Effect of organic fertilisers on glomalin content and soil organic matter quality

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Abstract: Glomalin is one of the factors with an important role at forming and stabilising soil aggregates. Long-term stationary experiments were carried out to observe the influence of various fertilisation treatments on the content of glomalin in topsoil. The content of easily extractable glomalin (EEG) and total glomalin (TG) were determined. Moreover, glomalin was also determined by using the near-infrared reflectance spectroscopy (G_{NIRS}). Both mineral and organic fertilisation significantly increased the content of glomalin compared to the unfertilised control. However, observed differences among individual fertilisation treatments were not significant. A significant correlation was determined between the content of EEG, TG, G_{NIRS}, and the content of humic substances as well as humic acids. Both methods used (EEG, TG) can equally reflect soil organic matter quality. A significant correlation was also recorded between the G_{NIRS} and extraction methods (EEG, TG).

Keywords: long-term experiments; mineral fertilisation; humic substances fractionation; glycoprotein; decomposition; quality indicator

Besides a range of soil organic matter quality indicators, a great attention has been recently paid to the glomalin content observations (Bedini et al. 2013). Glomalin is produced by arbuscular mycorrhizal fungi and is one of the most important soil proteins. This compound was discovered and named in 1996 by Wright and Upadhyaya (1996). Glomalin is a glycoprotein excreted by fungi *Glomeromycota* (glomales). The exact molecular composition has not been determined yet (Singh et al. 2013), because the glomalin fraction obtained by extraction is not pure enough (Schindler et al. 2007). This is the reason that some studies use the term "glomalin-related soil protein" or GRSP (Rillig 2004).

Glomalin is hydrophobic and thermally stable (the extraction is carried out in an autoclave at 121 °C). It is significantly resistant to decomposition in soil

(Rillig 2004, Gadkar and Rillig 2006, Nichols and Wright 2006).

The content of glomalin is influenced by the amount and type of organic fertilisation (Alguacil et al. 2008, Gispert et al. 2013, Singh et al. 2013). Lower glomalin concentrations were recorded in arable land compared to permanent grasses, which is probably related to higher aerification of cultivated soils and, thus, more intensive soil organic matter mineralisation (Smatanová and Komprsová 2019). Glomalin concentration is significantly lower in tilled soils compared to no-till systems (Curaqueo et al. 2011).

Glomalin is one of the factors with an important role at forming and stabilising soil aggregates. It thus significantly influences soil organic matter stability (Wright and Upadhyaya 1998, Wright and Anderson 2000, Rillig 2004, Driver et al. 2005, Emran et al.

2012, Galazka et al. 2017), as soil aggregates slow down soil organic matter decomposition (Jastrow 1996, Six et al. 2000). The presence of glomalin in the soil further increases water retention, prevents erosion, supports the development of roots, affects the activity of soil enzymes, and stimulates plant growth (Wang et al. 2015). Reduced soil glomalin concentration has been usually reported at sites where the soil structure was disturbed, e.g., by tillage or drought (Wright and Anderson 2000).

Glomalin content also varies in relation to the soil profile depth, as reported in the results of Galazka et al. (2017); they determined the highest content of easily extractable glomalin (EEG), total glomalin (TG), and glomalin-related soil protein (GRSP) in the 5–20 cm layer, whereas it was significantly lower in the 30–35 cm layer. Gispert et al. (2013) determined higher glomalin content in larger soil aggregates (2.0–5.6 mm) compared to the smaller ones (0.25–2.00 mm).

The impact of rotated crops selection is also significant: in long-term experiments in Skierniewice (Poland), the TG content was lower at plots with rye monoculture compared to those with rotated crops (potato/rye) (Wojewódzki and Cieścińska 2012). Steinberg and Rillig (2003) reported slight seasonal oscillations of glomalin content in the soil; it is thus possible that it may also vary during the vegetation period. For instance, Galazka et al. (2017) recorded significantly higher TG content in the soil during the maize growth compared to the soil glomalin content before sowing.

Generally, it may be concluded that the use of mineral and organic fertilisers increases the glomalin content in soils compared to the unfertilised control (Curaqueo et al. 2011, Dai et al. 2013, Turgay et al. 2015, Sandeep et al. 2016).

The evaluation of glomalin content as an indicator of changes in quality of soil fertility is due to its positive correlation with the changes of soil organic matter carbon content (C_{SOM}) (Wright and Upadhyaya 1996, 1998, Wilson et al. 2009, Dai et al. 2013, Xie et al. 2015). Contrariwise, results published by Galazka et al. (2017) show a negative correlation between the content of GRSP and C_{SOM}.

The aim of this paper was to study the effect of various fertilisation systems on soil organic matter quality using the glomalin content as an indicator, and further to quantify the relationship between the glomalin content and the content of humic substances, humic acids, and fulvic acids.

MATERIAL AND METHODS

The effect of fertilisation on the content of glomalin in soil was observed in long-term field trials established in 1996 at the experimental base Červený Újezd – 50°4'22''N, 14°10'19''E. Except for low potassium content, the content of other available nutrients (P, Mg, Ca) in soil (Mehlich 3) was sufficient (Table 1).

Within these trials, three crops were rotated in the following order: maize, winter wheat, and spring barley. Each year, all of the crops were grown. The size of the experimental plots was 80 m^2, every plot was divided into four sub-plots during harvest (20 m^2 each). The trial included seven treatments: (1) no fertilisation (control); (2) sewage sludge (SS1); (3) high dose of sewage sludge (SS3); (4) farmyard manure (FYM1); (5) half dose of farmyard manure + N in mineral nitrogen fertiliser (FYM1/2 + N); (6) mineral nitrogen fertiliser (N); (7) spring barley straw + N in mineral nitrogen fertiliser (N + St) (Table 2). Organic fertilisers (farmyard manure, sewage sludge, and straw) were always applied in autumn (October) to maize and were immediately incorporated in the soil to approximately 28 cm depth with ploughing. The remaining treatments were ploughed to the same depth at the same time. Mineral nitrogen fertilisers in the form of calcium ammonium nitrate were applied to maize and spring barley in spring, prior to the crop establishment. In the case of winter wheat, the dose of nitrogen was divided in half, with the first half applied at BBCH 21 and the second at BBCH 31–32. The content of nitrogen was 140 kg N/ha for wheat and 70 kg N/ha for spring barley; FYM1/2 + N

Table 1. Characteristics of the Červený Újezd experimental site

Altitude (m a.s.l.)	410
Mean annual temperature (°C)	7.7
Mean annual precipitation (mm)	493
Soil type	Luvisol
Soil texture	loam
pH$_{CaCl_2}$	6.5
C_{SOM} (%)	1.2
Cation exchange capacity (mmol$_+$/kg)	145
P – Mehlich 3 (mg/kg)	100
K – Mehlich 3 (mg/kg)	80
Mg – Mehlich 3 (mg/kg)	110
Ca – Mehlich 3 (mg/kg)	3 600

C_{SOM} – soil organic matter carbon content

Table 2. Experimental design

Treatment	Maize	Wheat	Barley	Organic fertiliser	
	N (kg/ha/year)			C (kg/ha/year)	C/N
Cont	0	0	0	0	0
SS1	330[1]	0	0	813	7.4/1
SS3	990[1]	0	0	2 439	7.4/1
FYM1	330[1]	0	0	1 482	13.4/1
FYM1/2 + N	165[1]	115[2]	50[2]	741	13.4/1
N	120[2]	140[2]	70[2]	0	0
N + St	120[2] + St	140[2]	70[2]	712	79.3/1

[1]Total dose of nitrogen applied in organic fertiliser; average yearly dose based on the analysis of farmyard manure and of sewage sludge; [2]Applied as follows: N – calcium ammonium nitrate (27% N); FYM – farmyard manure – average dose of fresh FYM was as follows: 51.42 t/ha/3 years (15.82 t of dry matter/ha/3 years); SS1 – sewage sludge – average dose of fresh sewage sludge was: 30.18 t/ha/3 years (9.38 t of dry matter/ha/3 years); SS3 – high dose of sewage sludge – average dose of fresh sewage sludge was: 90.54 t/ha/3 years (29.49 t of dry matter/ha/3 years); straw was applied in doses of 5 t of dry matter/ha/3 years

was applied at the rate of 115 kg N/ha for wheat and 50 kg N/ha for spring barley.

Soil analysis. Topsoil (depth of 30 cm) analyses were performed with air-dried soil samples ($\leq$ 2 mm) collected after harvest in September 2018. The content of total organic carbon in air-dried samples of soils, in farmyard manure, and in sewage sludge was determined using oxidation on the CNS analyser Elementar Vario Macro (Elementar Analysensysteme, Hanau-Frankfurt am Main, Germany).

Fractionation of humic substances (C_{HS}) was done according to Kononova (1963) to obtain the pyrophosphate extractable fraction, which represents the sum of the carbon in humic acids (C_{HA}) and fulvic acids (C_{FA}). In brief, C_{HA} and C_{FA} were extracted from a 5 g soil sample with a mixture of 0.1 mol/L NaOH and 0.1 mol/L $Na_4P_2O_7$ (1:20, v/v) solution. The carbon of humic substances C_{HS} and C_{HA} was determined by an oxidimetric titration method. The content of C_{FA} was calculated as the difference between C_{HS} and C_{HA}.

NIRS measurement of glomalin (G_{NIRS}) was performed, according to Zbíral et al. (2017). The NIRS spectra were recorded by an FT-NIR instrument Nicolet Antaris II (Thermo Fisher Scientific, Waltham, USA). The reflectance spectra were measured from 4 000 to 10 000/cm, resolution 2/cm. The spectra were processed using the TQ Analyst 8 instrument software (Thermo Electron Corporation, Waltham, USA). The NIR spectrometer was calibrated for TG at a completely different data set than the samples of our long-term trials.

Easily extractable glomalin (EEG) and total glomalin (TG) fractions were performed according to Wright and Upadhyaya (1998), i.e., to 1 g of ground dry-sieved soil 8 mL of sodium citrate (20 mmol/L of pH 7.0 – EEG, 50 mmol/L of pH 8.0 – TG) was added, followed with autoclaving at 121 °C (30 min – EEG, 60 min – TG), cooling down and centrifugation at 5 000 rpm (10 min – EEG, 15 min – TG). In the case of the TG, the centrifugation of supernatant of the same sample was repeated 5 times, until the supernatant no longer showed the red-brown colour typical for glomalin.

The results were assessed using the ANOVA statistical analysis with Tukey's test. The Statistica program (StatSoft, Tulsa, USA) was used.

RESULTS AND DISCUSSION

Individual treatments varied significantly in the amount of organic matter supplied by the fertilisers (Table 2). The highest amount of organic matter (expressed as the carbon content) was supplied in treatment SS3 (high dose of sewage sludge), 2 439 kg C/ha/year. Over the experimental period of 22 years, the total amount of carbon supplied there was 53 676 kg C/ha. In treatment N + St, it was 15 660 kg C/ha in total (e.g. 29% compared to SS3). In treatment with manure, it was 32 604 kg C/ha (e.g., 60% compared to SS3). An important quality indicator of organic fertilisers is the C/N ratio; the lowest ratio was in the case of sewage sludge (7.4/1), manure had a medium value (13.4/1), and straw the highest (79.3/1).
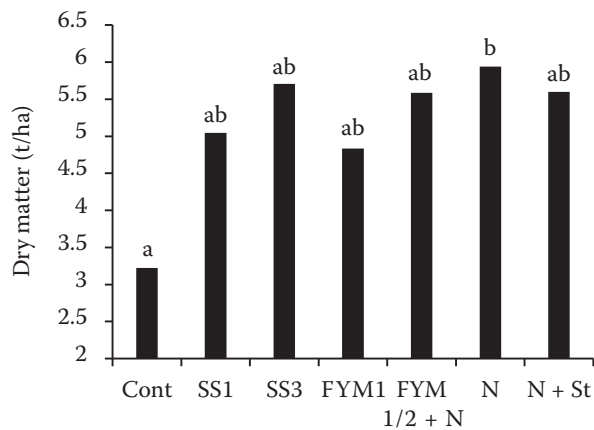
Figure 1. The yield of winter wheat grain (average of 22 years). Cont – control (no fertilisation); SS1 – sewage sludge; SS3 – high dose of sewage sludge; FYM – farmyard manure; FYM1/2 + N – half dose of farmyard manure + N in mineral nitrogen fertiliser; N – mineral nitrogen fertiliser; N + St – spring barley straw + N in mineral nitrogen fertiliser

To illustrate the yields obtained in individual treatments, the average yields of winter wheat grain over a period of 22 years are presented (Figure 1). It clearly shows the lowest yields obtained at the unfertilised control plot (3.22 t/ha). Fertilised plots gave the yields higher by 57–100%. However, differences among the fertilisation treatments were not significant. A simi-

lar trend was also determined in the case of barley and maize. These results indirectly inform us about the relations between individual treatments, as to the amount of postharvest residues and root mass.

Organic fertilisation and the amount of organic matter from postharvest residues and roots were subsequently reflected in the content of soil organic matter (Table 3). The original content of C_{SOM} in topsoil was 1.26%. Over a period of 22 years, it decreased to 1.10% at the unfertilised control. Other fertilised plots show a slightly increasing tendency. Fertilised plots did not differ significantly in the content of C_{SOM}, except for an extremely high dose of sewage sludge (SS3).

An important qualitative indicator of soil organic matter is humic to the fulvic acid ratio (Kononova 1963). Generally, the higher the ratio, the higher the quality of soil organic matter. Even though the content of humic acids was significantly lower at the control unfertilised treatment, significant differences among fertilised treatments were not recorded (Table 3). Other fertilised plots did not vary. The C_{HA}/C_{FA} ratio was the lowest at control (0.812), which did not significantly differ from the sewage sludge treatments. It clearly shows that sewage sludge contributes to the formation of relatively unstable humic substances with a low C/N ratio. The C/N ratio of the applied sewage sludge is nearing the "fast-effect" organic fertilisers, such as slurry. The content of humic substances is

Table 3. The contents of different organic carbon fractions and the content of glomalin determined using different methods

	Cont	SS1	SS3	FYM1	FYM1/2 + N	N	N + St	Average
Carbon (%)								
C_{SOM}	1.099[a]	1.365[bc]	1.568[c]	1.388[b]	1.314[b]	1.331[b]	1.404[b]	1.353
C_{FA}	0.145[a]	0.178[c]	0.208[d]	0.173[c]	0.160[bc]	0.160[b]	0.148[ab]	0.167
C_{HA}	0.118[a]	0.150[b]	0.183[b]	0.175[b]	0.193[b]	0.175[b]	0.178[b]	0.167
C_{HS}	0.263[a]	0.328[b]	0.390[c]	0.348[bc]	0.353[b]	0.335[b]	0.325[b]	0.335
C_{HA}/C_{FA}	0.812[a]	0.842[ab]	0.882[ab]	1.011[b]	1.209[b]	1.096[b]	1.213[b]	1.009
C_{SOM}/N_t	9.601[a]	9.778[ab]	9.661[ab]	9.966[ab]	10.02[ab]	9.731[a]	10.21[b]	9.852
Glomalin (mg/g)								
EEG	0.364[a]	0.427[b]	0.482[bc]	0.508[c]	0.493[c]	0.477[bc]	0.484[bc]	0.462
TG	2.185[a]	2.403[b]	2.764[c]	2.639[bc]	2.717[c]	2.795[c]	2.439[bc]	2.563
G_{NIRS}	1.308[a]	1.673[b]	2.100[d]	1.863[c]	1.730[b]	1.668[b]	1.795[bc]	1.734

Cont – control (no fertilisation); SS1 – sewage sludge; SS3 – high dose of sewage sludge; FYM – farmyard manure; FYM1/2 + N – half dose of farmyard manure + N in mineral nitrogen fertiliser; N – mineral nitrogen fertiliser; N + St – spring barley straw + N in mineral nitrogen fertiliser; C_{SOM} – soil organic matter carbon; C_{FA} – carbon in fulvic acids; C_{HA} – carbon in humic acids; C_{HS} – carbon of humic substances; N_t – soil total nitrogen; EEG – easily extractable glomalin; TG – total glomalin; G_{NIRS} – glomalin determined using the near-infrared reflectance spectroscopy

the sum of $C_{HA} + C_{FA}$. The highest C_{HS} content was again at the SS3 treatment (0.39%).

Different fertilisation systems used in the experiment also affected the change of the C_{SOM}/N_t (soil total nitrogen) ratio in topsoil (Table 3). Even though a high amount of carbon was supplied in the SS3 treatment, no significant increase of the C_{SOM}/N_t ratio in topsoil was observed. The reason is the high amount of nitrogen supplied to the soil within this treatment. The highest C_{SOM}/N_t ratio was recorded at the treatment N + St, which corresponds to the high C/N ratio of the input straw (79.3/1).

The content of C_{SOM} correlated significantly with C_{FA} ($r = 0.539$***) and C_{HA} ($r = 0.438$***). An even tighter linear correlation was calculated for the amount of humic substances ($r = 0.572$***). At the same time, no significant correlation was found between C_{SOM} and the C_{HA}/C_{FA} ratio ($r = 0.174$). The obtained results confirm that as to the C_{SOM} quality, it is a very diverse set; it was subsequently used for testing of the glomalin content as an indicator of soil organic matter quality.

Soil samples taken after harvest of crops in autumn 2018 were analysed. The set comprised all three blocks; the crops were maize, wheat, and barley. It followed the results of Steinberg and Rillig (2003), who reported slight seasonal oscillations of the glomalin content in soils; thus, the soil analyses were not carried out during vegetation. Table 3 presents the contents of easily extractable glomalin and total glomalin. The average content of TG was 2.563 mg/kg; for EEG, it was 0.462 mg/kg (i.e., only 18% of TG). The determined TG values correspond to the results of many other authors (Wuest et al. 2005, Wilson et al. 2009, Luna et al. 2016). The average G_{NIRS} content of all treatments was 1.734 mg/kg. This result corresponds to the values obtained with the same method for soils in the Czech Republic; Smatanová and Komprsová (2019) reported the results of large monitoring of arable soils in the Czech Republic (13 083 samples) with the median value of 2.51 mg/kg.

Results of the three methods used (EEG, TG, G_{NIRS}) suggest that significantly lowest glomalin content was obtained at the unfertilised control. In compliance with the results published by Curaqueo et al. (2011), a positive effect of sole mineral fertilisation on the glomalin content in topsoil was observed in our study, too. Also, the combination of mineral and organic fertilisation positively influenced the content of glomalin, which corresponds to the results by Dai et al. (2013) or Sandeep et al. (2016).

In accordance with our results, increased glomalin content was also observed by Dai et al. (2013) after the manure application, or by Nie et al. (2007) and Zhang et al. (2014) after the straw application. Even though Sandeep et al. (2016) recorded an increase in glomalin content after the application of sewage sludge, the SS1 treatment achieved significantly lower glomalin content compared to treatments with the highest glomalin contents. Based on the statistical evaluation in Table 3, it was not possible to definitely determine fertilisation treatment with the highest impact on the glomalin content. One of the reasons may be the glomalin stability and slow cycle of its decomposition in soil (Harner et al. 2004). It may also be presumed that the influence of individual fertilisation systems on the glomalin content will manifest more significantly during the longer time horizon of the experiments; it is similar to the content of organic matter in the soil.

As follows from Table 4, the correlation between G_{NIRS} and C_{SOM} was tight. Surprisingly the relation between TG and C_{SOM} was insignificant, although a positive correlation of glomalin content and C_{SOM} was reported by many authors (Wright and Upadhyaya 1998, Wilson et al. 2009, Dai et al. 2013, Xie et al. 2015). Contrariwise, Galazka et al. (2017) published the results with a negative correlation between the content of TG and C_{SOM}.

A very important finding is a tighter correlation of glomalin content (EEG, TG, G_{NIRS}) with humic substances than with C_{SOM}. A significant correlation was also determined between the content of glomalin (EEG, TG, G_{NIRS}) and C_{HA}, which corresponds to the results published by Šarapatka et al.

Table 4. Pearson correlation coefficients of glomalin content changes in relationship with different organic carbon fractions

	EEG	TG	C_{NIRS}
C_{SOM}	0.379**	0.250	0.766***
C_{HS}	0.472***	0.563***	0.834***
C_{HA}	0.524***	0.452***	0.683***
C_{FA}	0.155	0.449***	0.716***
C_{HA}/C_{FA} ratio	0.460***	0.213	0.297*

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; EEG – easily extractable glomalin; TG – total glomalin; G_{NIRS} – glomalin determined using the near-infrared reflectance spectroscopy; C_{SOM} – soil organic matter carbon; C_{HS} – carbon of humic substances; C_{HA} – carbon in humic acids; C_{FA} – carbon in fulvic acids

(2019). Similarly, the relationship of the content of TG, G_{NIRS}, and C_{FA} was insignificant. The weakest correlation was found between the glomalin content and the C_{HA}/C_{FA} ratio.
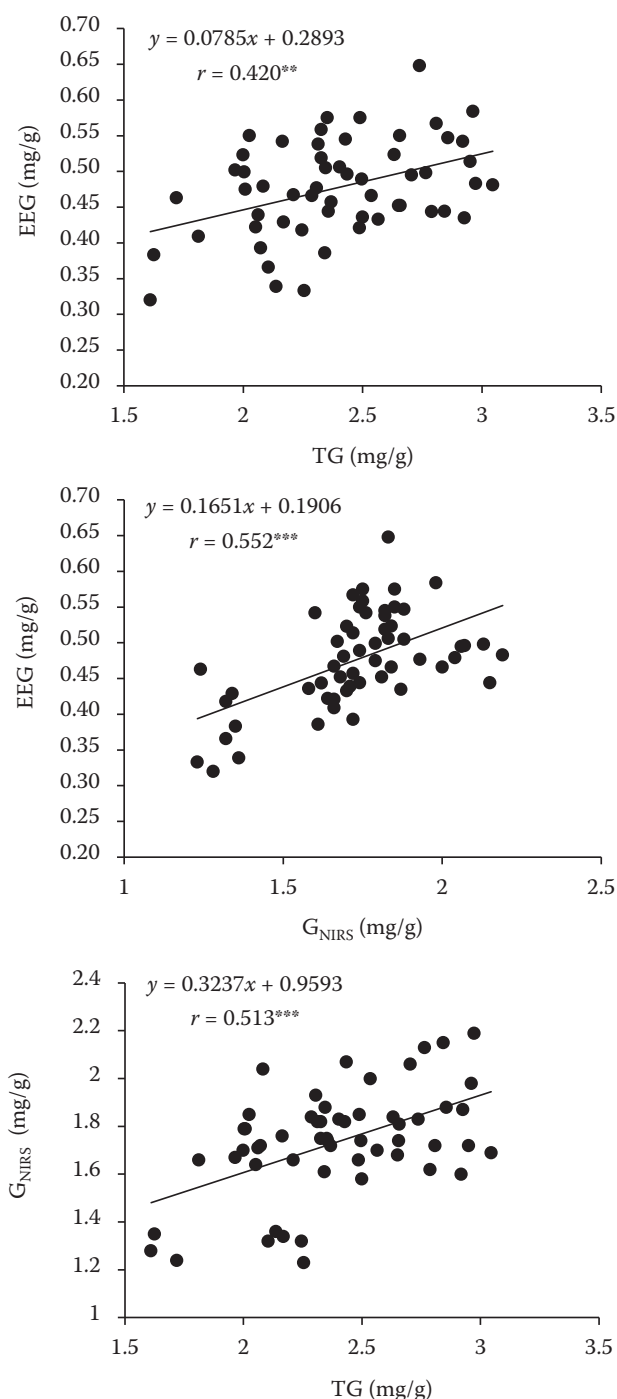

Figure 2. Relationships among different procedures to the determining of glomalin. EEG – easily extractable glomalin; TG – total glomalin; G_{NIRS} – glomalin determined using the near-infrared reflectance spectroscopy; **$P \leq 0.01$; ***$P \leq 0.001$

Positive correlations of glomalin with humic substances, humic acids, and fulvic acids confirm the hypothesis that the glomalin content may be used as an indicator of changes of soil organic matter quality. This finding is important, especially in the case of easily extractable glomalin and total glomalin.

To determine G_{NIRS} by the NIR spectroscopy method, the essential prerequisite is the proper calibration of the machine and thorough validation of this method, as reported by Zbíral et al. (2017); the importance is confirmed by a significant correlation of G_{NIRS} with easily extractable glomalin and total glomalin determined in our study (Figure 2). Surprisingly, lower tightness values were determined for the content of easily extractable glomalin and total glomalin.

As shown in Table 4, it is not possible to determine which glomalin pool (EEG or TG) is more suitable to evaluate the quality of soil organic matter (SOM). The EEG method may be preferred for its lower labour intensity. However, the determined EEG contents were low, which means a possibility of higher analytical uncertainty. In our case, a conclusive correlation of EEG with C_{HA}/C_{FA} increased the perspective of the EEG method to indicate soil organic matter quality. The relative response of the EEG to fertilisation was slightly higher than of the TG. Moreover, in case of appropriate validation, NIR spectroscopy may also be used, especially for larger data sets. A significant positive factor is especially the lower labour intensity of the analysis.

Suitability of glomalin content as a possible indicator of soil organic matter quality thus appears rather perspective. Yet, this conclusion must be confirmed by the evaluation of a large data set. It is also necessary to remind that long-term experiments have a greater potential to obtain positive results than smaller sets of less differentiated data. On the other hand, the changes of glomalin content caused by different fertilisation treatments may be expected after a longer time interval (min. 10 years). The content of glomalin in the soil is a result of several factors. Besides fertilisation, it is, e.g., the influence of cultivated crops (Wojewódzki and Cieścińska 2012). As reported by Wright and Anderson (2000) and Curaqueo et al. (2011), changes in the glomalin content are more dynamic at improper soil cultivation or under drought conditions.

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