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Influence of number of samples on prediction error of visible and near infrared spectroscopy of selected soil properties at farm scale

Boyan Kuang* and Abdul Mounem Mouazen

Environmental Science and Technology Department, Cranfield University, MK43 0AL, United Kingdom,

Correspondence, b.kuang@cranifeld.ac.uk

Running Head: vis-NIR calibration

Summary

Although visible and near infrared (vis-NIR) spectroscopy has proved to be a fast, inexpensive and relatively accurate tool to measure soil properties, considerable research is required to optimise the calibration procedure and establish robust calibration models. This paper reports on the influence of the number of samples used for the development of farm-scale calibration models for moisture content (MC), total nitrogen (TN) and organic carbon (OC) on the prediction error expressed as root mean square error of prediction (RMSEP). Fresh (wet) soil samples collected from four farms in Czech Republic, Germany, Denmark and the UK were scanned with a fibre type vis-NIR, AgroSpec spectrophotometer (tec5 Technology for Spectroscopy, Germany) with a spectral range of 305 - 2200 nm. Spectra were divided into calibration (two-third) and prediction (one-third) sets and the calibration spectra were subjected to a partial least squares regression (PLSR) with leave-one-out cross

validation using Unscrambler 7.8 software (Camo Inc., Oslo, Norway). The RMSEP values of models with large sample number (46 - 84 samples from each farm) were compared with those of models developed using small sample number (25 samples selected from the large sample set of each farm) for the same variation range. Both large set and small set models were validated by the same prediction set for each property. Further PLSR analysis was carried out on samples from the German farm, with different sample number of the calibration set of 25, 50, 75 and 100 samples. Results showed that the large-size dataset models resulted in lower RMSEP values than the small-size dataset models for all the soil properties studied. The results also demonstrated that with the increase in sample number used in the calibration set, RMSEP decreased in almost linear fashion, although the largest decrease was between 25 and 50 samples. Therefore, it is recommended to chose the number of samples according to accuracy required, although 50 soil samples is considered appropriate in this study to establish calibration models of TN, OC and MC with smaller expected prediction errors as compared with smaller sample numbers.

Introduction

The urgent need to double farm production over the next 25 years using smaller land and water resources, through further intensification of agriculture will inevitably involve substantial social, economical and environmental cost. One of the strategies to increase productivity and economic profits while conserving the environment is Precision Agriculture (PA). PA is an environmental friendly strategy, in which farmers can vary input use and cultivation methods including application of seed, fertilizers, pesticides, water, planting and tillage to respond to variable soil and crop conditions across a field (Srinivasan, 2006).

Conventional measurement of soil spatial variability needed for the implementation of PA usually involves manual soil sampling, sample pre-treatment, laboratory chemical and physical analyses and mapping. This procedure is very expensive and time consuming because the implementation of PA needs analysis of numerous soil samples to characterise the soil spatial variability in the field. Therefore, the development of fast, cost effective and environmental friendly methods for the measurement of soil spatial variability is a preliminary task for the implementation of PA.

Visible and near infrared (vis-NIR) spectroscopy recently became a proven technique for a fast, inexpensive and relatively accurate alternative method to the laboratory analyses of soil properties (Viscarra Rossel & McBratney, 1998; Shepherd & Walsh, 2002; Mouazen *et al*, 2010; Stenberg *et al*., 2010). Today, intensive research is being carried out to establish new approaches, improve existing methods and combine several techniques of modelling to enhance the calibration accuracy of vis-NIR spectroscopy. Research indicates that there is a debate on the optimal size of sample set to be used to build calibration models with the largest possible accuracy. This requires careful consideration, as recent reports showed this to affect the robustness and accuracy of the calibration models developed (Kuang & Mouazen, 2011). Although a large number of soil samples might be a better option to characterise the soil variability than a small sample set, the cost of analysing a larger number of samples would be significantly higher. In general, the spectral libraries need to include sufficient soil samples to account for the soil variability in the new target site, where the prediction will be carried out (Viscarra Rossel *et al*., 2008; Guerrero *et al*., 2010). However, this requirement is not always fulfilled due to the large number of soil samples needed (Shepherd and Walsh, 2002; Brown *et al*., 2006). Spiking the local (target site) soil samples into the global or regional models proved to be an efficient way to improve the prediction accuracy of target field for some soil constituents (Shepherd & Walsh, 2002; Janik *et al*., 2007, Viscarra Rossel

76 *et al*., 2008, Guerrero *et al*., 2010). However, these studies did not compare the error resulting
77 from calibration models established with a large number of samples with that of significantly
78 smaller number of samples for *in situ* measurement conditions using fresh soil samples.
79 Considering dried soil samples collected from one area in Spain, Guerrero *et al*. (2010)
80 claimed that spiking could increase the prediction accuracy. The authors observed the
81 important influence of the number of samples in the calibration set, which controls the
82 adaptability of calibrations to target sites. They observed that a small-size model provided a
83 better prediction accuracy of soil total nitrogen (TN) than large-size model. This conclusion
84 is disputable because the authors did not use the same range of variation of soil properties for
85 both the large-size and small-size models. Comparing the accuracy of general calibration
86 models based on samples collected from three farms across Europe with that of farm specific
87 calibration models for total carbon (TC), TN and organic carbon (OC), pH and P, Kuang &
88 Mouazen (2011) found that larger standard deviations (SD) and wider variation ranges
89 resulted in larger coefficient of determination (R^2) values and ration of prediction deviation
90 (RPD), but also larger root mean square errors of prediction (RMSEP). However, those
91 authors did not test the effect of sample number on the calibration accuracy of these
92 properties for farm-scale modelling. Therefore, it will be interesting to establish how the
93 number of samples affects the farm-scale model accuracy when the range of properties is kept
94 constant. To our knowledge there is no report on the influence of the number of soil samples
95 used for farm-scale calibration on the prediction error of models developed for the same
96 range of variation of a soil property.
97 The aim of this study was to investigate the effects of the number of soil samples on
98 the prediction error of farm-scale calibration models of TN, OC and moisture content (MC)
99 developed using fresh soil samples collected from four farms in the Czech Republic,
100 Germany, Denmark and the UK. This was important to evaluate, since a smaller number of

calibration samples to be analysed by means of conventional chemical/physical techniques would mean a reduction in cost of analysis with vis-NIR spectroscopy.

Material and methods

Soil samples

A total of 399 soil samples were used in this study. They were collected from four different farms in Europe, namely Mespol Medlov, A.S. (Czech Republic, http://farmsubsidy.org/CZ), Wimex (Germany, http://www.wimex-online.de), Bramstrup Estate (Denmark, http://www.bramstrup.dk) (Kuang & Mouazen, 2011) and Silsoe Farm (the UK). Bulked samples from 16 cores were collected from the upper soil layer (0-30 cm) in the spring of 2008 (Czech Republic and Germany), spring of 2009 (Denmark) and summer of 2009 (UK) and represented a diverse range of soil conditions. A total of 111, 70 ,128 soil samples were, respectively, collected from six fields in Mespol Medlov, A.S. farm in Czech Republic, five fields in Bramstrup Estate farm in Denmark, five fields in Silsoe farm in the UK and 36 fields at the Wimex farm in Germany. The number of samples taken from each field was depending on the field size and was collected within the field with the aim of covering the whole field area. Table 1 provides information about the samples collected from different farms of this study.

About 200 g of soil was collected for each sample, which was carefully mixed, split into two halves and stored deep frozen at -18 °C. Half of each of the 200 g samples from the Czech Republic, Germany and Denmark was sent to Leibniz Centre for Agricultural Landscape Research (ZALF) in Germany for soil chemical analyses for TN and OC and the

second half was sent to Cranfield University for optical measurement and data analysis. Samples collected from the UK were subjected to both optical and chemical measurement at the National Soil Resources Institute (NSRI), Cranfield University.

Chemical analysis

The measurements of OC (total carbon) and TN were done by a TrusSpecCNS spectrometer (LECO Corporation, St. Joseph, MI, USA) using the Dumas combustion method. The OC was measured by Dumas combustion method after removing the soil carbonate by using Hydrochloric acid. The MC was measured by oven drying the samples at 105°C for 24 hour.

Optical measurement

Each soil sample was placed in a glass container and mixed well, after big stones and plant residues were removed. Then each soil sample was placed into three Petri dishes, which were 2 cm deep and 2 cm in radius. The soil in the Petri dish was shaken and pressed gently before levelling with a spatula. A smooth soil surface ensures maximum light reflection and high signal-to-noise ratio (Mouazen *et al*., 2005).

The soil samples were scanned in diffuse reflectance mode by an AgroSpec mobile, fibre type, vis-NIR spectrophotometer (tec5 Technology for Spectroscopy, Germany), with a measurement range of 305-2200 nm. Although this spectrophotometer does not cover the entire wavelength range in the NIR region, it was selected in this study as it uses diode array detectors, which proved to be stable under on-line measurement conditions (Mouazen *et al*., 2009). A 20 watt halogen lamp was used as a light source. A 100 % white reference was used

150 before scanning, which was repeated every 30 minutes. Three replicates were considered for
151 each sample and a total of 10 scans were collected from each replicate.
152 *Data pre-treatment and establishment of calibration models*
153
154 The triplicate raw spectra were averaged to one spectrum, followed by noise cut on both sides
155 arriving at a wavelength range of 371 - 2150 nm. A three-point (wavelength) average was
156 applied in the visible range and 10-point average was applied in the NIR range. This was
157 followed successively by maximum normalisation, Savitzky-Golay (S-G) first derivative and
158 S-G smoothing. Normalisation is typically used to get all data to approximately the same
159 scale, or to get a more even distribution of the variances and the average values. The
160 maximum normalisation method is a normalisation that "polarizes" the spectra. The peaks of
161 all spectra with positive values scale to +1, while spectra with values negative values scale to
162 -1. Since all the soil spectra in this study had positive values, the peaks of these spectra scaled
163 to +1 (Mouazen *et al*., 2005). Spectra were then subjected to the S-G first derivative (Martens
164 and Naes, 1989). This method enables computing the first or higher-order derivatives,
165 including a smoothing factor, which determines how many adjacent variables will be used to
166 estimate the polynomial approximation used for derivatives. A second order polynomial
167 approximation was selected. A 2:2 smoothing was carried out after the first derivative to
168 remove noise from the measured spectra. The same pre-treatment was used for all properties,
169 using Unscrambler 7.8 software (Camo Inc.; Oslo, Norway).
170 The pre-treated spectra and the laboratory measurement were used to develop
171 calibration models for the three soil properties, namely, OC, TN and MC. To study the effect
172 of the number of samples of the calibration set on the prediction error, two types of
173 calibration models were developed, namely large-size and small-size dataset models. For the
174 former models, two-thirds of soil samples from each farm were randomly selected. From

these two-third sample sets, 25 samples were randomly selected to develop the small-size dataset models. Particular attention was paid to ensure that the selection of 25 samples was carefully done to cover the full range of variation in soil properties on a farm. To eliminate the effect of different ranges of concentration of a property on the prediction error, the ranges were kept identical in both the calibration and prediction sets for both the large-size and small-size dataset models. The selection of calibration-prediction samples and the validation of PLS regression models were performed in the following sequences (Fig. 1):

1- The entire soil spectra for each farm were divided into calibration (two-third) and prediction (one-third) sets. The sample statistics for these two sets are provided in Tables 2 and 3, respectively.

2- A subset was selected from calibration samples, which was used as the small-size calibration set.

3- The calibration spectra of the large-size and small-size datasets were subjected to a partial least squares regression (PLSR) with leave-one-out cross validation using an Unscrambler 7.8 software (Camo Inc.; Oslo, Norway).

4- Both the small-size and large-size dataset models were validated on the same prediction set, extracted in step 1.

Further analysis was considered to evaluate the effect of different-size datasets of the calibration set models on prediction accuracy. This was done for the Wimex farm only, which was considered as an example. The number of samples in the calibration set was 25, 50, 75, and 100. Those 4 different-size dataset models were validated using the same 28 samples used as prediction set.

The accuracy of models developed was evaluated by comparing values of the root mean square error of cross validation (RMSECV), RMSEP and bias in prediction. Bias was

also considered to evaluate whether differences in RMSEP is due to bias or to the number of samples used in the calibration set.

Results and discussion

Prediction accuracy of models developed with large-size datasets

Table 4 summarises values of R^2, RPD, RMSEPCV and RMSEP, resulted from the large- and small-size dataset models for the prediction of TN, OC and MC. The accuracy of calibration and prediction varies from property to property and farm to farm. Examining values of R^2 and RPD, which is the standard deviation divided by RMSEP obtained from the large-size dataset models in all four farms, reveals that the prediction of soil MC is the most successful, as compared to OC and TN with R^2 = 0.80-0.96 and RPD = 1.98-4.69 for the calibration set and R^2 = 0.74-0.92 and RPD = 1.63-4.57 for the prediction set. The accuracy of predicting OC (R^2 = 0.58-0.90 and RPD = 1.30-3.08 for the calibration set and R^2 = 0.47-0.90 and RPD = 0.97-3.28 in the prediction set) is similar to that of TN (R^2 = 0.61-0.88, RPD = 1.27-3.33 for the calibration set and R^2 = 0.54-0.90, RPD = 1.19-3.33 in the prediction set), because OC and TN are strongly correlated (Table 5). Using crushed and air-dried soils samples, Brunet et al. 2007 reported farm scale calibration models accuracy for TN with square error of prediction (SEP) = 0.03-0.39 g kg^{-1}, which is comparable or higher than the majority of RMSEP values in this study (Table 4) even when fresh soil samples are used. Similarly, Moron and Cozzolino (2002) have built a farm scale organic carbon calibration model with RMSEP =5 g kg^{-1}, which is higher than most of farm scale models' RMSEP values reported in this study. The values of the RMSECV and RMSEP reported in Table 4 for MC are smaller than those

reported by other researchers with similar scale of calibration (Dalal *et al*., 1986; Slaughter *et al*. 2001; Mouazen et al. 2005).

All calibration models developed provide small RMSE, not only in the calibration set, but also in the prediction set (Table 4). The lowest prediction error (the smallest RMSEP) for the three properties studied, in both the calibration and prediction sets is for the farm in Denmark (Table 4), which is successively followed by the results for the Czech Republic, UK and German farms. These results confirm the conclusion obtained from a previous study (Kuang and Mouazen, 2011) that a larger range in concentration or a larger SD of samples in the calibration set result not only in higher R^2 and RPD, but also in higher RMSEP values. The smaller variation ranges of the three properties (Tables 2 and 3) reported for the Danish farm is the reason explaining why this farm had the smallest RMSEP, as compared to the other three farms. For a successful calibration, it is recommended to cover the widest possible concentration range in soil properties, so that the prediction is feasible for any new field or farm with any concentration that falls within the range of concentration of the calibration model. However, when the range of concentration of a property has to be decided in advance, the most meaningful accuracy criteria is RMSEP, as end users are interested to work with the smallest errors. A compromise selection of the range of concentration should be made to cover a wide range while preserving low RMSEP. Because the range of concentration of soil properties was kept identical between the small-size dataset and the large-size dataset models in the calibration and prediction sets for all four farms, the only criterion that could be used to assess accuracy was the RMSEP.

Comparison between large- and small-size dataset models

Comparing the accuracy of the large-size dataset models with corresponding small-size dataset (25 samples) models, estimated as R^2 values, provide mixed results for all four farms in both the calibration and prediction, although the former results in a higher accuracy in majority of farms. Regarding RPD, large-size models provided higher RPD values than the small-size models in both the calibration and prediction sets. Also, since the range was kept identical in the calibration and prediction sets, the RMSEP is the most valuable parameter to consider, as this reflects the error. Figure 2a, 2b and 2c show the RMSEP values calculated for TN, OC and MC, respectively, to be lower for the large-size dataset models than for the small samples-size models (in both the calibration and validation sets). However, the error differences between the two sets vary from farm to farm. Comparing the number of latent variables (LV) used during PLS in both the large- and small-size dataset calibration sets (Table 4), slight increases in the number of LVs for the former, as compared to the latter set can be observed. In some cases, the same LVs are used for both cases, whereas in another case a larger number of LVs is used to develop the small-size dataset model (e.g. for OC model in the German farm). Furthermore, a similar conclusion can be drawn if values of the bias of the two modelling procedures are compared (Table 4). The absolute values of bias are very small compared to RMSEP, thus the influence of bias is not important.

Furthermore, examining the scatter plot of the measured versus predicted values, no samples can be observed as outliers that might explain the higher RMSEP values of small-size datasets (Figures 3, 4 and 5). Therefore, it can be concluded that differences in RMSEP values resulted from the two-size dataset models can be barely attributed to the number of LVs, the presence of outliers in the data sets or bias, which confirms that these differences are mainly due to different number of samples considered during the development of calibration models for MC, OC and TN. There are two subgroups of soil samples in the data set, which

are because the physical and chemical values of MC, OC and TN in one field are significantly higher than that of other fields.

For global and local calibration schemes using dry soil samples, Shepherd and Walsh (2002) reported a decrease in R^2 and an increase in RMSEP values with the reduction in the number of soil samples used for calibration, which is in line with the results obtained in this study for farm-scale modelling under *in situ* measurement conditions. Guerrero *et al*. (2010) achieved more accurate calibration (higher R^2 and lower RMSEP) for local, small-size dataset models, after spiking with a few local samples, than models derived from very large libraries. This was attributed to the fact that, among the large number of samples in the very large library, there were only few soil samples that could describe the variability of the target fields. Since Guerrero *et al*. (2010) did not consider the same range of variation when comparing the performance of the small sample model with the large sample model, the lower RMSEP of the former model, as compared to that of the latter model, might result from the narrow range of concentration (Kuang and Mouazen, 2011). Wetterlind *et al.* (2008) reported a successful farm scale calibration model for soil organic matter using 25 soil samples only, but with a relatively high RMSEP of 3.2 gkg^{-1}, high R^2 of 0.89 and RPD of 3.0. Authors did not make it explicitly clear whether or not RMSEP could be improved by increasing the number of soil samples used for model development. Compared to other reports, the current study proves that large-size dataset models (> 25 samples) for TN, OC and MC result in smaller RMSEP values, compared to small-size dataset models of 25 samples, when the same concentration range is considered in both calibration sets.

Effect of dataset size on prediction error

Although, the increase in the number of samples can improve the prediction accuracy in terms of RMSEP, analysing large number of samples results in a significant increase in the cost of model development. To optimise the number of soil samples to be considered in the calibration set, so as to achieve accurate results at minimal cost, 25, 50, 75 and 100 soil samples from Wimex farm (Germany) were used to build four calibration models for MC, OC and TN. These models were validated on the same prediction set of 28 samples. Table 6 and Fig. (6) illustrate the decrease in RMSEP with increasing size of the data set. However, the rate of decrease varies, with the largest decrease occurring between models of 25 and 50 samples. This is particularly true for TN (Fig. 6a) and OC (Fig. 6b), whereas linear decrease is observed for MC. Although, no tipping point between sample number and RMSEP can be deducted, a minimum of 50 soil samples is recommended to obtain a reasonable accuracy at a minimal cost for farm-scale calibration of vis-NIR spectroscopy for MC, OC and TN. A balance between accuracy and cost has to be made to select the optimal number of samples in the calibration set, which will be governed by the degree of accuracy required for a given application of model output.

Conclusions

This study investigated the influence of size of the calibration set on prediction error of MC, OC and TN with the vis-NIR spectroscopy. The analyses were carried out under *in situ* (using fresh samples) measurement conditions at farm-scale in four different European countries. The following conclusions can be drawn from the results:

1. Individual farm-scale models for the four European farms can be successfully established with good accuracy.

2. When the same range of variation of a given soil property was preserved, the RMSEP is the best indicator of accuracy; this is important because farmers and land managers are interested in the error of measurement of soil properties.
3. The large sample data set models produced lower RMSEP than the small sample data set models (25 soil samples), in both the calibration and prediction sets for the three soil properties studied.
4. The RMSEP decreases with sample number in linear fashion for MC, whereas for OC and TN the largest decrease occurred between models with 25 and 50 samples.

Overall, the number of samples to be used in farm scale calibration models for MC, OC and TN depends on the accuracy required. However, using around 50 soil samples to establish calibration models for MC, OC and TN at farm-scale modelling is considered appropriate, as it will result in smaller prediction errors than other models with smaller sample numbers. Increasing the number of samples beyond 50 samples would lead not only to increase accuracy but also to increase cost. In the future, field scale models might need to be developed to establish a quantitative relationship between number of samples and RMSEP.

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