

## PAPER



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# Direct quantification of total organic carbon in soils using native CN and C<sub>2</sub> molecular emissions by laser-induced breakdown spectroscopy (LIBS)

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The soil carbon cycle plays a central role in global warming, making accurate mapping of Total Organic Carbon (TOC) in soils essential for climate change mitigation. Conventional TOC determination methods are often time-consuming, costly, error-prone, and environmentally unsustainable due to the use of chemical reagents and extensive sample preparation. This study introduces a proof of concept for a chemically grounded LIBS-based approach that enables the direct quantification of TOC in soils by exploiting the emission of native CN and C<sub>2</sub> molecular species. Two Laser-Induced Breakdown Spectroscopy (LIBS) systems, Spark Discharge-assisted LIBS (SD-LIBS) and a handheld LIBS device (hLIBS), were evaluated using soil samples with different textures and TOC levels. Argon purging ensured inert plasma conditions favoring CN and C<sub>2</sub> native species in the plasma, while Ar emission lines were used to perform spectral normalization. For SD-LIBS, a Partial Least Squares (PLS) model based on 12 CN and C<sub>2</sub> wavelengths achieved an  $R = 0.90$  in calibration and an  $R = 0.82$  (MAE = 0.33%) in validation. The hLIBS model, combining 12 CN and C<sub>2</sub> emission bands and 8 more correlation-selected wavelengths, yielded  $R = 0.96$  for calibration and  $R = 0.79$  (MAE = 0.38%) for validation. Both systems delivered comparable analytical performance, demonstrating the feasibility of rapid, reagent-free, and *in situ* TOC quantification in soils. The proposed approach paves the way toward sustainable soil monitoring and carbon management strategies.

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## 1. Introduction

Total Organic Carbon (TOC) content in soils plays a fundamental role in the global carbon cycle. Consequently, reliable and accurate determination of TOC is crucial for the development of effective soil management strategies, as it provides essential insights into carbon sequestration processes in both environmental and agricultural contexts.<sup>1,2</sup> Conventional methodologies, including dry combustion and wet chemical approaches, frequently demand sample pre-treatment, most notably acid application for carbonate removal, which may compromise analytical accuracy. Furthermore, these methods are labor-intensive, expensive, and environmentally harmful.<sup>3</sup> Such limitations have stimulated the pursuit of alternative analytical techniques that are faster, reagent-free, and more suitable for *in situ* applications.<sup>3,4</sup>

Laser-Induced Breakdown Spectroscopy (LIBS) has emerged as a promising alternative technique for soil TOC

determination, offering several advantages over conventional methods. LIBS enables rapid, direct, and minimally destructive analysis while eliminating the need for chemical reagents and sample preparation.<sup>5,6</sup> Beyond its application to soil analysis, the versatility of LIBS has been widely demonstrated in diverse fields such as environmental monitoring, food safety, medical diagnostics, aerospace engineering, and agriculture.<sup>7–11</sup> In recent years, the development of handheld LIBS (hLIBS) systems has further enhanced the technique's applicability, making it particularly attractive for soil analysis. Studies have shown that nowadays hLIBS can achieve a performance comparable to laboratory-based systems, making it a valuable tool for in-field measurements.<sup>12,13</sup>

Although LIBS has been extensively investigated for carbon determination in soils,<sup>3,11,14</sup> most reported approaches focus primarily on Total Carbon (TC). Considerable efforts have also been directed toward developing methods to discriminate among TC, inorganic carbon (IC), and organic carbon (OC). For instance, Brickley *et al.*<sup>15</sup> combined LIBS with machine learning to propose an indirect strategy for OC estimation, relying on the quantification of TC and IC. Other LIBS-based methods have also been reported, where emission signals from various elements were employed as key predictors. These approaches, however, depend strongly on assumptions of

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complex stoichiometric biogeochemical correlations and chemical matrix effects,<sup>16,17</sup> which may limit their robustness and general applicability.

The organic composition of samples can be probed by LIBS through the detection of diatomic molecular emissions such as C<sub>2</sub>, CN, CH, NH, and OH, which may originate from the direct release of organic molecules or large primary organic fragments, often referred to as native species.<sup>18</sup> In particular, with respect to CN and C<sub>2</sub>, Mousavi *et al.*<sup>19</sup> reported that the intensity of C<sub>2</sub> Swan bands is strongly correlated with the presence of organic carbon in organic materials, supporting the hypothesis that the C<sub>2</sub> detected in plasma arises predominantly from the fragmentation of complex organic structures.<sup>19</sup> Some molecular emissions, however, can result from recombination processes involving plasma species or atmospheric interactions (recombinative species). In order to measure the emission of native molecular species, specific experimental strategies are required to minimize recombinative species. For instance, Dib *et al.*<sup>20</sup> successfully mitigated the contribution of recombined CN in Spark Discharge-assisted LIBS (SD-LIBS) analysis by applying an Ar purge (10 L min<sup>-1</sup>) over the surface of samples.

In light of the above considerations, the present work introduces a conceptual innovation for TOC determination in soils by LIBS: the use of the emission bands of the diatomic species CN and C<sub>2</sub> as direct markers of TOC. Unlike indirect methods that rely on empirical correlations, this strategy is chemically grounded: the emission signal directly reflects the presence of organic precursor molecules, since the molecular fragments of complex organic compounds formed during laser ablation are intrinsically linked to the organic matter in the soil sample. This feature characterizes the method with a truly innovative character, overcoming the selectivity limitations of previous approaches and opening new perspectives for rapid, direct, and robust TOC analysis in soils.

Furthermore, as a proof of concept to validate the feasibility of directly quantifying TOC in soils through the emission of native CN and C<sub>2</sub> molecular bands, two LIBS systems with distinct instrumental characteristics, a laboratory-based SD-LIBS setup and a handheld LIBS (hLIBS) device, were evaluated and compared for the direct determination of TOC in soils.

## 2. Materials and methods

### 2.1. Soil samples

A set of 54 soil samples of different chemical and physical compositions was used in this study. The samples originated from the proficiency test conducted by the Agronomic Institute of Campinas (AIC), which involves soils from different regions of Brazil.

Three pellets were prepared for each sample by transferring 200 mg of soil sieved at 2 mm mesh sample to a C steel mold (13 mm diameter), and applying a pressure of 10 tons for 2 min using a hydraulic press (Solab SL- 10/1, Piracicaba, Brazil).

Soil texture (clay, silt and sand, fractions contents) was determined by 89 different laboratories participating in the AIC proficiency test, using either the pipette or hydrometer method. Outlier laboratories were excluded according to the program

Table 1 Clay, silt, sand, and TOC contents of studied soil samples<sup>a</sup>

Sample identification	TOC* (%)	Clay* (g kg <sup>-1</sup> )	Silt* (g kg <sup>-1</sup> )	Sand* (g kg <sup>-1</sup> )
237	1.53	297	71	636
238	2.08	485	147	362
239	2.86	313	149	537
240	0.94	328	88	589
241	1.54	130	110	758
242	3.20	632	210	145
243	3.06	312	141	540
244	1.12	394	175	429
245	1.67	539	187	272
246	3.34	656	301	40
248	1.71	531	191	274
249	2.18	487	148	365
250	3.08	630	213	147
251	5.71	409	187	396
252	1.63	555	158	275
253	3.02	318	142	536
254	2.13	488	148	363
255	3.08	640	208	146
256	1.75	542	184	273
257	3.13	603	209	181
259	1.53	421	95	480
260	1.37	337	92	570
262	2.13	309	125	572
263	1.13	341	64	592
264	1.42	339	88	574
265	2.88	612	202	184
266	2.71	505	348	148
268	2.95	610	203	183
270	1.51	417	101	484
271	1.81	565	309	112
272	1.06	342	71	594
273	1.54	416	104	481
274	1.02	337	74	591
275	1.24	441	100	458
276	1.44	336	92	573
277	2.28	436	152	412
278	2.74	610	204	191
280	1.38	456	153	394
281	1.58	334	97	569
282	1.76	220	114	664
283	1.76	336	144	520
284	6.63	453	242	307
285	2.96	615	192	194
286	2.22	546	160	294
287	2.23	437	144	419
288	1.68	311	103	591
289	1.92	525	138	331
290	1.41	460	149	394
291	1.66	332	138	525
292	2.77	616	184	195
293	2.16	434	139	421
294	1.63	332	143	522
295	1.67	449	128	425
296	1.31	454	149	395

<sup>a</sup> TOC typical range of standard deviation according to ISO 10694 is 0.3 to 2%; standard deviation range given by AIC is: clay = 3 to 14%, silt = 3 to 26%, sand = 2 to 13%.

protocol. The mean values are presented in Table 1, and the ranges of standard deviations for each texture components are provided in the table footnotes.

TOC (Table 1) was determined using an Analytik Jena multi N/C 2100 analyzer after removal of inorganic carbon with 1% HCl. This analysis was carried out at the Environmental BioGeoChemistry Laboratory, UFRGS, Brazil, following similar guidelines of ISO 10694, a validated analytical procedure for quantifying organic carbon in soils.<sup>21</sup> Single measurements were performed for each sample; therefore, standard deviations could not be calculated. However, precision data reported in ISO 10694, indicate typical interlaboratory precision between 0.3 and 2%, depending on soil texture and TOC content.

## 2.2. Spark discharge and handheld LIBS instrumentations and analytical procedure

The benchtop LIBS system used in this study consisted of a Q-switched Nd:YAG laser (Quantel, Big Sky Ultra 50, USA), which emits pulses at a wavelength of 1064 nm, with an energy of 48 mJ and a pulse duration of 20 ns. The laser pulse was focused onto the sample surface using a plano-convex converging lens, resulting in a fluence of approximately  $68 \text{ J cm}^{-2}$  and an irradiance of  $3.40 \text{ GW cm}^{-2}$ , estimated based on the measured average diameter of the ablation craters (approximately  $300 \mu\text{m}$ ). The plasma emission was collected by a lens and transmitted through optical fibers to four spectrometers (Ocean Optics, LIBS HR2000+, USA), covering the spectral range from 200 to 625 nm. The optical resolution of the spectrometers is 0.1 nm full width at half maximum (FWHM). The Q-switch delay relative to the laser pulse was set at  $2 \mu\text{s}$ , and the instrumentally fixed spectrometer integration time was 1 ms. The entire system control and data acquisition were achieved using a microcomputer running the OOILIBS software (Ocean Optics, USA). In the sample chamber, the sample was moved in the  $x$ - $y$  directions by the operator using a joystick, which allowed dispersed rastering across the surface of the pellets. A video camera was used to monitor the laser pulses.

To enhance the analytical sensitivity, a previously developed high-voltage spark discharge (SD) device<sup>22</sup> was coupled to the LIBS system. This device consisted of a primary electrical circuit and two pure tungsten cylindrical electrodes, each 100 mm in length and 2.6 mm in diameter, arranged so that their tips were separated by a 4-mm gap and positioned 2 mm above the sample surface. Sixty spectra were acquired for each sample, distributing the laser pulses over different regions of the three pellets (20 spectra per pellet), using a discharge voltage of 4000 V. Furthermore, an Ar flow of  $5 \text{ L min}^{-1}$  was used in order to conduct the analyses in an inert atmosphere and minimize the formation of recombinative CN fragments resulting from atmospheric  $\text{N}_2$  interference.

The hLIBS instrument used in this study was a SciAps Z-903 (Woburn, MA, USA) powered by an on-board rechargeable Li-ion battery<sup>13</sup> which included a proprietary Nd-YAG diode-pumped, and a solid-state pulsed nanosec laser at 1064 nm wavelength that delivers a 5-6 mJ laser pulse of 1 ns pulse duration with a nominal  $100 \mu\text{m}$  beam size at a 10 Hz firing rate. The plasma light was collected and transferred by fiber optic cables to three spectrometers equipped with time-gated, charge-coupled detectors (CCD), which allowed to record the spectra in a wavelength acquisition range from 190 to 950 nm, *i.e.* from the ultraviolet (UV) to visible and near-infrared (VNIR) range.

The SciAps Z-903 instrument incorporated a rechargeable canister screwed to the instrument, which allowed to purge the sample with the inert gas Ar during the measurement, and permitted plasma confinement and enhancement of signal emission. Furthermore, the instrument used a raster grid by which the laser beam fired across the sample surface, and could be configured to optimize the number of points to hit. This feature is quite useful as the initial laser shots, the number of which can be adjusted prior to data acquisition, can be used to “clean” the sample surface from dust and burn contamination. A miniature camera in the nose of the instrument enabled to view and optimize the correct location of the sampled points and their documentation/archiving. All hLIBS analyses were performed at ambient air under a constant Ar purge with a fixed

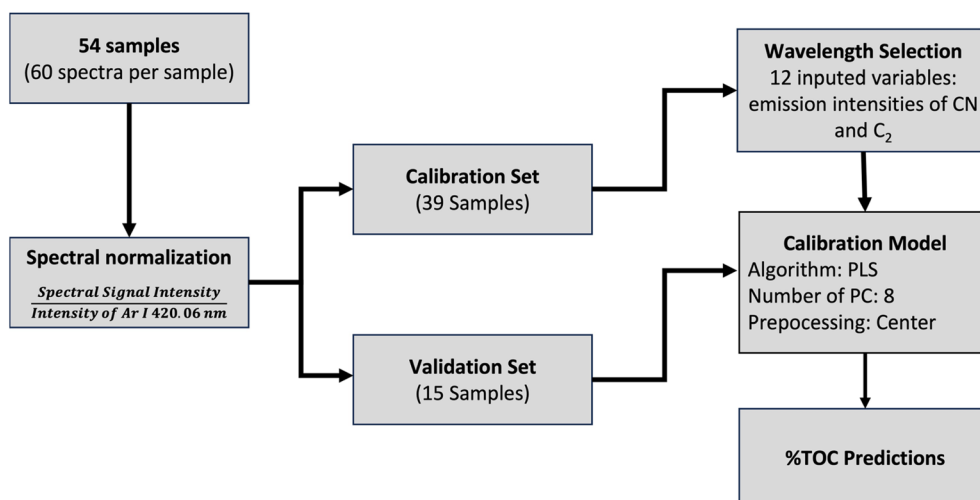


Fig. 1 Schematic representation of data processing applied to SD-LIBS spectra.

delay time of 650 ns from the beginning of the acquisition of the LIBS emission with an acquisition time window fixed to 3 ms.

The analysis was performed by firing one prior laser cleaning shot and then four measuring laser shots on the twelve points located in three randomly selected rectangular areas where the LIBS grid pattern was positioned on the sample surface. This procedure resulted in the acquisition of 64 averaged spectra for each pellet, *i.e.*, 192 spectra per sample, as three pellets were analyzed for each sample.

### 2.3. Analysis and modeling LIBS spectral data

The final averaged spectra achieved by each LIBS system used in this study were divided into two sets, *i.e.*, a calibration set consisting of 39 samples and a validation set consisting of 15 samples. The samples were separated using a random ~70:30 split, while ensuring that both the minimum and maximum TOC levels of the dataset were included in the calibration set so that the calibration model encompassed the full concentration range. The TOC content ranged from 0.7% to 3.5% in the calibration set and from 1.3% to 3.3% in the validation set.

To develop the calibration models for TOC prediction, spectral preprocessing, variable selection, and the determination of the optimal number of principal components for the Partial Least Square (PLS) model fitting were evaluated. The best-performing models, selected on the basis of the correlation coefficient ( $R$ ) and the Mean Absolute Error (MAE), were applied to predict the TOC % in the validation samples. The predictive performance was assessed using MAE. A summary of the experimental procedure adopted for SD-LIBS and hLIBS data analysis is provided in Fig. 1 and 2, respectively.

Twelve input variables acquired from the spectral range of emission bands of  $C_2$  and  $CN^{14}$  were selected to fit the calibration models of SD-LIBS and hLIBS data. In particular, for the SD-LIBS, the band intensities selected were 385.03, 385.67, 386.31, 388.32, 415.52, 416.53, 423.58, 460.35, 460.41, 473.69, 505.50, and 516.80 nm; whereas for the hLIBS, the selected

wavelengths were: 358.12, 385.60, 386.58, 387.83, 388.67, 413.22, 414.32, 466.80, 468.26, 469.18, 474.32, and 516.79 nm. Furthermore, for hLIBS, eight additional wavelengths, *i.e.*, 350.19, 351.24, 356.21, 359.63, 359.79, 360.11, 360.18, and 362.40 nm, which showed the highest correlation coefficients ( $R > 0.7$ ) with TOC, were included as variables.

## 3. Results and discussion

### 3.1. LIBS spectral preprocessing

The fluctuations of laser energy and its interaction with the sample can lead to variations of the plasma characteristics, which increase signal uncertainty and impact negatively the accuracy and precision of LIBS measurements. Thus, the reduction of uncertainty is essential for obtaining reliable results.<sup>23</sup> Preliminary investigations using SD-LIBS and hLIBS data supported this consideration, as poorly fitted calibration models were obtained when spectra were used without preprocessing. To address the challenge of spectral variability, one commonly adopted strategy in spectrometry is the so-called internal standardization, which consists in the use of a reference spectral signal to normalize the others.<sup>24</sup> However, the identification of a suitable emission line that fulfills the criteria for an internal standard across multiple spectral signals results particularly complex in the context of heterogeneous soil samples exhibiting very different compositions (Table 1).

In this context, a consistent and effective strategy was developed in this work to minimize spectral fluctuations. Given the need to maintain an inert analytical atmosphere to minimize the formation of molecular CN from recombination processes that could interfere with TOC assessment by enabling the detection of inorganic C, the Ar gas was employed at a constant flow-rate in both LIBS systems evaluated. As a result, the Ar emission lines exhibited consistent intensities across all samples. Thus, leveraging this factor, a novel spectral preprocessing approach was proposed which consisted in the

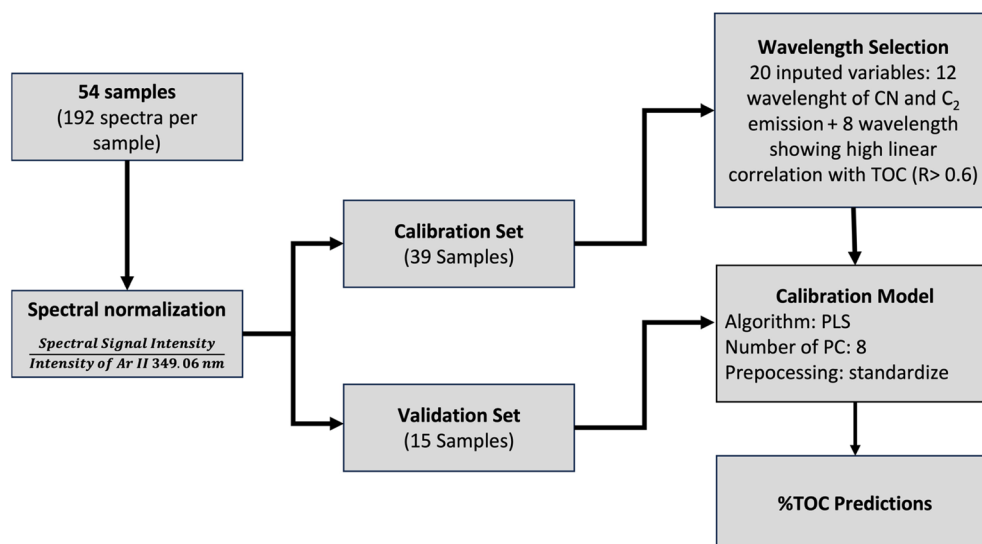


Fig. 2 Schematic representation of data processing applied to hLIBS spectra.

normalization of all spectra using the intensity of a selected Ar emission line.

The selection criteria for the Ar emission line included the absence of spectral interferences, sufficient intensity above the noise level, and signal strength intermediate relative to other spectral lines. A previous evaluation of the performance of Ar lines for spectral normalization led to select the Ar atomic line at 420.06 nm for SD-LIBS data normalization, while for hLIBS, a good performance was achieved using the ionic Ar line at 349.06 nm. As an example, preprocessed spectra and the corresponding raw spectra are compared in Fig. 3, clearly showing that preprocessing was able to minimize intrinsic fluctuations inherent to the analytical procedure. This led to reduced baseline shifts and, consequently, decreased variability in the intensity of the variables. Thus, the preprocessed spectra that resulted by applying this approach were used in this study.

### 3.2. SD-LIBS

LIBS spectra from complex matrices like soil exhibit a rich pattern of emission lines and molecular bands resulting in

high-dimensional data. Therefore, a variable selection for calibration model fitting is crucial to avoid overfitting.<sup>16</sup> Although various data mining strategies have been proposed for the analysis and modeling of complex systems, the variable selection based on the analyst's expertise of cause-and-effect relationships remains fundamental in building robust and interpretable predictive models.<sup>25</sup> Thus, with the aim of determining organic C from CN and C<sub>2</sub> molecular fragments derived from incomplete breakdown processes of soil organic C, the CN and C<sub>2</sub> spectral bands detected in a representative soil sample (Fig. 4) were assigned based on the literature.<sup>18</sup> Then, twelve molecular bands showing a satisfactory spectral profile (Fig. 4 insets) were selected to be further used as input variables in the calibration model developed using the PLS algorithm.

Based on model optimization, the optimal number of latent variables was set to eight, which explained approximately 81% of the variance in TOC for the calibration set. The PLS model yielded  $R_{\text{cal}} = 0.90$ ,  $\text{MAE}_{\text{cal}} = 0.39\%$ , and root mean square error of calibration (RMSEC) = 0.51%, indicating good calibration performance. When applied to the 15 samples in the validation set, the

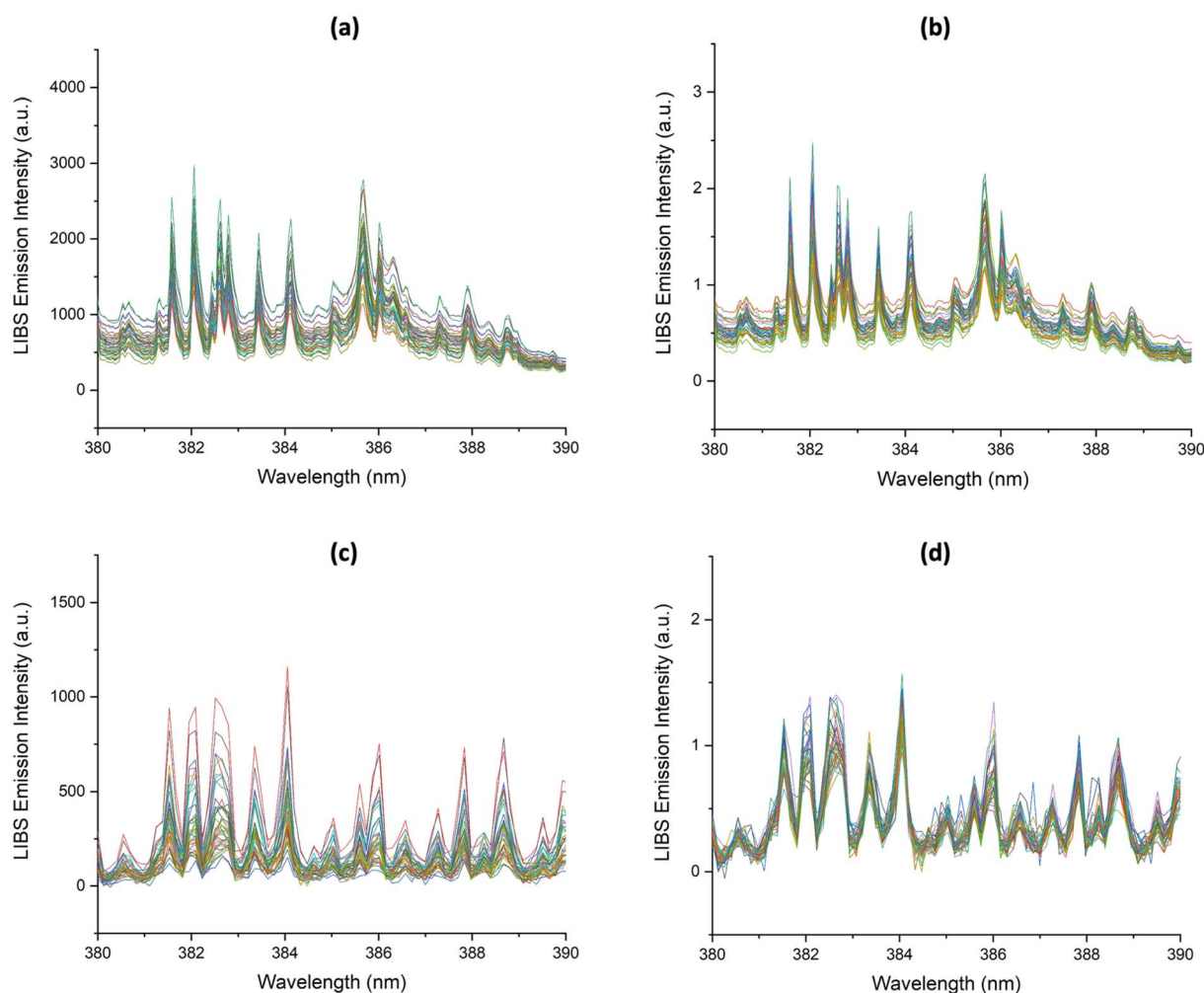


Fig. 3 Spectral profiles in the 380–390 nm range acquired from 40 measurements on a soil sample (n. 237), before and after preprocessing based on Ar emission lines: (a) and (b) SD-LIBS spectra without and with preprocessing, respectively; (c) and (d) hLIBS spectra without and with preprocessing, respectively.

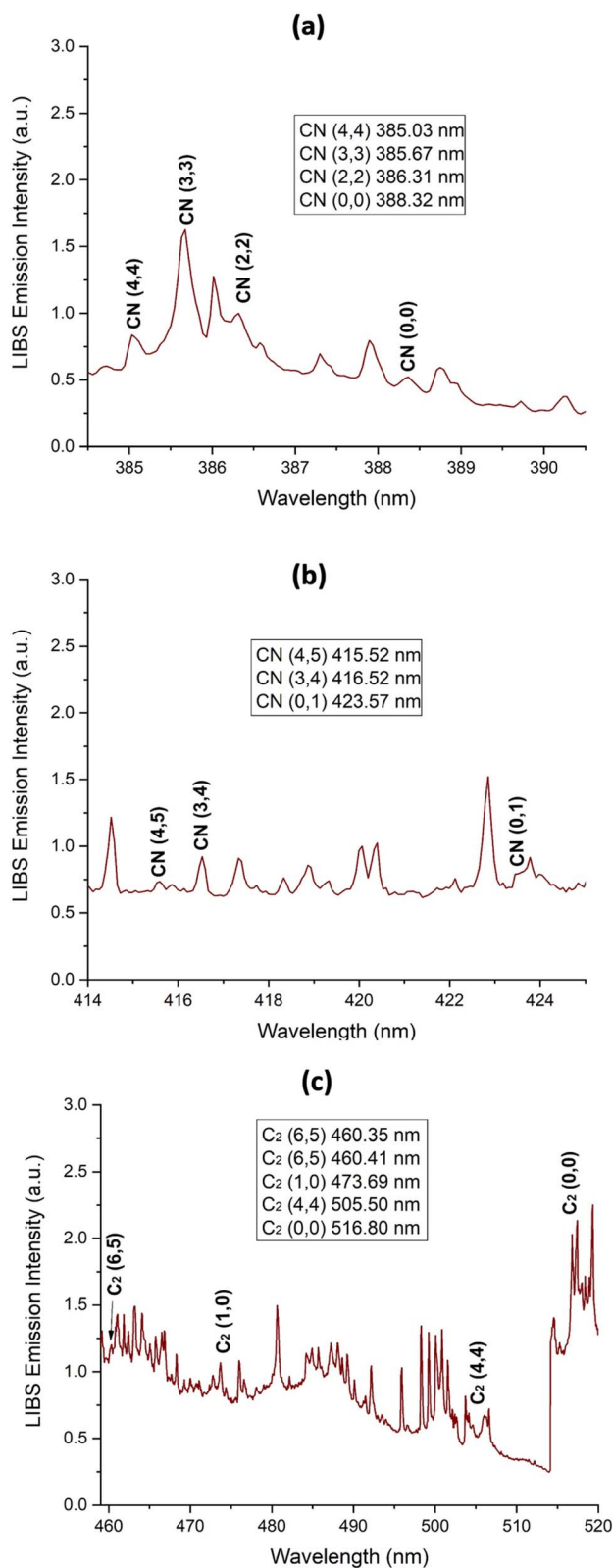


Fig. 4 Spectral regions of CN (a and b) and C<sub>2</sub> (c) emission bands with the selected input variables listed in the insets.

model produced  $R_{\text{val}} = 0.72$ ,  $\text{MAE}_{\text{val}} = 0.39\%$ , and root mean square error of prediction (RMSEP) = 0.52%. The corresponding ratio of performance to deviation (RPD  $\approx 1.4$ ), calculated as the ratio between the standard deviation of the reference TOC values and the RMSEP, confirms that the model provides a useful, although moderate, predictive ability for TOC within this heterogeneous soil dataset. Fig. 5 displays this relationship, where each point corresponds to a single soil sample; therefore, error bars are not shown, as model uncertainties are already represented by the statistical parameters provided above.

In particular, the highest relative prediction error (81%, indicated by the arrow point in Fig. 5) was achieved for a sample very rich in sand ( $569 \text{ g kg}^{-1}$ , sample n. 281, Table 1), which may have contributed to the spectral variability observed. Due to their inherently low compactness, sandy soils tend to generate more dust during laser ablation, compromising plasma stability.<sup>26</sup> This effect can, in turn, affect the intensity and reproducibility of CN and C<sub>2</sub> molecular emissions, thereby impacting the reliability of total organic carbon (TOC) predictions based on these signals. If this sample is excluded, the average relative prediction error decreased from 21% to 17%,  $R_{\text{val}}$  increased to 0.82 and  $\text{MAE}_{\text{val}}$  decreased to 0.33%.

Considering the variability of the studied soil sample set, the method achieved good performance in predicting TOC levels, with a limit of detection (LOD) of 1.9% (calculated according Allegrini & Oliviere<sup>27</sup> as  $\text{LOD} = 3.3 \times \text{RMSEP}/\text{slope}$ , where the slope is obtained from the validation regression between predicted and reference TOC values). Because multivariate PLS models rely on combined information from many wavelengths, the LOD is derived from validation-based prediction variance rather than from a single blank signal as in univariate calibration. Therefore, the LOD does not define the minimum concentration that can be predicted by the model, and samples with TOC levels below 1.9% can still be predicted, albeit with higher uncertainty.

The method enables a rapid analysis without the need of extensive sample preparation and use of chemical reagents,

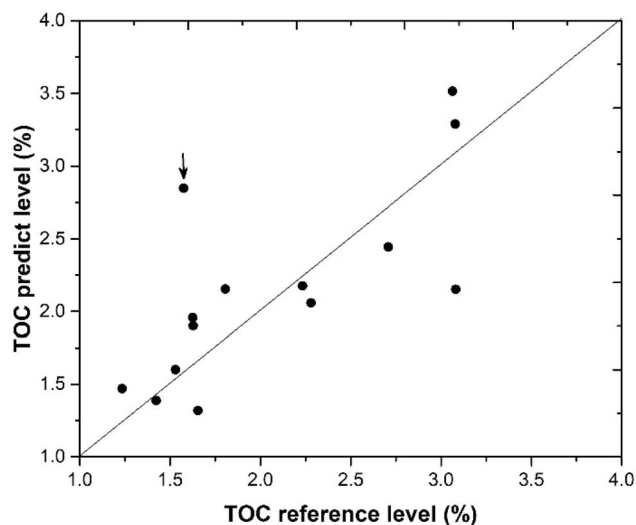


Fig. 5 Predictive performance of the SD-LIBS PLS-adjusted model for TOC prediction.

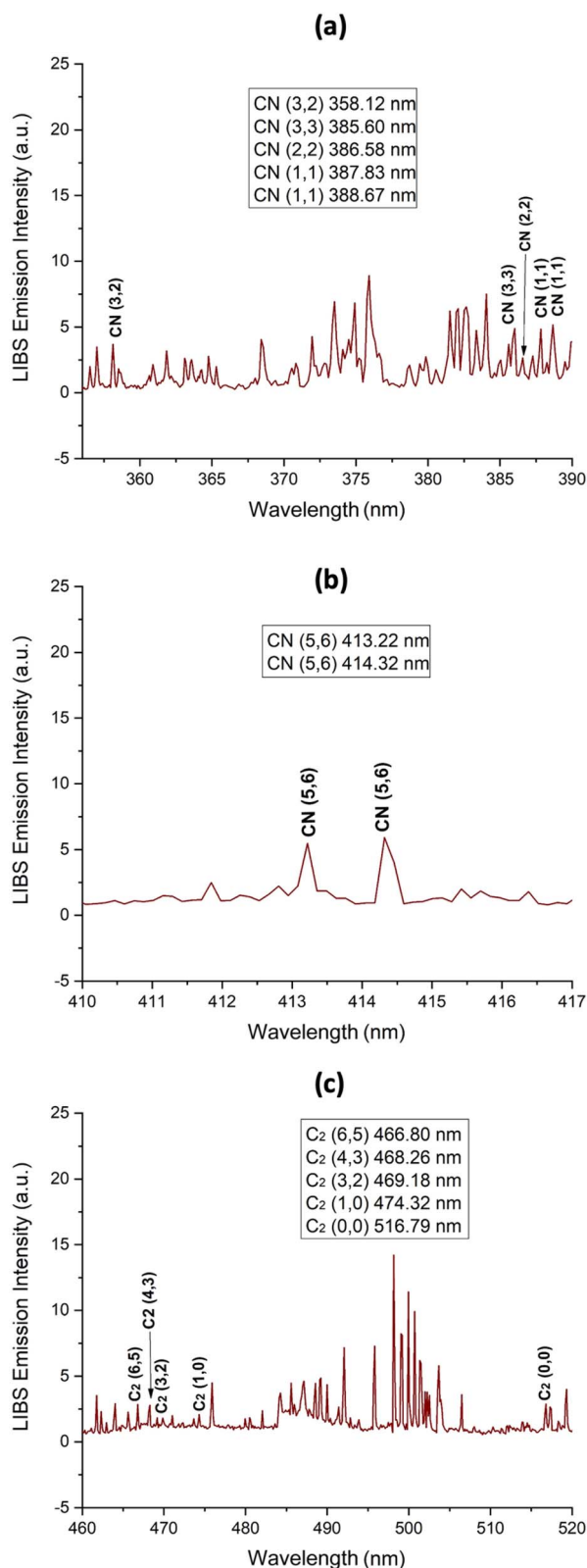


Fig. 6 Spectral regions of CN (a and b) and C<sub>2</sub> (c), and emission bands with the selected input variables highlighted listed in the insets.

thus being environmentally friendly with no generation of chemical wastes, and providing valuable data to support decision-making, so contributing to swift strategies for mitigating global warming.

### 3.3. hLIBS

Also, in the hLIBS experiment twelve peak emission intensities corresponding to CN and C<sub>2</sub> bands were identified<sup>18</sup> (Fig. 6), but their wavelengths slightly differed from those determined in the SD-LIBS experiment. This divergence can be ascribed to the two different instruments used, as well as to the application of the selection criterion adopted (molecular bands with good spectral definition and free from spectral interferences). Based on the twelve selected variables, the predictive model built using the PLS algorithm showed a satisfactory calibration performance, with  $R_{\text{cal}} = 0.93$  and  $\text{MAE}_{\text{cal}} = 0.38\%$ .

Despite the good calibration fit, the model performed poorly when applied to the prediction of TOC in the validation samples, yielding a  $R_{\text{val}} = 0.27$  and a  $\text{MAE}_{\text{val}} = 0.77\%$ . Therefore, it was necessary to select additional variables to improve the robustness and applicability of the model. To this purpose, a variable selection strategy was tested based on ranking the linear correlations between the TOC content and individual wavelengths within the CN and C<sub>2</sub> emission regions. Using this approach, eight additional variables were selected, all exhibiting correlation coefficients above 0.7.

A new PLS model was then developed using eight latent variables, which explained approximately 91% of the variance in the calibration set. This model yielded improved performance and a lower calibration error, with  $R_{\text{cal}} = 0.96$ ,  $\text{MAE}_{\text{cal}} = 0.25\%$  and  $\text{RMSEC} = 0.35\%$ , and a  $\text{LOD} = 1.9\%$ . When applied to the validation set, the optimized model produced satisfactory results, with  $R_{\text{val}} = 0.79$ ,  $\text{MAE}_{\text{val}} = 0.38\%$  and  $\text{RMSEP} = 0.45\%$ . The corresponding ratio of performance to deviation ( $\text{RPD} \approx 1.6$ ), calculated as the ratio between the standard deviation of the reference TOC values and the  $\text{RMSEP}$ , confirms that the model achieves a useful, although still moderate, predictive ability for TOC in the studied soils.

Fig. 7 shows the correlation between the reference and predicted TOC values obtained with the optimized PLS model. Each data point represents a single soil sample; therefore, error bars are not shown, as prediction uncertainties are already expressed by the statistical parameters reported above.

These results indicate that, despite being a portable system, hLIBS can still provide good performance for TOC determination in soil, especially when combined with variable selection strategies based on relevant spectral correlations. The inclusion of additional variables led to a significant improvement in model accuracy and generalization capacity, indicating that hLIBS is a promising tool for rapid and sustainable analysis of TOC in soils.

### 3.4. SD-LIBS versus hLIBS

The predictive performance of the TOC models for different soil samples using two distinct LIBS systems is compared in Table 2. For this comparison, the SD-LIBS validation data considered

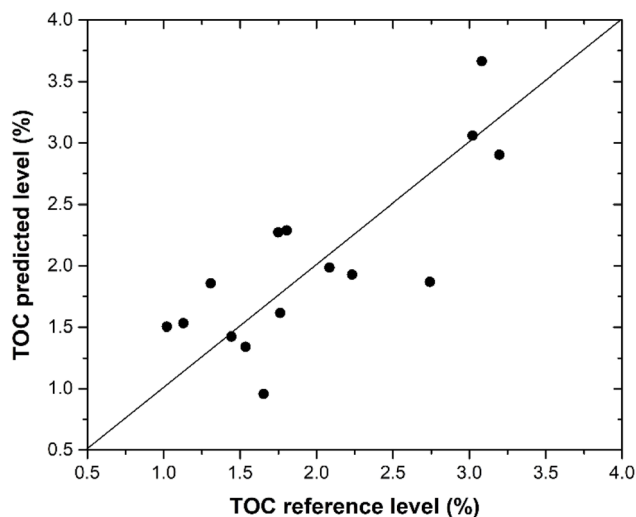


Fig. 7 Predictive performance of the hLIBS PLS-adjusted model for TOC prediction.

Table 2 Comparison of the predictive performance of SD-LIBS and hLIBS

Performance parameters	SD-LIBS	hLIBS
$R_{\text{val}}$	0.82	0.79
$\text{MAE}_{\text{val}}$ (%)	0.33	0.38
RMSEP (%)	0.46	0.45
RPD	1.6	1.6

excluded the sample that showed a highly discrepant prediction error (81%). This sample had a TOC content of 1.58%, which is not an outlier in terms of concentration, but may exhibit particular matrix effects or spectral anomalies that affected the model performance. The removal of this sample emphasizes the need of further investigation of the model robustness and potential sources of error, such as heterogeneity in soil texture, moisture, or mineral content.

The results indicated that both LIBS systems produced consistent PLS models capable of predicting TOC levels across a heterogeneous soil dataset. Notably, in the validation process both models achieved  $\text{MAE}_{\text{val}}$  values below 0.40%, which is particularly relevant considering that the lowest TOC content observed in the dataset was 0.71%. For SD-LIBS, the validation performance after sample removal yielded  $R_{\text{val}} = 0.82$ ,  $\text{MAE}_{\text{val}} = 0.33\%$ ,  $\text{RMSEP} = 0.46\%$  and an RPD of approximately 1.6. The hLIBS model showed comparable performance, with  $R_{\text{val}} = 0.79$ ,  $\text{MAE}_{\text{val}} = 0.38\%$ ,  $\text{RMSEP} = 0.45\%$  and  $\text{RPD} \approx 1.6$ .

Overall, although SD-LIBS remains slightly more precise due to the controlled conditions of a benchtop system, hLIBS stands out as a viable alternative for sustainable and rapid soil analysis. The close agreement between the two instruments, particularly after variable selection for hLIBS, supports its feasibility for routine environmental monitoring and field-based assessment of soil organic carbon, contributing to

practical and scalable strategies for carbon management in agricultural landscapes.

## 4. Conclusion

The results of this study confirm the successful validation of a chemically grounded approach for the direct quantification of total organic carbon (TOC) in soils using LIBS. By exploiting the emission of native CN and  $\text{C}_2$  molecular species as analytical markers, the method establishes a direct and selective relationship between the spectroscopic signal and the organic carbon content of the sample, thus overcoming the indirect and empirical character of conventional LIBS quantification based on atomic lines. This molecularly driven strategy imparts genuine novelty to the approach, enhancing its selectivity and robustness while opening new perspectives for rapid, direct, and reliable TOC determination in soils.

Both evaluated LIBS configuration (SD-LIBS and hLIBS) confirmed the robustness and reproducibility of this molecular approach. Their analytical performances were comparable, with MAEs below 0.4% and consistent validation results. This consistency across distinct instrumental setups reinforces the reliability of the proposed concept and highlights its adaptability for both controlled laboratory conditions and *in situ* applications.

The success of this molecular emission-based approach also demonstrates the effectiveness of the variable selection strategy, which enhanced model performance by integrating spectroscopic insight with multivariate modeling. Beyond its technical achievements, this work establishes a new, greener, and more accessible paradigm for soil carbon monitoring. By enabling rapid, reagent-free, and environmentally responsible analysis, LIBS, particularly in its handheld format, emerges as a promising solution for precision agriculture and environmental diagnostics, contributing meaningfully to climate change mitigation and global sustainability goals.

## Author contributions

Rafael Rodrigues Balbino: data curation, methodology, validation, writing-review & editing. Yusef Sadik Gavrilo: formal analysis, writing-review & editing. Giorgio Saverio Senesi: conceptualization, formal Analysis, writing-original draft, review & editing. José Anchieta Gomes Neto: conceptualization, writing-review & editing. Edilene Cristina Ferreira: conceptualization, funding acquisition, project administration, supervision, writing-original draft, review & editing.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

Data for this article are available at Institutional Repository UNESP at <https://hdl.handle.net/11449/258542>.

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