National-Scale Forest Soil Carbon Characterizing Using Reflectance Spectroscopy

Asa Gholizadeh^{a,*}, Raphael A. Viscarra Rossel^b, Mohammadmehdi Saberioon^c, Josef Kratina^a, Lubos Boruvka^a, Lenka Pavlu^a

^a*Department of Soil Science and Soil Protection, Faculty of Agrobiological Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Suchbátka, Prague 16500, Czech Republic*

^b*Soil and Landscape Science, School of Molecular and Life Sciences, Faculty of Science and Engineering, Bentley Campus, Curtin University, G.P.O. Box U1987, Perth WA 6845, Australia*

^c*Helmholtz Centre Potsdam GFZ German Research Centre for Geosciences, Section 1.4 Remote Sensing and Geoinformatics, Telegrafenberg, Potsdam 14473, Germany*

Abstract

Any strategy to change the Carbon (C) pool has a substantial effect on the functionality of numerous ecosystem functions, the detachment of Soil Organic Carbon (SOC), the atmospheric carbon dioxide (CO₂) concentration, and climate change mitigation. As the largest amount of the world's C is stored in forests soils, the importance of forest SOC management is highlighted. The total SOC in a forest varies not only laterally, but also vertically (i.e., with depth). However, the SOC storage of different forest soil horizons has not been investigated in a national scale thoroughly, despite their potential to frame our understanding of soil function. Visible–Near Infrared (vis–NIR) reflectance spectroscopy enables rapid examination of the horizontal distribution of forest SOC, overcoming the limitations of traditional soil assessment methods. This study aims to evaluate the potential of

*Corresponding author; Email address: gholizadeh@af.czu.cz (Asa Gholizadeh)

vis-NIR spectroscopy in characterizing and predicting the SOC content of organic and mineral horizons in forests. We investigate 1080 forested sites across the Czech Republic at five individual soil layers, representing the Litter (L), Fragmented (F), and Humus (H) organic horizons, as well as the A₁ (depth of 2–10 cm) and A₂ (depth of 10–40 cm) mineral horizons (for a total of 5400 samples). We, then, use Support Vector Machines (SVMs) to classify the soil horizons based on their spectra and also to predict the SOC content of (i) the profile (all organic and mineral horizons together), (ii) the combined organic horizons, (iii) the combined mineral horizons, and (iv) each individual horizon separately. The models are validated using 10-repeated 10-fold cross validation. The results show that there is at least more than seven times as much SOC in the combined organic horizons, compared to the combined mineral horizons, with more variation in the deeper layers. The SVM with radial based kernel is a reliable classifier for classification of soil horizons, with Correct Classification Rate (CCR) of 70% and Kappa coefficient of 0.63. All individual horizon SOC values are successfully predicted with low error and with R² values higher than 0.63. However, the prediction accuracies of the F and A₁ models are greater, compared to others (R² > 0.70 and very low-biased spatial estimates). We conclude that the modelling of SOC with vis-NIR spectra in different soil horizons of highly heterogeneous forests in the Czech Republic is practical. This study provides an example of how general pedological knowledge can be used to define depth functions of SOC for forested sites.

Keywords: Soil organic carbon, Climate change, Forest soil, Soil horizons, National scale, Reflectance spectroscopy

1. Introduction

Concerns regarding Carbon (C) loading of the atmosphere from soil have significantly increased scientific interest in soil, as it is the largest terrestrial pool for long-term C storage (Batjes, 1996; Deluca & Boisvenue, 2012). In particular, the potentially major effects of soil C on the atmospheric carbon dioxide (CO₂) concentration (Schlesinger, 1995), as well as its ability to sequester Soil Organic Carbon (SOC) and mitigate climate change (Cienfiala et al., 2008; Viscarra Rossel et al., 2019) has attracted the attention of environmental research and policy agendas. Soil contains more C than the atmosphere (Bellassen & Luyssaert, 2014; Lal, 2017; Zdruli et al., 2017) and is the main component of the C cycle on Earth. Thus, SOC is crucial to preserving soil quality and health, assuring food security, and mitigating climate change (Lal, 2004, 2016). Climate change causes an increased biomass production as well as CO₂ concentrations and, as a result, causes an unbalanced C cycle (England & Viscarra Rossel, 2018). In this context, SOC management must be a part of an international plan to understand the SOC pools and the role of soil in the global C cycle.

Forest soil has high SOC content, well-developed organic horizons, excessive spatial variability, and features discernible variations in SOC with depth (IPCC, 2000). The SOC amount in forest soil is a function of the alteration between the C in surface litter and the below-ground biomass, as well as root exudates to the soil profile. It decreases due to decomposition, erosion, and leaching (Black et al., 2014). SOC in a forest ecosystem is stored in different horizons (Lal, 2008; Brahma et al., 2018) with different concentrations, due to the different stages of decomposition (Gibson et al., 2002). Accordingly,

26 evidence has been presented which suggests that measurements should ex-
27 tend to different horizons (Wendt & Hauser, 2013; Lorenz & Lal, 2014; Fan
28 et al., 2016). Since the Climate Summit (COP21) in Paris in December 2015,
29 there has been much interest in the storage of SOC in the top 40 cm of soil
30 (Lal, 2017), which has been categorized into organic and mineral horizons by
31 Zanella et al. (2017).

32 The spatial variability of forest SOC content is scale-dependent (Goidts
33 et al., 2009; Hutengs et al., 2019). Small-scale variation of SOC occurs due
34 to site-specific topography, erosion, drainage, different lithology and weath-
35 ering intensities, biological activity, temporal effects of soil moisture, and
36 soil management (Ellert et al., 2001; Conen et al., 2004; Saby & Arrouays,
37 2004; Kirwan et al., 2005; Yoo et al., 2006; Vanguelova et al., 2016). Large-
38 scale SOC variability is related to topography and land use (Jian-Bing et al.,
39 2006; Smith, 2008; Griffiths & Madritch, 2009; Webster et al., 2011; Barcena
40 et al., 2014; Fernandez-Romero et al., 2014). At regional or national scales,
41 it is necessary to evaluate the impact of the number of observations and as-
42 sociated soil variability on SOC content detection (Saby & Arrouays, 2004;
43 Bellamy et al., 2005; Chamberlain et al., 2010). However, the characteriza-
44 tion and prediction of SOC in organic and mineral horizons of forests at a
45 national scale with conventional analytical methods is laborious and costly,
46 as a large number of samples are needed in such a large heterogeneous soil C
47 pool (Palmer et al., 2002; Conen et al., 2004; Vanguelova et al., 2016), par-
48 ticularly for depth measurements (England & Viscarra Rossel, 2018). Thus,
49 it is crucial to measure and monitor SOC in forest soil effectively and with
50 sufficient accuracy using reliable, quick, and low-cost techniques not only

51 vertically, but also horizontally.

52 Visible–Near Infrared (vis–NIR) spectrometers allow many more mea-
53 surements to be made across the landscape at depth and in time, such that
54 the data can sufficiently characterize the spatial (vertical and lateral) and
55 temporal variability of soil (Viscarra Rossel et al., 2017). Reflectance spec-
56 troscopy can also provide rapid, inexpensive, accurate, and non-destructive
57 measurements of SOC content and composition at different scales (Ben-Dor
58 & Banin, 1995; Stenberg et al., 2010; Knadel et al., 2012; Viscarra Rossel &
59 Hicks, 2015; Gholizadeh et al., 2018; Liu et al., 2019). There have been a
60 few local-scale studies which investigated the use of reflectance spectroscopy
61 for assessing forest SOC in different horizons (Jiang et al., 2017; Wang et al.,
62 2019; Zhao et al., 2019), although the spectroscopic models established by
63 these studies usually need to be recalibrated repetitively across different re-
64 search locations, which limits the capability of vis–NIR spectroscopy ex-
65 tremely. Characterizing and developing large-scale (e.g., at a national or
66 global scale) SOC models may provide an approach for improving the effec-
67 tiveness of the technique (Viscarra Rossel et al., 2016); however, we did not
68 find any published literature on the application of vis–NIR spectroscopy for
69 classifying and predicting forest SOC in different soil horizons at a national
70 scale.

71 To this end, we intend to test whether vis–NIR spectroscopy can be used
72 to characterize and determine forest soils in the Czech Republic for the pur-
73 pose of more informed management of forest soils. Our hypothesis is that
74 vis–NIR spectroscopy can help us to better understand the composition of
75 SOC in forest soils and to accurately and cost-effectively classify and pre-

dict SOC concentrations. Thus, our aims were (i) to characterize forest soils using only the information content of the spectra, (ii) to predict the SOC content of the forest soil profile, combined organic, and combined mineral horizons, as well as individual horizons, using the spectra, and (iii) to assess the prediction ability of the models.

2. Materials and Methods

2.1. Study Sites, Soil Sampling, and Measurements

The Czech Republic has a total land area of 78,865 km², of which 34% is covered by forest (Czech Statistical Office, 2019) (Figure 1).

Figure 1: Location of Czech forests and soil sampling sites.

The soil sampling sites in this study were distributed over all the forested areas of the country, in order to proportionally represent various forest stands (e.g., forest composition, soil classes, and altitudes). The proportions of various tree species, soil classes, and altitudes are shown in Table 1. Sites with

89 prevailing coniferous trees formed about two thirds of the total set of sam-
 90 pling locations (65.6%), Norway spruce (*Picea abies* L. Krast.) being the
 91 most frequent species (51.5%), followed by Scots pine (*Pinus sylvestris* L.,
 92 13.8%). Among sites with prevailing broad-leaved trees (34.4%), oak (*Quer-*
 93 *cus sp.*, 17.9%) and European beech (*Fagus sylvatica* L., 16.6%) dominated.
 94 Cambisols were the dominant soil class (70.1%), mainly Dystric (36.7%),
 95 Hyperdystric (28.9%), and Eutric (22.6%). Podzols (15.4%) and Stagnosols
 96 (5.2%) were also quite abundant; the other soil classes (e.g., Arenosols, Lep-
 97 tosols, Retisols, Luvisols, and Fluvisols) were less frequent. The largest
 98 proportion of sampling sites were located between 400 m and 600 m a.s.l.
 99 (47.1%). With increasing and decreasing altitudes from this range, the num-
 100 ber of sites was decreased. This corresponds to relative proportion of various
 101 altitudes in the Czech Republic and the distribution of forests.

102 The samples originated from the Forest Soil Survey (Fiala et al., 2013)
 103 from the years 2000–2008 and were collected from 1080 points in forested sites
 104 across the whole Czech Republic (Figure 1). At each site, soil samples were
 105 collected separately from five individual layers (for a total of 5400 samples);
 106 namely, organic horizons Litter (L), Fragmented (F), and Humus (H), as well
 107 as mineral horizon A (with thickness ranging usually from 2–10 cm) (A_1) and
 108 subsurface mineral horizon (usually to the depth of 40 cm) (A_2). At each
 109 sampling site, composite soil samples were created from several subsamples
 110 collected in a circle of 10 m in diameter.

111 The soil samples were air-dried, ground, sieved (≤ 2 mm), and thor-
 112 oughly mixed before the soil analyses. SOC was determined using the rapid
 113 dichromate oxidation technique (Sparks, 1996).

Table 1: Proportion of sites with various prevailing tree species, soil classes, and altitudes in the set of sampling sites.							
Prevailing tree species	Number of sites	%	Soil classes	Number of sites	%	Altitudes (m a.s.l.)	Number of sites
Coniferous	709	65.5	Cambisols	758	70.1	<200	28
Spruce	557	51.5	Podzols	166	15.4	200-300	61
Broadleaved	372	34.4	Stagnosols	56	5.2	300-400	170
Oak	193	17.9	Leptosols	23	2.1	400-500	266
Beech	179	16.6	Arenosols	21	1.9	500-600	243
Pine	149	13.8	Retisols	19	1.8	600-700	111
Fir	2	0.2	Luvisols	17	1.6	700-800	65
Larch	1	0.1	Fluvisols	17	1.6	800-900	65
			Histosols	2	0.2	900-1000	43
			Gleysols	1	0.1	>1000	29
			Phaeozems	1	0.1		

114 2.2. Organic and Mineral Horizons

115 The study was designed to test variation in SOC as a function of soil
116 horizon. Organic and mineral horizons representatives of various SOC con-
117 tents were selected for the current study. Based on the World Reference Base
118 for Soil Resources (IUSS Working Group WRB, 2015), the main criteria for
119 distinguishing between organic and mineral horizons is C content, which is
120 higher than 20% for the organic horizons. According to Zanella et al. (2017),
121 as coloured by organic matter, organic horizons (L, F, and H) are generally
122 darker (H is almost black) in colour than the lower mineral horizons of the
123 soil profile (divided into the individual horizons A₁ and A₂), which depend on
124 animal and fungal activities, as well as the effects of soil forming processes.

125 2.3. Spectroscopic Measurements and Preprocessing

126 Spectroscopic measurements were made in the range of 350–2500 nm using
127 an ASD FieldSpec Pro FR spectroradiometer (ASD Inc., Denver, Colorado,
128 USA) with a high intensity contact probe. The spectral resolution of the
129 spectroradiometer was 2 nm for the region of 350–1050 nm and 10 nm for
130 the region of 1050–2500 nm. Moreover, the radiometer bandwidth from 350–
131 1000 nm was 1.4 nm, while it was 2 nm from 1000–2500 nm. The instrument
132 ran for about 30 minutes, in order to allow the spectrometer and lamp to
133 warm. Soil samples were placed in 9 cm diameter petri dishes, forming 2 cm
134 layers of soil. This was done to avoid beam reflectance from the bottom of
135 the dish, due to down-welling solar and sky radiation penetrating into the
136 soil at approximately 1/2 the wavelength (Jensen, 2007), which could have an
137 unwanted effect (i.e., modifying the soil spectra). Samples were levelled off
138 using a stainless steel blade to guarantee a flat surface (flush with the top of

139 the petri dish), as a smooth soil surface ensures maximum light reflection and
140 a high Signal-to-Noise Ratio (SNR) (Mouazen et al., 2005). We measured
141 all spectral readings in the center of the samples (with 3 replicates each) in
142 a dark room to avoid interference from stray light. The spectroradiometer
143 was optimized using a white SpectralonTM (Lab-sphere, North Sutton, New
144 Hampshire, USA) prior to the first scan and after every six measurements
145 (Shi et al., 2016). For each soil measurement, 30 spectra were averaged to
146 improve the SNR.

147 Before proceeding with our analysis, the spectral reflectance (R) was
148 transformed into apparent absorbance spectra through $\log_{10} (1/R)$ and then
149 preprocessed using Savitzky–Golay smoothing with a second-order polynomial
150 fit, a window size of 10 wavelengths, and first derivatives (Savitzky &
151 Golay, 1964). Due to the high collinearity of the spectra, we retained only
152 every tenth wavelength from 350 nm to 2500 nm inclusive to reduce dimensionality
153 (Viscarra Rossel & Webster, 2012). Principal Component Analysis
154 (PCA) and the scores of Principal Components (PCs) were performed. Scatterplots
155 of these components were used to visualize the structure of the data
156 and to emphasize the SOC variations in different horizons (Viscarra Rossel
157 et al., 2016; Dotto et al., 2018), as SOC contents seemed clearly distinct
158 at various depths. It was also a requirement to analytically identify reference
159 data outliers in the data sets (Martens & Naes, 1992). We checked
160 the histograms of data and tested for outliers, both visually and using the
161 Cook distance, which has been used in regression analysis to find influential
162 outliers in a set of predictor variables (Cook, 1977; Kim, 2017). Wavelet
163 decomposition was performed to keep only those coefficients that would be

164 useful in the models. For this purpose, we followed the methodology pre-
 165 sented in Viscarra Rossel & Lark (2009), which involves the selection of the
 166 wavelet coefficients based on their variance, regardless of wavelet scale. The
 167 selected coefficients were then used as the predictors. The resulting spectra
 168 from Continuum Removal (CR) technique (Clark & Roush, 1984) were used
 169 to assist in the spectral interpretation in the database.

170 *2.4. Spectroscopic Modelling and Assessment Statistics*

171 To train and then validate our models, the data was randomly split into a
 172 training set (75%) and a testing set (25%). The training set was used to build
 173 the classification and regression models and the test set was used to verify the
 174 generalization ability of the developed models (Kooistra et al., 2003). For the
 175 spectroscopic classification and prediction, we used the Support Vector Ma-
 176 chine (SVM) algorithm, which is a supervised, non-parametric, and kernel-
 177 based learning method from statistical learning theory (Vapnik, 1998). It has
 178 been identified to strike the correct balance between the accuracy gained from
 179 a given limited amount of training patterns and the generalization capability
 180 to handle unseen data. Viscarra Rossel & Behrens (2010) and Gholizadeh
 181 et al. (2016) have described the implementation of SVM for spectroscopic
 182 modelling in detail. All of the spectroscopic models were validated using 10-
 183 repeated 10-fold cross validation (Hastie et al., 2009; Viscarra Rossel et al.,
 184 2017, 2019) and the validation statistics reported are the means of the 10
 185 repeats. Non-parametric bootstraps were used to quantify the uncertainty of
 186 the SVM models in SOC estimation, in which we sampled with replacement
 187 from the training data (Hastie et al., 2009; Viscarra Rossel, 2007); this means
 188 that the method used the raw data to generate new data sets (Hastie et al.,

2009).

The potential of the SVM classification was evaluated through the analysis of Correct Classification Rate (CCR) and Cohens Kappa coefficient in the validation set (Saberioon et al., 2018). Moreover, we assessed the performance of the prediction models by comparing the predicted values on the independent test data set with the observed ones using standard model evaluation statistics; the coefficient of determination (R^2) between the observed and predicted values of SOC, the Root Mean Squared Error (RMSE), and the Mean Error (ME) or bias (Viscarra Rossel et al., 2006). R^2 is defined as the proportion of variation in the response that can be explained by the regression model. RMSE is the parameter commonly used in vis-NIR spectroscopy literature to describe the prediction ability of a model. The bias or ME appears as the error of means and is independent (Bellon-Maurel et al., 2010; Gomez et al., 2016). Table 2 shows the statistical metrics for models evaluation.

Table 2: Statistical metrics to evaluate SVM calibration.

Statistics	Formula
Correct Classification Rate (CCR)	$\frac{N_1}{N_0} \times 100\%$
Kappa coefficient	$\frac{Pr(a) - Pr(e)}{1 - Pr(e)}$
Coefficient of determination (R^2)	$1 - \frac{\sum_{i=1}^N (\hat{y}_i - y_i)^2}{\sum_{i=1}^N (y_i - \bar{y})^2}$
Root Mean Squared Error (RMSE)	$\sqrt{\frac{\sum_{i=1}^N (\hat{y}_i - y_i)^2}{N}}$
Mean Error (ME) or bias	$\frac{1}{N} \sum_{i=1}^N (\hat{y}_i - y_i)$

The resulting spectroscopic models yielded from SVM was used to classify

soil horizons and predict the SOC content of the forested areas, at different organic and mineral horizons, where we measured spectra. Thus, we made a total of 5400 estimates at 1080 sites.

3. Results

3.1. Descriptive Statistics of SOC at Different Horizons

Table 3 summarizes the statistics of the estimated SOC, including Mean, Minimum (Min), Maximum (Max), Standard Deviation (SD), Coefficient of Variation (CV), and skewness for data from all sampling locations for the profile, combined organic and combined mineral horizons, and the individual layers. There was a large variation in SOC content in the forest soil profile, as well as between all individual horizons, which reflects the different depths from which the samples originated (Viscarra Rossel & Behrens, 2010). The whole profile results indicate that the average SOC content of all samples was $23.54\% \pm 17.12\%$ (SD), with a distribution that shows high variability (CV = 73%) and an approximate normal distribution (skewness = -0.06).

Table 3: Statistical summary of SOC (%) for each horizon.

Soil horizon	Samples n.	Mean	Median	Min	Max	SD	CV	Skewness
Profile	5400	23.54	27.03	0.18	52.71	17.12	73	-0.06
Combined organic horizons	3240	36.12	39.00	3.85	52.71	9.18	25	-1.06
Combined mineral horizons	2160	4.62	4.11	0.18	25.20	3.26	70	1.59
L	1080	42.91	43.57	25.90	52.71	3.50	8	-0.50
F	1080	38.67	39.35	4.25	48.79	6.02	16	-1.83
H	1080	26.79	27.83	3.85	45.98	8.05	30	-0.49
A ₁	1080	6.49	6.22	0.82	25.20	3.20	49	1.56
A ₂	1080	2.74	2.33	0.18	23.75	1.99	73	2.99

220 The average SOC content in the L horizon was high (mean = 42.91%); it
221 varied from 25.90% to 52.71%. In the horizons F and H, the SOC contents
222 were lower (mean = 38.67% and 26.79%), with ranges of 4.25–48.79% and
223 3.85–45.98%, respectively. The SOC content in the A₁ horizon ranged from
224 0.82% to 25.20%, with an average value of 6.49%; while the A₂ horizon showed
225 an average SOC content of 2.47% and minimum and maximum of 0.18%
226 and 23.75%, respectively. On average, there was at least more than seven
227 times as much SOC (concentration) in the combined organic horizons (mean
228 = 36.12%), compared to the combined mineral horizons (mean = 4.62%).
229 The data distribution characteristic was found using the value of the SD. For
230 instance, the SD values of the SOC in the top organic sets (L, F, and H) were
231 3.50%, 6.02%, and 8.05%, respectively, and were thus higher than those of the
232 lower mineral sets A₁ and A₂ (3.20% and 1.99%, respectively). The SOC data
233 for all horizons had skewed distributions. The individual organic horizons
234 (L, F, and H) statistical distributions were negatively skewed, where horizon
235 F had a strongly skewed distribution (with a skewness of <-1), while L and
236 H were mildly skewed. The distribution of SOC in the individual mineral
237 horizons A₁ and A₂ was more skewed than those of the organic horizons and
238 they were strongly positive, with higher means than medians. In this study,
239 a comparison of layer CVs showed that, among all horizons, A₂, as a mineral
240 horizon, had the highest CV (73%). In contrast, the organic horizon L had
241 the lowest CV (8%), which shows that its distribution was more homogeneous
242 than other individual horizons, partly caused by its (highest) mean.

243 3.2. vis-NIR Spectral Data at Different Horizons

244 The continuum-removed spectra with their SDs for the soil samples from
 245 organic and mineral horizons are shown in Figure 2, which illustrates some
 246 minor spectral changes in the shape of the spectra and more apparent differ-
 247 ences in the depth of absorption bands from the upper to the lower horizons.

Figure 2: Continuum-removed spectra of forest soils for different horizons.

248 The spectra of horizons, particularly of those for L, F, and H, were sim-
 249 ply related. They followed a progression which shows a reduction in the
 250 organic matter content. Compared with the spectra that represented small
 251 SOC contents (i.e., the mineral horizons), the spectra that represented high
 252 SOC (i.e., the organic horizons) reflected less and had a remarkably concave

253 shape in the range of 400–1000 nm, which has been commonly seen in soils
 254 with abundant C content. The spectra for the mineral horizons A₁ and A₂
 255 were typical of soils with higher mineral content and lower C concentration,
 256 with absorptions due to iron oxides, water, and clay minerals. Noticeable
 257 absorption features in the spectra of all target horizons can also be observed
 258 at the wavelengths 1400 nm, 1900 nm (Figure-2), and 2200 nm, which are
 259 different depending on depth.

260 3.3. Information Content of Forest Soil Spectra

261 PCA was performed on the organic and mineral horizon soil spectra to
 262 investigate the differences in SOC variations at the target horizons (Figure 3).

Figure 3: Scoreplot for the first two PCs in PCA analysis carried on SOC for different horizons.

263 As shown in Figure 3, the plot of PC₁ and PC₂, accounting for about
 264 67% of variance, confirms that some samples were scattered away from the
 265 majority of the remaining samples, which were recognized as outliers and
 266 excluded from further investigation. The scoreplot of PCA indicates the

267 data compression and its dimensionality reduction. As expected, there was
 268 an obvious separation of data (L, F, H, A₁, and A₂) by soil horizon (Figure 3).
 269 This occurred along the continuum of decomposition process, agreeing with
 270 what we had previously seen in the continuum-removed spectra (Figure 2).

271 The PCs of the data sets for each soil layer were different, as the samples
 272 from the organic and mineral horizons included different contents and types
 273 of SOC. The scores for the organic horizons L, F, and H occupied more PC
 274 space all over the plot, although mostly in the left half. Conversely, the
 275 mineral horizons (A₁ and A₂) were particularly concentrated in the lower
 276 right half. Indeed, the results were different for A₁ and A₂, compared to the
 277 organic horizons (L, F, and H), as in the mineral horizons, some overlapping
 278 parts of the PCs were observed, which indicates that the data from A₁ had
 279 some identical conditions to A₂ in the corresponding feature space.

280 Figure 4 presents confusion matrix generated for the forest soil horizons
 281 classification using the SVM classifier, which delivers a visualization for the
 282 classification performance of spectral data for each soil horizon.

		Reference				
		A1	A2	F	H	L
Prediction	A1	92.5	19	2.5	33.9	0
	A2	7.5	78.5	0	1.7	0
	F	0	0.3	73.3	3.5	48.7
	H	0	0.3	23.3	60.8	6.85
	L	0	0.3	0.9	0	44.4

Figure 4: Confusion matrix percentage for soil horizons classification using SVM with national spectral data.

283 The correctly classified cases and true positive rates are indicated in the
 284 diagonal of each matrix, although the misclassified cases and false negative
 285 rates are shown in the shaded grids outside the diagonal (Figure 4). The
 286 mineral horizons of A₁ and A₂ were the best classified horizons using vis-
 287 NIR spectroscopy with high true positive percentages of 92.5% and 78.5%,
 288 respectively. However, the organic horizons, especially the layer L provided
 289 the lowest true positive percentage of 44.4%. It can also be noticed that the
 290 highest false negative rate occurred for the horizon L, as 48.7% were classified
 291 as the F horizon.

292 The accuracy of the horizons classification was evaluated by Correct Clas-
 293 sification Rate (CCR) and Kappa coefficient. The SVM with radial kernel
 294 had the potential to correctly classify forest soil horizons based on their spec-
 295 tra with CCR of 70% and Kappa coefficient of 0.63 for testing set.

296 3.4. Spectroscopic Prediction of SOC Content

297 Figure 5 highlights the results of the national SVM models for estimating
 298 SOC content at profile (organic and mineral horizons combined), combined
 299 organic, and combined mineral horizons of the Czech forest soils. The results
 300 of the prediction indicate that the national model developed for SOC at soil
 301 profile and combined organic horizon levels could be explained as providing
 302 good prediction accuracy, with $R^2 = 0.76$ and 0.78 and $RMSE = 1.63\%$ and
 303 1.83% , respectively (Figures 5A and 5B); however, the profile model was to-
 304 tally unbiased ($ME = 0\%$). The results for the SOC estimation in combined
 305 mineral horizons (Figure 5C), which contained considerably lower SOC con-
 306 tent than the combined organic horizons, provided a relatively similar RMSE
 307 but a considerably lower R^2 value ($R^2 = 0.53$). Nevertheless, according to

308 Chang et al. (2001), the results for these horizons were still fair (R^2 be-
309 tween 0.5 and 0.7). In general, the model's performance under the combined
310 organic and combined mineral horizons showed a descending trend as soil
311 depths increased; however, the assessment statistics, in terms of the model
312 biases and SDs (small values), indicated that the models were still capable
313 of SOC prediction (Figure 5). According to Figure 5B, an equal distribution
314 across the entire range of SOC is an advantage in model performance (Ja-
315 coní et al., 2019), which was the case for the whole profile and the combined
316 organic horizons models.

317 For further assessment, we compared the estimated SOC content at in-
318 dividual layers for both organic and mineral horizons. A comparison of the
319 scatterplots with predicted versus measured SOC for the five individual hori-
320 zons (L, F, H, A_1 , and A_2) is presented in Figure-6.

321 The estimation accuracy ranges were $R^2 = 0.63$ – 0.72 and $RMSE = 0.98$ –
322 4.2% for individual horizons. Horizons F and A_1 showed the best prediction
323 results among the others, with higher R^2 and lower ME (low-biased spatial
324 estimates) values. We noted that these comparisons conformed well to the
325 horizon spectral analysis shown in Figure-2. The predictions of SOC content
326 from the other individual horizons (L, H, and A_2) also produced low error and
327 R^2 , between 0.5 and 0.7, despite their more scattered plots in the predicted
328 to the measured values. There were no noticeable differences between the
329 predictions of SOC in these horizons, in terms of their R^2 and ME values,
330 although the H horizon provided a higher RMSE (4.2%) and was relatively
331 more biased (Figure-6), which may be attributed to its higher SD (Table-3)
332 as a higher SD results in larger RMSE value (Kuang & Mouazen, 2011).

333 It can also be seen that, in the individual organic horizons (L, F, and H),
 334 the RMSE increased with depth but, for all three layers, the RMSE values
 335 were somewhat smaller than the SDs of the SOC in the sampled soil profiles
 336 (Table 3 and Figure 6).

Figure 5: Predicted SOC plotted against measured SOC for profile (A), combined organic (B), and combined mineral (C) horizons in Czech forest soils.

Figure 6: Predicted SOC plotted against measured SOC for L (A), F (B), H (C), A₁ (D), and A₂ (E) horizons in Czech forest soils.

337 According to the results presented here, vis-NIR spectroscopy coupled
338 with SVM could classify and predict the SOC at both organic and mineral
339 horizons (together and individually) of forest soils at a national scale in the
340 Czech Republic. The horizons classification was better performed in mineral
341 horizons; however, the prediction accuracy was higher in organic horizons
342 with shallower depth and considerably higher SOC content. The values of
343 assessment metrics (CCR, Kappa coefficient, R^2 , RMSE, and ME) indicated
344 that the SOC could be characterized and predicted well, whereas the accuracy
345 for each horizon varied.

346 4. Discussion

347 The mean SOC content in our study (Table 3) showed that forest soils of
348 the Czech Republic have high C storage in general (mean = 23.54%), which
349 decreases with depth. Jiang et al. (2017) mentioned that, over time, micro-
350 organisms gradually decompose the organic composition of litter, which en-
351 riches the top soils. In a study on different horizons of soil in Japanese forests,
352 Ugawa et al. (2012) showed that soil C stock is usually decreased with depth
353 as a result of C influx from decomposing litter and dead roots in the upper
354 soil layer and low decomposition rates in the lower soil layer. Moreover, ac-
355 cording to Verje et al. (2003) and Ping et al. (2010), in Cambisols, the most
356 dominant soil class of the Czech Republic as well as in our sample set (Table-
357 1), there is little C under the organic horizons of these soils. They lack any
358 significant soil development with depth, but can support thick organic rich
359 surface horizons (Deluca & Boisvenue, 2012). In general, our results of SOC
360 content with depth (Table 3) were consistent with the prevailing trends in

361 SOC depth distribution for soils across the globe (Li et al., 2015). Routh
362 et al. (2016) showed a sharp decrease in SOC values of Swedish forests with
363 increasing soil depth, from upper to lower horizons, and related it to lower
364 rate of C sequestration in deeper soil layers. Jia et al. (2017), Wang et al.
365 (2019), and Zhao et al. (2019) obtained similar results on vertical SOC pat-
366 terns in different forested sites of China. They attributed it to reduction of
367 below-ground plant biomass with soil depth, which has a considerable effect
368 on SOC content. Table 3 also indicates the increase of SOC heterogene-
369 ity with depth, which was comparable with the conclusions of Hobley et al.
370 (2018), who connected this large variance to high variability of SOC in lower
371 horizons.

372 Soil spectral signature, like other soil properties, varies continuously and
373 the resulting spectra represent complex compositional mixtures of soil mate-
374 rials from diverse origins, which are also affected by their environments. It is
375 clear that vis-NIR spectra contain useful information about soil in general,
376 and about SOC in particular (Gholizadeh et al., 2017), due to the different
377 chemical bonds (Li et al., 2015). Figure 2 shows that all spectra, especially
378 the L, F, and H horizon spectra, had a similar general form. They reflected
379 less and had a concave shape in the range of 400–1000 nm, which has been
380 shown to be typical for soils with high C content (Viscarra Rossel & Hicks,
381 2015). The observed spectral features in the range of 400–1000 nm are in-
382 dicative of iron and C concentration (Sherman & Waite, 1985). The spectral
383 patterns of horizons L and F, with accumulation of less-decomposed organic
384 matter (e.g. leaves, needles, and little branches) in this range (400–1000 nm)
385 are mainly functions of the remaining photosynthetic pigments (e.g. chloro-

386 phyll, carotenoids, and xanthophylls) and cell structures (Gitelson et al.,
387 2002). These results mirrored the results of Ogen et al. (2017), who used
388 the vis–NIR range for soil classification and concluded that the accumulated
389 roots and other plant residuals at upper soil layers affected the soil spec-
390 tral pattern. In the NIR range, at the wavelengths 1400 nm, 1900 nm, and
391 2200 nm, there were sharp absorption bands that resulted from overtones and
392 combination vibrations of the organic functional groups methyl, methylene
393 and aryl, carbonyl, ester and carboxyl C=O, cellulose C–C/C–H, C–H, and
394 O–H groups (Viscarra Rossel & Hicks, 2015). Ben-Dor & Banin (1995) also
395 mentioned that absorption at these specific wavelengths (1400 nm, 1900 nm,
396 and 2200 nm) are generally associated with minerals and water bound in the
397 interlayer lattices. However, the process of air-drying and soil grinding re-
398 duced the absorbance spectra and amplitude characteristic peaks (Jia et al.,
399 2017). Buitrago et al. (2016) and Gholizadeh & Kopackova (2019) stated
400 that the dominant factors controlling the pattern of vegetation spectra at
401 these wavelengths (for organic horizons L and F, with mostly plant matter)
402 are the biochemical constituents of fresh plants (e.g. lignin, cellulose, starch,
403 and protein). The soil horizon colour, which varies due to soil organic matter
404 content (Ben-Dor et al., 2008) and structure (Zanella et al., 2017), can also
405 influence soil spectra (Gholizadeh et al., 2020). This is probably the reason
406 for the general strong absorption peaks in horizon H (Figure 2), which had
407 a very dark colour and a fine and well-decomposed structure (Zanella et al.,
408 2017).

409 The plot of the PCA scores shows that SOC was coherent with differ-
410 ences in its content and variability, although the results were quite different

411 between shallower samples in organic horizons and deeper samples in mineral
412 horizons (Figure 3). This may be consistent with the variation of SOC values
413 in the samples. There are also likely complex interactions reflecting pedo-
414 genesis processes behind this and in this regard, the forest soils (as major
415 sources of C) go beyond simple soil types and classes and are, thus, suitable
416 for showing variations in SOC content properly. In order to confirm these
417 findings, the potential of the SVM classifier for classification of forest soil
418 organic and mineral horizons based on their vis-NIR spectra was performed,
419 which provided promising discrimination among all studied horizons. SVM-
420 based classification can actually strike balance between acquired from a given
421 finite amount of training patterns and the ability to generalize to unseen data
422 (Mountrakis et al., 2011).

423 According to the prediction statistics for the vis-NIR spectra of the soil
424 profile, the combined organic and combined mineral horizons, as well as their
425 individual layers (Figures 5 and 6), it was apparent that, in general, the pre-
426 diction capability was weakened (lower R^2 and relatively similar error) with
427 increasing depth. This is presumably because the spectral reflectance from
428 the soil becomes less effective as a predictor regarding the sharp decline of
429 SOC concentration (Jia et al., 2017). However, in spite of this descend-
430 ing trend, the technique still cost-effectively estimated SOC concentration,
431 even in deeper horizons of forested sites. At a large scale (i.e., regional or
432 global), the relationships between the soil spectra and soil properties are
433 usually spatially dependent (Hong et al., 2018), which limits the efficiency
434 of spectroscopic models over large areas (Liu et al., 2019). Given such vari-
435 ation at a large national scale, our results were comparable with the results

436 of some previous studies on different soil types and geographical scales (Li
437 et al., 2015; Viscarra Rossel et al., 2016; Jia et al., 2017), though the statis-
438 tical metrics of the aforementioned studies showed broad ranges. Regarding
439 the important criteria of geographical scale of the research (i.e., field, local,
440 regional, or national), it should be noted that soil spectroscopic models es-
441 tablished in local-scale studies usually performed better (Stevens et al., 2013;
442 Guerrero et al., 2016), possibly because soils sampled at a local scale have
443 lower variability and more similar spectral features.

444 Considering SOC prediction in individual horizons (Figure 6), superior
445 results for horizons F and A₁ can be noticed, with higher R² and lower ME
446 (low-biased spatial estimates) than other target horizons. This can probably
447 be explained by expanding our knowledge about the possible contribution of
448 SOC type, as well as its correlation with other available soil properties in
449 these horizons, which has been reported in some studies (Telles et al., 2003;
450 Gholizadeh et al., 2018). Horizon H had the highest RMSE (4.2%), along
451 with the highest SD value among others (Table 3), which was noticeable
452 in Figure-6C. According to research by Kuang & Mouazen (2011) and Jia
453 et al. (2017), high SD and variability in the range of SOC content are crucial
454 factors affecting the accuracy of calibration models. Therefore, their results,
455 similarly to ours, highlighted that a larger SD and wider range explained
456 the variability of SOC content and led to a larger RMSE. This is in contrast
457 with Gaudinski et al. (2000), who stated that, as the residence time of SOC
458 enhances with depth, the maximum changes and higher SDs are more likely
459 to occur in the organic horizons. In the current study, the RMSE in organic
460 horizons increased with depth, although it was smaller than the SDs of the

461 SOC in the sampled soil profiles. These results agree with the findings of
462 Viscarra Rossel et al. (2016) at three soil depths across grazing lands.

463 Overall, this study provides an example of how general pedological knowl-
464 edge can be used to define depth functions of SOC for forested sites. Im-
465 portantly, the outcomes of this work indicate that vis-NIR reflectance spec-
466 troscopy, as an effective technique, could rapidly and cost-effectively clas-
467 sify forest soil horizons and also predict SOC content at a large number of
468 national-scale samples from the upper to lower horizons. The technique al-
469 lows us to create massive soil depth data, which can facilitate SOC mapping,
470 accounting, and management. This information is not typically available
471 through conventional approaches.

472 Conclusions

473 SOC in forest varies by depth due to different decomposition rates, which
474 has a significant influence on total C pool alteration and, therefore, on de-
475 tachment of SOC, atmospheric CO₂ concentration, and climate change mit-
476 igation. This study showed that there was a large variation in SOC content
477 among the forest soil horizons (from 42.91% in L to 2.74% in A₂), depend-
478 ing on the depth from which the samples were derived and, further, due to
479 the most dominant soil class of the study area (Cambisols), which are soils
480 with little C under the organic horizons. Soil spectra forms and trends were
481 generally similar across the horizons, particularly in the L, F, and H lay-
482 ers; however, the spectra of horizon H showed noticeably stronger absorption
483 peaks, probably because of its darker colour and well-decomposed structure.
484 The soil horizons were satisfactorily characterized with accuracy of CCR =

485 70% using their reflectance spectra. The SOC spectroscopic models of the
486 combined organic horizons were more accurate than those of the combined
487 mineral horizons. The prediction accuracy of the models based on the indi-
488 vidual horizons F and A₁ were greater than the others, with R² values higher
489 than 0.70 and low-biased spatial estimates. However, SOC content in hori-
490 zons L, H, and A₂ were also well-estimated, with low error and R² > 0.63.
491 The findings of the current study indicate that vis-NIR spectroscopy coupled
492 with SVM could characterize and predict SOC content of different horizons
493 across the forests of the Czech Republic, which are representative of the
494 forests of temperate Europe.

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