR spectrometer system fitted with Pike AutoDiff-Automated diffuse reflectance system. Spectra were acquired over 8000 – 400 cm^{-1}; however, in the subsequent analyses the spectra were truncated to 6000 – 1030 cm^{-1} and baseline corrected using a baseline offset transformation in which the lowest point of each spectrum is subtracted from all other spectral values. All transformations and subsequent analyses were completed using Unscrambler 9.8. Principal components analysis with full cross validation was used to assess the influence of grinding time on the homogeneity of the acquired spectra. A partial least squares analysis followed by prediction was used to define the influence of grinding duration on the prediction of SOC content and its allocation to fractions.

Results

The diffuse reflectance MIR spectra acquired for all 384 samples (soil type x grinding time x grinding rep x analysis rep = 3 x 8 x 4 x 4) are presented in Figure 5.
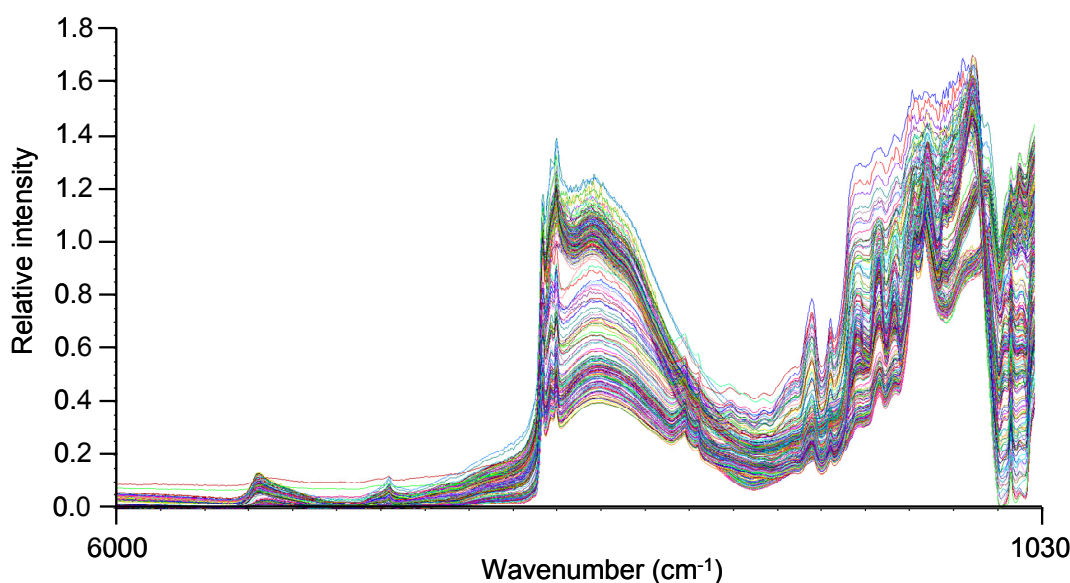

Figure 5. Diffuse reflectance MIR spectra acquired for all soil samples.

Application of principal component analysis of the spectra showed that 99% of the spectral variation could be explained using 4 principal components (Table 2). For all three soils the variability in acquired spectra decreased with increasing grinding duration, with the influence of grinding being more pronounced for the sandy soil than either of the other two soils (Figure 6). Little change in spectral variation occurred between 150 and 180 s of grinding. As a result, and to err on the conservative side, 180 s of grinding on the Restch MM400 grinding mill used to prepare the samples for MIR analysis.

To assess the impact of grinding on MIR prediction of total carbon and carbon fractions, these variables were predicted from the acquired MIR spectra using a previously derived PLS calibration (Janik *et al.* 2007). Figure 7 presents the predicted values obtained for total organic carbon (TOC), particulate organic carbon (POC) and the resistant charcoal fraction. As for the PCA analysis, the WA soil required a longer grinding duration to stabilise the predicted values. To ensure representative MIR/PLS predicted values, a grinding duration of >120 s was required for some samples.

Table 2. Percentage of spectral variance explained by the addition of each principal component to the overal model.

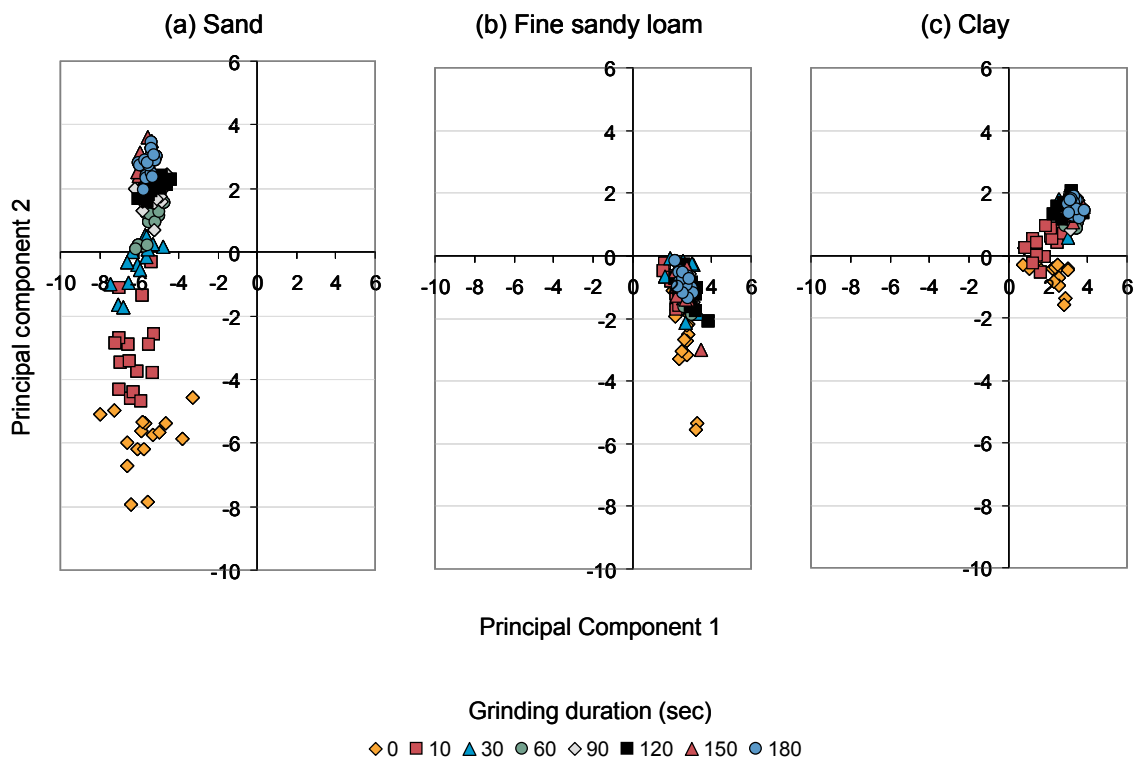| PC	Percentage of spectral variance explained
PC1	70.7
PC2	89.6
PC3	98.2
PC4	99.1

Figure 6. Principal component analysis of the impact of grinding time on variance in spectra for (a) a sand, (b) a fine sandy loam and (c) a clay.
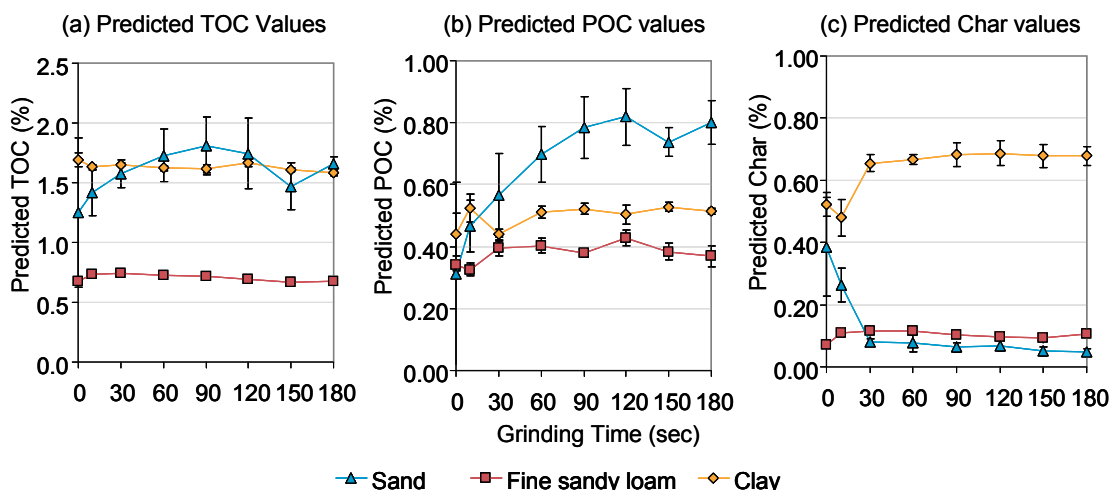

Figure 7. MIR/PLS predicted contents of (a) total organic carbon (TOC), (b) particulate organic carbon (POC), and (c) charcoal carbon as a function of grinding time.

Conclusions

The use of MIR spectra coupled to PCA and PLS analyses, provided an excellent methodology for defining the sample grinding time required to minimise MIR spectral variability and predictions of the contents of SOC and its component fractions. The response of the different soils to grinding duration varied, with the sandy soil requiring the longest times. Exposing the other soils to longer grinding durations did not adversely affect the MIR spectra or SOC predictions. Therefore when setting up a standard grinding protocol to be applied across many different samples, a duration of 180 s has been selected.

References

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