Characterization of soil organic matter by near infrared spectroscopy - determination of glomalin in different soils

Jiří Zbíral[1], David Čižmár[1], Stanislav Malý[1] and Elena Obdržálková[1]

[1] Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic

Correspondence to: J. Zbíral, (jiri.zbiral@ukzuz.cz)

Abstract

Determining and characterizing soil organic matter (SOM) cheaply and reliably can help to support decisions concerning sustainable land management and climate policy. Glomalin, a glycoprotein produced by arbuscular mycorrhizal fungi, was recommended as a ~~promising~~ possible indicator of SOM quality. Characterization of glomalin as an individual constituent of SOM was found to be difficult therefore glomalin-related soil protein (GRSP) was proposed as an operationally defined parameter. ~~But e~~Extracting ~~glomalin~~ GRSP from and determining ~~glomalin~~ GRSP in soils using classical chemical methods is too complicated and time consuming and therefore limits the use of this parameter in large scale surveys. Near infrared spectroscopy (NIRS) is a very rapid, non-destructive analytical technique that can be used to determine many constituents of soil organic matter including glomalin.

Representative sets of 84 different soil samples from arable land and grasslands and 75 forest soils were used to develop reliable NIRS calibration models for glomalin. One calibration model was developed for samples with a low content of ~~glomalin~~ GRSP (arable land and grasslands), the second for soils with a high content of ~~glomalin~~ GRSP (forest soils), and the third calibration model for all combined soil samples. Calibrations were validated and optimized by leave-one-sample-out-cross-validation (LOSOCV) and by the external validation using eight soil samples (arable land and grassland), and six soil samples (forest soils) not included in the calibration models.

Two different calibration models were recommended. One model for arable and grassland soils and the second for forest soils. No statistically significant differences were found between the reference and the NIRS method for both calibration models. The parameters of the NIRS calibration model (RMSECV = 0,70 and R = 0,90 for soils from arable land and grasslands and RMSECV = 3,8 and R = 0,94 for forest soils) proved that ~~glomalin~~ GRSP can be determined directly in air-dried soils by NIRS with adequate trueness and precision.

1. Introduction

Glomalin, a glycoprotein produced by arbuscular mycorrhizal fungi, was discovered and partially characterized in 1996 (Wright and Upadhyaya, 1996; Wright et al., 1996). It is assumed that the first function of glomalin is to protect hyphae from water and nutritient loss. But glomalin is also one of the factors that plays an important role in the formation and stabilization of soil aggregates (Wright and Upadhyaya, 1998; Rillig, 2004; Purin and Rillig, 2007). Glomalin presence increases water retention, nutrient cycling and reduces soil erosion. Glomalin 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 et al., 2001; Rillig, 2004). Therefore glomalin 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 (Fokom et al., 2012, Vasconcellos et al., 2013) and as one of the criteria to define agricultural management strategies (Fokom et al, 2013). Glomalin was assumed to be a sensitive indicator of soil carbon changes (Rillig et al., 2003).

Characterizing glomalin as a separate and unique fraction of soil organic matter is a complicated task (Nichols, 2003; Nichols and Wright, 2005; 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 ~~closely~~ to soil quality.

~~Glomalin~~ GRSP is usually extracted from soils using 50 mM sodium citrate at pH 8 at 121°C in several one hour cycles. Comparison of the efficacy of three different extractants was studied by Wright et al. (2006). The authors found that sodium pyrophosphate or sodium borate were more effective at extracting glomalin than sodium citrate. Rosier et al. (2006) showed that the extraction process cannot 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. Janos et al. (2007) studied autoclaving duration and delayed centrifugation, extraction ratio, spike recovery and denaturation of proteins during autoclaving. The authors concluded that although glomalin is partially denaturated by heat and pressure during extraction, autoclaving is necessary to efficiently remove glomalin from soils. They suggest paying attention to the extraction procedure (extraction volume, autoclaving time) and to the contact of soil with the extract after extraction (they recommend immediate centrifugation). On the contrary to these results Lu et al. (2011) found that for the total glomalin content there is no significance difference between centrifugation delays of up to two hours. Pérez et al. (2012) compared two extractants with different extraction effectivity for glomalin. They concluded that more aggressive extractants than citrate and extractants with a higher concentration are more likely to remove some additional fractions of humic substances.

Interferences in the Bradford assay caused by co-extracted humic substances for forest soils were studied by Jorge-Araújo et al. (2014). The authors concluded that the exact quantification of soil protein is complicated by the positive interference of non-proteins and the negative interference in the Bradford assay by co-extracted humic substances even though determination of GRSP can be useful for comparison of soil protein in different soils or for studies of variations for a given soil due to season, climate or land use.

Soil extraction followed by the Bradford assay determination has been 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 can effectively predict ~~glomalin~~ 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 is mainly used to analyse food, feed, pharmaceuticals etc. but it has proved to be a very effective method for the basic characterization of some soil constituents (Shepherd et al., 2005; McCarty and Reeves, 2006; Cheng-Wen Chang and Laird, 2002; Jia et al., 2014), for the prediction of some chemical and biological soil properties (Heinze et al., 2013), and for a 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 (Martens and Nas, 1989; Nas et al., 2002; Barnes et al., 1989; Stone, 1974; Williams and Norris, 2001). Central Institute for Supervising and Testing in Agriculture (UKZUZ) has developed and optimized the NIRS method for determining carbon and nitrogen in soils and prepared this method for international standardization in ISO TC 190 Soil quality (ISO, 2014). It was assumed that more information, including information about GRSP content, could be retrieved from the same NIRS soil spectra simultaneously.

We decided to focus our research mostly on these questions:

Can the measurement and calibration procedure described in the ISO standard for carbon and nitrogen determination by NIRS also be applied for the determination of GRSP?

Is the reference method and the NIRS method applicable for the whole range of agriculture and forest soils and contents of GRSP?

2. Materials and methods

2.1. 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~~ Albeluvisol 6 samples, ~~cambisol~~ Cambisol 26 samples, ~~chernozem~~ Chernozem 8 samples, ~~fluvisol~~ Fluvisol 1 sample, ~~gleysol~~ Gleysol 6 samples, ~~haplic~~ Haplic

luvisol Luvisol 25 samples, leptosol Leptosol 3 samples, phaeozem Phaeozem 1 sample, planosol Planosol 6 samples, regosol Regosol 1 sample, technosol 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. Eighty four samples were used for calibration and eight soil samples were used for external validation (Table 2).

Eighty one forest soil samples from the F+H horizons represented the variability of forest ecosystems across the Czech Republic (soil types: fluvisol Fluvisol 28 samples, cambisol Cambisol 28 samples, albic Albic podzol Podzol 20 samples, and stagnosol Stagnosol 5 samples). Tree species forest composition and elevation, soil type, distance from cities and distance from the forest edge were the main criteria of the sampling sites for the study (Borůvka et al., 2015). Seventy five soil samples were used for the calibration model (Table 1) and six for external validation (Table 3).

2.2. Reference method - Soil extraction and protein determination

The soil samples were extracted following the procedure described by Wright and Upadhyaya (1996). Eight millilitres of a 50 mmol l^{-1} sodium citrate solution (pH = 8.00) were added to 1.00 g of soil sample in a 30ml 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, 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 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, 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^{-1}). 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-1). The mixture was incubated for 15 min. The absorbance was measured at 595 nm on a microplate reader (Versamax, Molecular Devices, 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.

2.3. NIRS determination

The NIRS spectra were recorded on a Nicolet Antaris II (Thermo Fisher Scientific, U.S.A.). The reflectance spectra were measured from 1000 to 2 500 nm, resolution 0,5 nm. 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 TQ Analyst 8 instrument software (Thermo Electron Corp., USA). The spectra were transformed in two ways:

(1) using the Savitzky-Golay algorithm with a 3rd order polynomial (Savitzky and Golay, 1964) and smoothed to reduce baseline variations and to enhance spectral features (Reeves et al.,2002)

(2) using a standard normal variate (SNV) to correct a light scattering for different particle sizes.

Principal component analysis (PCA) was then performed to check the spectral homogeneity of the dataset.

3. Results and Discussion

3.1. Calibration

The spectra and the results of the GRSP content determined by the reference method (2.2) were used to calculate the NIRS calibration model. A scatter plot of reference values and NIRS predicted values for arable and grassland soils is given in Fig. 1 and for forest soils in Fig. 2.

Two calibration sample sets (84 soil samples from arable land and grassland and 75 forest soil samples) were selected to gain an evenly distributed coverage of the basic soil properties (Table 1) and most of the possible spectral variability. Calibrations were performed by partial least square (PLS) regression. Leave-one-sample-out-cross-validation (LOSOCV) 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, 2014; Centner et al., 1998). 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 is the number of samples in calibration set, y_{ci} is the reference measurement value of sample *i*, and $\hat{y}_{ci}$ is the estimated value for sample *i* by the model constructed when the sample *i* is 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. A calibration model covering all soils can be also derived by the same procedure and the results are acceptable but the spectral properties of forest soils and agriculture soils were substantially different (Fig. 3) and therefore we finally decided to use two separate calibration models - one for soils from arable land and grassland with the content of ~~glomalin~~ GRSP up to 12 mg g^{-1} and the second for forest soils with the content of ~~glomalin~~

GRSP up to 60 mg g^{-1}. It can be seen in the Figure 1 that the soil sample set for arable land and grasslands consisted mostly from samples with the GRSP content lower than 8 mg g^{-1} and only two samples had the GRSP content around 10 mg g^{-1}. Therefore a separate calibration model was developed to check the influence of these two samples on the regression curve and RMSECV. The calibration model was only slightly worse (RMSECV = 0,71 and R = 0,87) if compared with the calibration model for all samples (RMSECV = 0,70 and R = 0,90) and the results of GRSP determination in samples used for external validation were practically identical for both calibration models (Table 3).

3.2. Validation of the calibration models

The prediction ability of both calibration models was tested on independent sample sets (eight different soil samples for arable land and grasslands and six samples for forest soils) by external validation. The main characterization of the samples used for external validation and the results of the estimation of ~~glomalin~~ GRSP content are given in Table 2 and Table 3. The content of GRSP was determined using a reference method (2.2) and NIRS (2.3) in triplicate. The results were compared using 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).

3.3. Routine measurement of GRSP by NIRS

In a routine measurement, a principal component analysis (PCA) and a cluster analysis are applied to prove that the developed calibration model is valid for each individual soil sample. Samples that do not fulfil the statistical criteria are analysed by a reference method and the results are used to improve the calibration model. Other soil parameters such as the content of oxidizable carbon (Cox), total carbon and total nitrogen are determined simultaneously from the same NIRS measurement (ISO, 2014).

4. CONCLUSIONS

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 to determine a wide range of soil properties with this method (e.g. Peltre et al., 2011; Heinze et al., 2013). The NIRS method has been tested in a large scale in the national soil testing survey ~~where, among others, the determination of the GRSP/Cox ratio was calculated as one of the possible markers of soil organic matter (SOM) quality~~. Our future work will focus on the improvement of the interpretation of the NIRS results as well as widening the scope of the NIRS calibration for other SOM quality markers.

Naformátováno: Zarovnat do bloku, Mezera Před: 6 b., Řádkování: 1,5 řádku

References

Askari, M. S., O'Rourke, A. M., and Holden, N. M.: Evaluation of soil quality for agricultural production using visible-near-infrared spectroscopy, Geoderma, 243–244, 80–91, 2015.

Barnes, R. J., Dhanoa, M. S., and Lister, S. J.: Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra, Applied Spectroscopy, 43, 772–777, 1989.

Bedini, S., Avio, L., Argese, E., and Giovannetti, M.: Effect of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein, Agriculture, Ecosystems and Environment, 120, 463–466, 2007.

Borůvka, L., Sáňka, M., Šrámek, V., Vácha, R., Ash, C., Čechmánková, J., Čupr, P., Drábek, O., Fadrhonsová, V., Fraňková, A., Hofman, J., Houška, J., Horváthová, V., Hubová, P., Němeček, K., Nikodem, A., Rotter, P., Sáňka, O., Skála, J., Tejnecký, V., Vašíčková, J., and Jurkovská, L.: Metody hodnocení zátěže lesních půd rizikovými látkami a identifikace ekologických rizik kontaminace lesních půd, Závěrečná zpráva o řešení výzkumného úkolu NAZV číslo QI112A201, 2015.

Bradford, M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Analytical Biochemistry, 72, 248–254, 1976.

Centner, V., Massart, D. L., and de Jong, S.: Inverse calibration predicts better than classical calibration, Fresenius Journal of Analytical Chemistry, 361, 2–9, 1998.

Chang, C-W. and Laird, D. A.: Near-infrared reflectance spectroscopic analysis of soil C and N Soil Science, 167(2), 110–116, 2002.

Fokom, R., Adamou, S., Teugwa, M. C., Boyogueno Begoude, A. D., Nana, W. L., Ngonkeu, M. E. L., Tchameni, N. S., Nwaga, D., Ndzomo Tsala, G., and Zollo Amvam, P. H.: Glomalin related soil protein, carbon, nitrogen, and soil aggregate stability as affected by land use variation in the humid forest zone of south Cameron, Soil and Tillage Research, 120, 69–75, 2012.

Fokom, R., Teugwa, M. C., Nana, W. L., Ngonkeu, M. E. L., Tchameni, N. S., Nwaga, D., Rillig, C. M., and Zollo Amvam, P. H.: Glomalin, carbon, nitrogen and soil aggregate stability as affected by land use

changes in the humid forest zone in south Cameron, Applied Ecology and Environmental Research, 11(4), 581–592, 2013.

Heinze, S., Vohland, M., Joergensen, R., and Ludwig, B.: Usefulness of near-infrared spectroscopy for the prediction of chemical and biological soil properties in different long-term experiments, Journal of Plant Nutrition and Soil Science, 176, 520–528, 2013.

ISO 17184:2014, Soil quality – Determination of carbon and nitrogen by near-infrared spectrometry (NIRS), ISO, Geneva, 2014.

IUSS Working Group WRB World reference base for soil resources 2006: World Soil Resources Reports No. 103. FAO, Rome, 2006.

Janos, D. P., Garamszegi, S., and Beltran, B.: Glomalin extraction and measurement, Soil Biology & Biochemistry, 40, 728–739, 2008.

Jia, S., Yang, X., Zhang, J., and Li, G.: Quantitative analysis of soil nitrogen, organic carbon, available phosphorus, and available potassium using near-infrared spectroscopy combined with variable selection, Soil Science, 179, 211–219, 2014.

Jorge-Araújo, P., Quiquampoix, H., Matumoto – Pintro, P. T., and Staunton, S.: Glomalin-related soil protein in French temperate forest soils: interference in the Bradford assay caused by co-extracted humic substances, European Journal of Soil Science, 1–9, 2014.

Koide, R. T. and Peoples, M. S.: Behavior of Bradford-reactive substances is consistent with preditions for glomalin, Applied Soil Ecology, 63, 8–14, 2013.

Lu, H. J., Liu, D. H., Dong, Y. H., and Li, J. G.: Determination of factors affecting glomalin-related soil protein with Bradford method, Journal of Ecology and Rural Environment, 27(5), 93–97, 2011.

Martens, H. and Nas, T.: Multivariate calibration, John Wiley & Sons, Chichester, 1989.

McCarty, G. W. and Reeves, J. B. III. : Comparison of near infrared and mid infrared diffuse reflectance spectroscopy for field-scale measurement of soil fertility parameters, Soil Science, 171(2), 94–102, 2006.

Naformátováno: Písmo: Times New Roman, 12 b., Barva písma: Černá, Angličtina (Spojené království)

Nas, T., Isaksson, T., Fearn, T., and Davies, T. A.: A user-friendly guide to multivariate calibration and classification, NIR Publications, Chichester, 2002.

Nichols, K. A.: Characterization of glomalin: A glycoprotein produced by arbuscular mycorrhizal fungi, Ph.D. thesis, University of Maryland, College Park, MD, 2003.

Nichols, K. A. and Wright, S. F.: Comparison of glomalin and humic acid in eight native US soils, USDA-ARS/UNL Faculty, Paper 161,

Available at http://digitalcommons.unl.edu/usdaarsfacpub/161, 2005.

Peltre, C., Thuriès, L., Barthès, B., Brunet, D., Morvan, T., Nicolardot, B., Parnaudeau, V., and Houot, S.: Near infrared reflectance spectroscopy: A tool to characterize the composition of different types of exogenous organic matter and their behaviour in soil, Soil Biology & Biochemistry, 43, 197–205, 2011.

Pérez, A. B., Etchevers, J. D., del Gonzáles Chávez, M. C., Moreno, C. H., Monrela, C. M., and Prat, C.: Extraction of glomalin and associated compounds with two chemical solutions in cultivated tepetates of Mexico, Communications in Soil Science and Plant Analysis, 43, 28–35, 2012.

Purin, S. and Rillig, M. C.: The arbuscular mycorrhizal fungal protein glomalin: Limitations, progress, and new hypothesis for its functions, Pedobiologia, 51, 123–130, 2007.

Rillig, M. C.: Arbuscular mycorrhizae, glomalin and soil quality, Canadian Journal of Soil Science, 84, 355–363, 2004.

Rillig, M. C., Wright, S. F., Nichols, K. A., Schmidt, W. F., and Torn, M. S.: Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils, Plant and Soil, 233, 167–177, 2001.

Rosier, C. L., Hoye, A. T., and Rillig, M. C.: Glomalin-related soil protein: assessment of current detection and quantification tools, Soil Biology & Biochemistry, 38, 2205–2211, 2006.

Savitzky, A. and Golay, M. J. E.: Smoothing and differentiation of data by simplified least squares procedures, Analytical Chemistry, 36(8), 1627–1639, 1964.

Schindler, F. V., Mercer, E. J., and Rice, J. A.: Chemical characteristics of glomalin-related soil protein (GRSP) extracted from soils of varying organic matter content, Soil Biology & Biochemistry, 39, 320–329, 2007.

Shepherd K. D., Vanlauwe, B., Gachengo, C. N., and Palm, C. A.: Decomposition and mineralization of organic residues predicted using near infrared spectroscopy, Plant and Soil, 277, 315–333, 2005.

Stone, M.: Cross-validatory choice and assessment of statistical prediction, Journal of Royal Statistical Society, B39, 111–133, 1974.

Treseder, K. K. and Turner, K. M.: Glomalin in Ecosystems, Soil Science Society of America Journal, 71, 1257–1266, 2007.

Vasconcellos, R. I. F., Bonfim, J. A., Naretta, D., and Cardoso, E. J. B. N.: Arbuscular mycorrhizal fungi (AMF) and glomalin related soil protein (GRSP) as potential indicators of soil quality in a recuperation gradient of the Atlantic forest in Brazil, Land Degradation and Development, doi: 10.1002/ldr. 2228, 2013.

Wang, S., Wu, Q.-S., and He, X.-H.: Exogenous easily extractable glomalin-related soil protein promotes soil aggregation, relevant soil enzyme activities and plant growth in trifoliate orange, Plant, Soil and Environment, 61, 66–71, 2015.

Williams, P. C. and Norris, K.: Variables affecting near-infrared spectroscopic analysis, in: Near-infrared Technology in the Agricultural and Food Industries, American Association of Cereal Chemists, St. Paul, Minnesota, 171–185, 2001.

Wright, S. F. and Upadhyaya, A.: Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi, Soil Science, 161, 575–586, 1996.

Wright, S. F., Franke-Snyder, M., Morton, J. B., and Upadhyaya, A.: Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots, Plant and Soil, 181, 193–203, 1996.

Wright, S. F. and Upadhyaya, A.: A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi, Plant and Soil, 198, 97–107, 1998.

Wright, S. F., Nichols, K. A., and Schmidt, W. F.: Comparison of efficacy of three extractants to solubilize glomalin on hyphae and in soil, Chemosphere, 64, 1219–1224, 2006.

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	Cox (%)	GRSP (mg g-1)	pH	Cox (%)	GRSP (mg g-1)
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

CEC – cation exchange capacity, Cox – oxidisable carbon, GRSP – content of glomalin

Naformátována tabulka

Table 2. Physico-chemical soil properties and the content of ~~glomalin~~ GRSP in samples used for external validation – arable land and grasslands

	pH	Cox (%)	GRSP (mg g^{-1})	NIRS (mg g^{-1})[a]	NIRS (mg g^{-1})[b]
Soil 1	6.8	1.4	1.9	1.9	2.1~~1.9~~
Soil 2	7.3	1.9	3.9	4.2	4.2~~4.2~~
Soil 3	5.9	2.0	3.5	3.2	2.9~~3.2~~
Soil 4	6.7	3.0	3.7	3.7	4.4~~3.7~~
Soil 5	5.7	1.7	2.5	3.3	3.0~~3.3~~
Soil 6	6.6	1.5	2.8	3.1	3.0~~3.1~~
Soil 7	5.2	1.9	3.1	2.8	2.7~~2.8~~
Soil 8	6.1	1.8	2.9	2.9	3.2~~2.9~~

CEC – cation exchange capacity, Cox – oxidisable carbon, GRSP – content of glomalin determined by high-pressure extraction and Bradford assay; NIRS - content of GRSP determined by NIRS method (NIRS[a] calibration model for all samples, content of GRSP < 12 mg g^{-1}; NIRS[b] calibration model without two high samples, content of GRSP < 8 mg g^{-1}).~~, NIRS – content of glomalin determined by NIRS method~~

Naformátována tabulka
Naformátováno: horní index
Naformátováno: horní index

Table 3. Physico-chemical soil properties and the content of ~~glomalin~~ GRSP in samples used for external validation – forest soils

	pH	Cox (%)	GRSP (mg g^{-1})	NIRS (mg g^{-1})
Soil 1	4.3	10.2	13.7	12.3
Soil 2	3.9	15.6	19.4	16.8
Soil 3	5.3	26.3	25.8	23.1
Soil 4	4.7	23.8	31.3	32.1
Soil 5	5.7	41.9	32.8	33.0
Soil 6	4.4	34.4	35.9	38.3

Cox – oxidisable carbon, Ctot – total carbon, GRSP – content of glomaline determined by high-pressure extraction and Bradford assay, NIRS – content of glomalin determined by NIRS method.

Naformátována tabulka

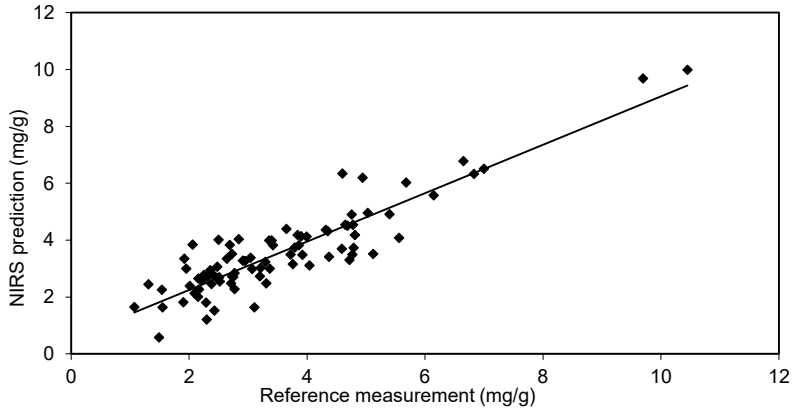

Figure 1. Scatter plot and r~~R~~egression curve for arable soils and grasslands. R = 0,90.

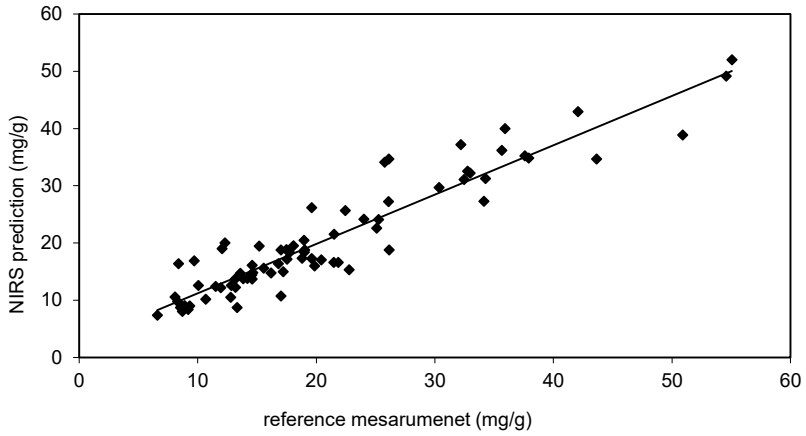

Figure 2. Scatter plot and r~~R~~egression curve for organic horizon of forest soils. R = 0,94.

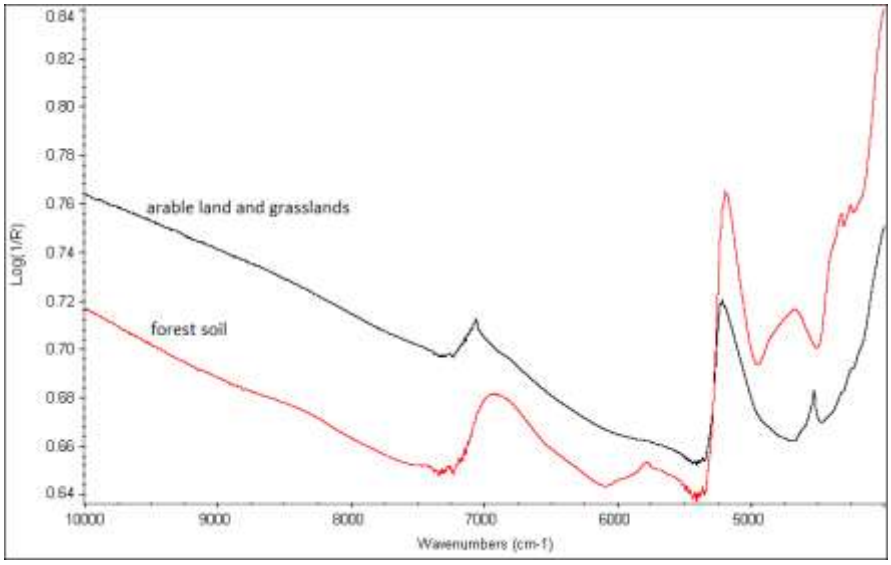

Figure 3. Example of ~~typical~~ NIRS spectra for a typical forest soil~~s~~ and ~~for~~ an arable and grassland~~s~~ ~~soils.~~soil.