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Determination of glomalin in agriculture and forest soils by near-infrared spectroscopy

Jiří ZBÍRAL*, David ČIŽMÁR, Stanislav MALÝ, Elena OBDRŽÁLKOVÁ

Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic
**Corresponding author: jiri.zbiral@ukzuz.cz*

ABSTRACT

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Determining and characterizing soil organic matter (SOM) cheaply and reliably can help to support decisions concerning sustainable land management and climate policy. Glomalin was recommended as one of possible indicators of SOM quality. Extracting glomalin from and determining it in soils using classical chemical methods is too complicated and therefore near-infrared spectroscopy (NIRS) was studied as a method of choice for the determination of glomalin. Representative sets of 84 different soil samples from arable land and grasslands and 75 forest soils were used to develop NIRS calibration models. The parameters of the NIRS calibration model (R = 0.90 for soils from arable land and grasslands and R = 0.94 for forest soils) proved that glomalin can be determined in air-dried soils by NIRS with adequate trueness and precision simultaneously with determination of nitrogen and oxidizable carbon.

Keywords: soil organic matter; glomalin-related soil protein; simultaneous determination; method validation

Glomalin, a glycoprotein produced by arbuscular mycorrhizal fungi, was discovered and partially characterized in 1996 (Wright and Upadhyaya 1996). It is assumed that the first function of glomalin is to protect hyphae from water and nutrient loss. However, glomalin is also one of the factors that play an important role in the formation and stabilization of soil aggregates (Wright and Upadhyaya 1998, Rillig 2004). Glomalin presence increases water retention, nutrient cycling, reduces soil erosion and also contributes to the improvement of soil porosity, development of root systems, relevant soil enzyme activities and plant growth (Wang et al. 2015). Glomalin contains approximately 37% carbon and, in the soil environment, is characterized by persistence ranging from several months to years (Rillig 2004). Therefore, it is supposed to be an important part of the terrestrial carbon pool reducing atmospheric carbon dioxide levels. The role of glomalin in ecosystems and the influence of land use on its content and stability was studied by Treseder and Turner (2007) and Bedini et al. (2007), among others. It was found that glomalin can be used as an effective indicator of soil quality (Vasconcellos et al. 2016) and as one of the criteria to define agricultural management strategies (Fokom et al. 2013).

Characterizing glomalin as a separate and unique fraction of soil organic matter is a complicated task (Nichols 2003, Schindler et al. 2007). The link between glomalin and various protein fractions in soil is not clearly defined. Co-extraction of non-glomalin proteins cannot be avoided and glomalin-related soil protein (GRSP) was proposed as an operationally defined parameter correlating with the ecosystem parameters of interest (Rillig 2004). Although GRSP is only operationally defined and influenced by the extraction procedure and the method of determination, it can be used as a parameter relating to soil quality.

GRSP is usually determined after extraction from soils using 50 mmol sodium citrate at pH 8 at 121°C in several one hour cycles. Rosier et al. (2006) showed that the extraction process cannot

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eliminate all non-glomalin protein sources also determined by the Bradford assay (Bradford 1976) because the Bradford assay detects all peptides larger than 3 kDa.

Soil extraction followed by the Bradford assay determination was studied also by Koide and Peoples (2013). The authors found that even if the Bradford assay suffers from many technical difficulties (quantification of non-glomalin soil proteins, interferences from co-extracted phenolic substances) the method could effectively predict GRSP content in hot citrate soil extracts in mineral soils.

Near-infrared reflectance spectroscopy (NIRS) is a very fast non-destructive and environmentally friendly analytical technique. This method has proved to be very effective for the basic characterization of some soil constituents (Shepherd et al. 2005, Jia et al. 2014), for the prediction of some chemical and biological soil properties (Heinze et al. 2013), and for rapid and cost-effective quantification of some soil quality indices (Askari et al. 2015). Calibration equations reflect the relationship between the constituents of the sample and the NIRS spectral information (Stone 1974, Nas et al. 2002). Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ) has developed and optimized the NIRS method for determining carbon and nitrogen in soils and prepared this method for international standardization in ISO 17184 (2014). It was assumed that more information, including information about GRSP content, could be retrieved from the same NIRS soil spectra simultaneously.

This research was decided to focus mostly on these questions:

– Can the measurement and calibration procedure described in the ISO standard for carbon and nitrogen determination by NIRS be also applied for the simultaneous determination of GRSP?
– Are the reference method and the NIRS method applicable for the whole range of agriculture and forest soils and contents of GRSP?

MATERIAL AND METHODS

Soil samples. Soil samples from the UKZUZ regular monitoring plots, 68 on arable land and 24 on grasslands, were used for the study (soil types: Albeluvisol – 6 samples; Cambisol – 26 samples; Chernozem – 8 samples; Fluvisol – 1 sample; Gleysol – 6 samples; Haplic Luvisol – 25 samples; Leptosol – 3 samples; Phaeozem – 1 sample; Planosol – 6 samples; Regosol – 1 sample; Technosol – 1 sample (IUSS Working Group WRB 2006). The soils covered a wide range of soils with different content of organic matter (Table 1). Air-dried soil samples, fraction < 2 mm, were used for the study. 84 samples were used for calibration and 8 soil samples were used for external validation (Table 2).

81 forest soil samples from the F + H horizons represented the variability of forest ecosystems across the Czech Republic (soil types: Fluvisol – 28 samples; Cambisol – 28 samples; Albic Podzol – 20 samples, and Stagnosol – 5 samples). 75 soil samples were used for the calibration model (Table 1) and 6 for external validation (Table 2).

Reference method – Soil extraction and protein determination. The soil samples were extracted following the procedure described by Wright and Upadhyaya (1996). 8 mL of a 50 mmol/L so-

Table 1. Physico-chemical soil properties of samples used for calibration (arable soils and grasslands – 84 samples; forest soils – 75 samples)

	Arable and grassland soils			Forest soils		
	pH	C_{ox} (%)	GRSP (mg/g)	pH	C_{ox} (%)	GRSP (mg/g)
Minimum	3.6	1.1	1.1	3.3	3.7	6.6
1st quartile	5.0	1.5	2.4	3.6	7.8	13.2
Median	5.6	1.9	3.2	4.0	13.3	17.8
Mean	5.6	2.2	3.6	4.2	16.2	21.0
3rd quartile	6.2	2.5	4.6	4.5	25.7	25.9
Maximum	7.5	6.1	10.5	6.6	35.3	55.1

C_{ox} – oxidizable carbon; GRSP – content of glomalin-related soil protein determined by the reference method

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Table 2. Physico-chemical soil properties and the content of GRSP in samples used for external validation

Soil	pH	C_{ox} (%)	GRSP (mg/g)	GRSP NIRS (mg/g)[a]
Arable land and grasslands				
1	6.8	1.4	1.9	1.9
2	7.3	1.9	3.9	4.2
3	5.9	2.0	3.5	3.2
4	6.7	3.0	3.7	3.7
5	5.7	1.7	2.5	3.3
6	6.6	1.5	2.8	3.1
7	5.2	1.9	3.1	2.8
8	6.1	1.8	2.9	2.9
Forest soils				
1	4.3	10.2	13.7	12.3
2	3.9	15.6	19.4	16.8
3	5.3	26.3	25.8	23.1
4	4.7	23.8	31.3	32.1
5	5.7	41.9	32.8	33.0
6	4.4	34.4	35.9	38.3

C_{ox} – oxidizable carbon, GRSP – content of glomalin related soil protein determined by high-pressure extraction and Bradford assay. GRSP NIRS – content of glomalin related soil protein determined by near-infrared reflectance spectroscopy method

dium citrate solution (pH = 8.00) were added to 1.00 g of soil sample in a 30 mL plastic autoclavable tube and extracted by autoclaving at 121°C and 1.4 kg/cm^2 for 60 min. A steam sterilizer (75 S, H + P Labortechnik, Oberschleissheim, Germany) was used for the extraction. Centrifugation at 3700 g for 15 min was started immediately after autoclaving. The supernatant was decanted and stored at 4°C until analysis but not more than three weeks. The soil was re-suspended and the extraction step was repeated until only a light yellow colour of the supernatant was reached. Not more than 10 extraction cycles were used.

The protein content in the extract was determined by the Bradford method (Bradford 1976) using the commercially available Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Irvine, USA). The analysis was performed according to the instructions provided by the manufacturer. Precipitation after addition of the Bradford reagent was observed for forest soils (not detected for soils with low content of organic matter) and the method had to be optimized. Dilution of one volume of the soil extract with two volumes of the extraction solution was finally found suitable for preventing precipitation. After this change of the procedure the spectrophotometric determination was possible. The standard curve was prepared with bovine serum albumin as a standard (0–300 µg/mL). Standard solutions or soil extracts diluted in phosphate-buffered saline (10 µL) in three replicates were mixed with 200 µL of diluted dye reagent in wells of a 96-well flat-bottomed microplates using a shaker (30 s, 600/min). The mixture was incubated for 15 min. The absorbance was measured at 595 nm on a microplate reader (Versamax, Molecular Devices, Sunnyvale, USA). The dye reagent was prepared by diluting one part Dye Reagent Concentrate with four parts water and filtering through a Whatman #1 filter.

All selected samples were extracted and determined by the reference method in triplicate and the mean was used as a reference value.

NIRS measurement. The NIRS spectra were recorded by a FT-NIR instrument Nicolet Antaris II (Thermo Fisher Scientific, Waltham, USA). The reflectance spectra were measured from 4000 to 10 000/cm, resolution 2/cm. The soil samples were transferred to the sample compressed cells with 3 cm diameter and the surface was levelled. The spectra of the samples were scanned in 120 scans under continuous sample rotation. Windows of the sample cups were carefully cleaned by a gentle stream of compressed air between the individual measurements. The spectra were processed using the TQ Analyst 8 instrument software (Thermo Electron Corporation, Waltham, USA).

RESULTS AND DISCUSSION

NIRS calibration. The spectra and the results of the GRSP content determined by the reference method were used to calculate the NIRS calibration model. A scatter plot of reference values and NIRS predicted values for arable, grassland, and forest soils are given in Figure 1. Both calibration models were developed using:

(1) The standard normal variate (SNV) – to eliminate differences in particle size produced significant variation in the spectra of standards. The SNV correction removes the effects of scattering by normalizing the spectra individually.

(2) Statistical spectra diagnostic for selection of important wavelength range in spectrum. This diagnostic generates the spectral regions that cor-

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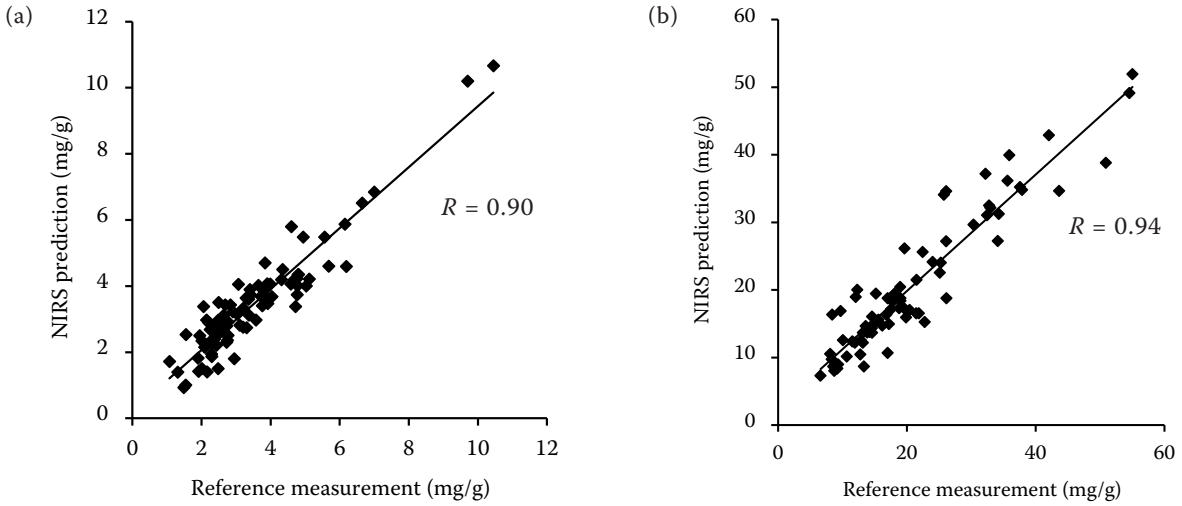

Figure 1. Scatter plot and regression curve for (a) arable soils and grasslands and (b) organic horizon of forest soils. NIRS – near-infrared reflectance spectroscopy

relate with changes in component concentration. For GRSP, the wavelength ranges of 4160–4468; 4610–5150 and 5338–9857/cm were found to bring maximum information and they were included into the final calibration model.

(3) Smoothing – the Savitzky-Golay algorithm was used with the 3rd order polynomial (Savitzky and Golay 1964) to reduce baseline variations and to enhance spectral features.

Calibrations were performed by partial least square (PLS) regression. Leave-one-sample-out-cross-validation was used to determine the optimum number of PLS components required to calibrate the models and then calculate the predicted values of the calibration subset in order to assess the robustness of the models. Eight PLS components were found to be an optimum for our calibration model. One sample was left out from the calibration set, and a model was built with the remaining samples. The left-out sample was predicted by this model, and the whole procedure was repeated by leaving out each sample in the calibration set (ISO 17184, 2014). The residuals of cross-validation predictions were pooled to calculate the root mean square error of cross validation (RMSECV). The RMSECV were calculated as:

$$RMSECV = \sqrt{\frac{\sum_{i=1}^{n_c}(\hat{y}_{ci} - y_{ci})^2}{n_c}} \quad (1)$$

Where: n_C – number of samples in calibration set; y_{ci} – reference measurement value of sample i, and $\hat{y}_{ci}$ – estimated value for sample i by the model constructed when the sample i – left out.

The final calibration model was chosen according to the global lowest RMSECV = 0.70 and R = 0.90 for soils from arable land and grassland and RMSECV = 3.8 and R = 0.94 for forest soils. The spectral properties of forest soils and agriculture soils were substantially different and therefore two separate calibration models were used – one for soils from arable land and grasslands with the content of GRSP up to 12 mg/g and the second for forest soils with the content of GRSP up to 60 mg/g.

Validation of the calibration models. The prediction ability of both calibration models was tested on independent sample sets (8 different soil samples for arable land and grasslands and 6 samples for forest soils) by external validation. The main characterization of the samples used for external validation and the results of the estimation of GRSP content are given in Table 2. The content of GRSP was determined using a reference method and NIRS in triplicate. The results were compared using the R 3.0.2 software by paired t-test. The analysis did not show any statistically significant difference between the reference method and the NIRS method (P = 0.55 for arable soils and grasslands, P = 0.54 for forest soils).

In conclusion, NIRS proved to be a very powerful technique to reliably and quickly determine GRSP. The method can substitute the relatively difficult and laborious determination of GRSP in soils by high-pressure extraction followed by Bradford protein determination. Our results support the results of many authors who used NIRS

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to determine a wide range of soil properties with this method (e.g. Heinze et al. 2013). Other soil parameters such as the content of oxidizable carbon (C_{ox}), total carbon and total nitrogen can be determined simultaneously from the same NIRS measurement (ISO 17184, 2014). Our future work will focus mainly on the widening the scope of the NIRS calibration for other SOM quality markers.

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