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METHODOLOGICAL CHALLENGES IN ESTIMATING SOIL ORGANIC MATTER: A REVIEW

SUMMARY

Soil organic matter (SOM) plays a crucial role in soil health, fertility, and carbon cycling, making its accurate estimation essential for sustainable agriculture and ecosystem management. However, the quantification of SOM is fraught with methodological challenges that can introduce variability and uncertainty into assessments. Traditional techniques may lack specificity and accuracy, while advanced methods pose challenges related to calibration and standardization. The selection of an appropriate method is critical and requires careful consideration of soil characteristics, land use, and research objectives. This article reviews the key methodological challenges associated with estimating soil organic matter, aiming to provide an understanding of the complexities involved, and provides insights on the latest instrumentation for SOM measurements.

Keywords: sustainable agriculture; soil organic matter (SOM); ecosystem management; land use; instrumentation

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INTRODUCTION

The relevance of a landscape-scale approach to address environmental concerns in agricultural areas is being increasingly recognized by soil scientists with an emphasis on estimation of soil C stocks (Kader *et al.*, 2022; Sestras *et al.*, 2023). In terrestrial ecosystems, the largest stock of carbon is comprised by the soil surface, contributing significant proportion of organic carbon (OC) (Li *et al.*, 2022). Of this, soil organic matter (SOM) offers a prime source of organic carbon in soils, paving a way for the establishment and restoration of soil elements, carbon balance, and climatic conditions and environmental sustainability (Santoiemma, 2018).

Being the key parameter of agricultural soils, SOM is a practical index of soil fertility and soil quality and a prime factor in governing the dynamics of different agrochemicals in soil (Dhaliwal *et al.*, 2019; Kader *et al.*, 2022). Furthermore, the physical, chemical and biological properties of different soil components are directly and indirectly affected by the SOM (Kader *et al.*, 2021; Kader *et al.*, 2023; Nannipieri and Eldor, 2009).

Comprehending the significance of organic matter, there is a call for regular monitoring of SOM for detection of changes in OC content, quality and its potential to sustain its purpose over time. Additionally, the SOM has proven an imperative parameter in directing soil fertilization as well as irrigation, thereby; a request for its determination by different fertility laboratories is apparent (Borase *et al.*, 2020; Kader *et al.*, 2022).

SOM encompasses a wide range of organic compounds, and no analytical method allows its accurate direct measurement. The determination of soil organic matter through conventional methodology relies on its oxidation, either by the utilization of strong oxidizing agents such as potassium dichromate in Tyurin or Walkley Black method with the use of conversion factor based on the assumption that SOM predominantly contains carbon (Shamrikova *et al.*, 2022).

However, the principal limitation in estimating the total carbon by Walkley Black method is that it results in the partial recovery of organic carbon (Nie *et al.*, 2021) and by dry combustion or EA method is the presence of inorganic carbon, most prominent problem in recently limed soils or calcareous soils. Therefore, accuracy of the analytical methods is critically important for the evaluation of short to medium term changes in soil organic carbon (SOC), such as, changes resulting from alternative agricultural management (Ramesh *et al.*, 2019).

Moreover, the assessment of soil organic matter on qualitative grounds in practical is still a challenging part. The involvement of humic acid to fulvic acid ratio and the absorbance ratio of alkaline soil extract at 400 and 600 nm wavelengths (E4/E6 or A400/A600) has been put forward for the assessment of SOM quality (Guillaume *et al.*, 2021), the fractionation being cantered on the principle of their differential solubility at distinct pH values (Kader and Jaufer, 2022).

However, majority of these determination methods are labour as well as time intensive. Therefore, there is a demand for employment of alternative

approaches in determination of soil organic matter for detection of changes in soil organic matter both quantitatively as well as qualitatively and for provision of thorough and timely information regarding soil organic matter content at low expenditure along with an acceptable level of reliability.

Spectroscopy in detection of SOM

Recent developments in reflectance spectroscopy have led to its emergence as a method to improve the efficiency of soil carbon analysis and stock estimation (Nayak *et al.*, 2019). Visible near-infrared and mid infra-red reflection spectroscopic methods are cost effective, rapid, non-destructive and reproducible (Currà *et al.*, 2019). Mid infra-red spectroscopic approach has attained popularity due to its comparatively simple procedure of sample preparation along with the capability of evaluating dry soils directly (Guerrero *et al.*, 2021). However, due to the appreciable sensitivity of the instrument regarding the homogeneity of the soil-matrix used, its applicability for determination of absolute concentrations is unsuitable. Usually, the outperformance of mid- infra-red spectroscopy to that of Vis- near infra-red has been concluded due to prevalence of more defined bands in case of mid-IR, thereby, performing better in quantification of soil organic matter as well as soil organic carbon (Mendes *et al.*, 2022).

An imperative requisite for the international databases have been marked through extensive reviews of all the accessible near-infra red techniques for soil quality assessment (Dharumarajan *et al.*, 2023; S. Kader *et al.*, 2023; Youssef *et al.*, 2023). The suitability of Vis-infra red spectroscopic technique has been proven even for the independent set of validation as well as under outdoor circumstances. The reliable estimates for quantification of soil organic matter and soil organic carbon during laboratory analysis have been produced for Vis- Infra red spectroscopy (Aqua, Santos, and Chiang, 2019). However, because of abandoned variations in surface conditions of soil, the direct measurement of soil spectra in the field or employment of air-borne imaging spectrometry remains challenging.

The spectral mechanism of prediction varies with the soil sample population due to its reliance on decomposition phase of soil organic matter, nature of existing compounds and the impact of other allied factors viz., soil moisture, iron oxides and soil texture. The average R-squared value for the prediction of soil organic matter was observed to be 0.96 for mid- infra-red, 0.81 for near infra-red and 0.78 via visible spectroscopy (Shi *et al.*, 2023), reflecting a less accurate predictability of visible spectra.

The utilization of mid-IR to determine soil organic matter on qualitative basis dealing with the band assignments has been put forth by (Nasonova *et al.*, 2022) after reviewing the applicability of mid infra-red spectroscopy for estimation of soil organic matter noted a general application of mid infra-red spectroscopy for both quantitative as well as qualitative analysis of soil. Spectrophotometric determinations of soil organic matter produced results with comparable precision and accuracy, improved performance, 60% fall in toxic

reagent consumption and 91% decline in the generation of residue volume (Souza *et al.*, 2016). Spectral quality being the weightiest constraint in calibration of Vis-infra red and mid infra-red, require a pre-treatment of soil spectra quite ahead of spectroscopy model calibration for the reduction of interference contributed by variation in particle size distribution. Numerous examinations put forward revealed an enhanced accuracy of predictions made by Vis-near infra-red and mid infra-red spectroscopy after the application of various pre-treatment methods for thinning of particle size and noise effects.

Laser induced breakdown spectroscopy (LIBS)

Laser induced breakdown spectroscopy (LIBS), relying on the atomic emission, detects the characteristic spectral signature of carbon. A laser beam possessing a certain wavelength is focused on the sample that is to be analyzed, via a lens of 50 mm focal length forming the micro-plasma that emits a characteristic wavelength for each sample's elemental composition (Ebinger *et al.*, 2003). A high correlation of 0.96 for soils exhibiting similar morphology was observed while comparing the data from LIBS with that of data acquired from dry combustion (Senesi and Senesi, 2016; Cremers *et al.*, 2001). The initial study of Cremers *et al.* (2001) was further outstretched through the exploitation of Q-switched Nd-YAG laser at 266 nm wavelength having 23-mJ pulse along with the provision of ICCD for the measurement of carbon and nitrogen contents of soil samples from Oak Ridge and southwest Virginia mined lands possessing a range of 0.16%–4.3% of total carbon concentrations which were earlier acid washed and dried. Simple and multiple linear regression models were used for the development of calibration curves and data validation for laser induced breakdown spectroscopy (Cama-Moncunill *et al.*, 2017). LIBS has proven an attractive and influential analytical tool unusual to conventional methodology due to some additive advantages such as no requisition of sample preparation, minimizing the disturbance of soil, its comparative simplicity, less analytical time of the order of minutes, and the capability of analysing large number of samples every day associated with the cost efficiency. However, the challenges still exist that need to be addressed for its strengthened applicability such as the management of ablation and plasma formation, advancement and interaction with the adjoining environment, the attainment of equilibrium conditions in case of plasma, and excluding the effects of self-absorption that may interfere in portraying the signal intensity for elements at higher concentrations, thereby, making their quantification impractical.

Inelastic neutron scattering (INS) for SOM estimation

The novel inelastic neutron scattering (INS) technique for analysis of soil carbon relies on the gamma ray spectroscopy acquired from the interaction of fast neutrons with the nuclei of elements in soil (Yakubova *et al.*, 2017a). The inelastic neutron scattering technique analyzes the acquired spectral data for different peak intensities (counts), utilizing an established calibration curve,

portrays the results instantaneously having units of kg C m^{-2} . An R-squared value of 0.99 has been observed while calibrating the INS system with synthetic soils containing a mixture of sand and a known quantity of carbon yielded. The significance of INS method to that of gold standard DC method has been confirmed where the speed of carbon content defining by the INS method was approximately 30-fold greater than the DC method (Yakubova *et al.*, 2017b). The inelastic neutron scattering technique has a potential of analysing intact samples of soil samples on a large spatial scale, however, the cost incurred on instrumentation and transport are quite huge and the licenses from radiological control and properly skilled technicians are required.

Recent developments in SOM estimation

Assessment of different proficient analytical techniques such as Elemental analysis paired with Isotope ratio mass spectrometry (EA-IRMS), Pyrolysis - gas chromatography in combination with mass spectrometry (Py-GCMS), and Nuclear magnetic resonance spectroscopy (NMR) to determine soil organic matter has been performed in view of recent methodologies for soil organic matter studies (Santoiemma, 2018). EA-IRMS being the bulk assessment technique furnishes the data on average composition of elements detected and isotopic signature of a soil sample (Bianchi and Careri, 2021). The estimation of $\delta^{13}\text{C}$ via EA-IRMS relates to the age of soil organic matter (Natali *et al.*, 2018) accounting it to the relationship existing between fractionation of isotopes and microbial respiration, rapid mineralisation of ^{13}C -poored constituents along with the decline in $\delta^{13}\text{C}$ of CO_2 in the previous years as a consequence of fossil combustion. However, the accessibility of IRMS standards along with standardized methodology is the foremost goal to be addressed in present (Khatri *et al.*, 2023). The coupling of analytical pyrolysis with gas chromatography-mass spectrometry (Py-GCMS) involves the decomposition of sample through pyrolysis/heating for the production of smaller compounds that can be separated by gas chromatography and detected by mass spectrometry (Bensidhom *et al.*, 2021). The identification of compounds present in the sample is done through a characteristic pattern of fragmentation, requiring huge database availability for recognition and elucidation of structure of chemical compounds. Py-GCMS improved the structural illustration of compounds, and with integration with other methods in multi-proxy approach, offered a high-resolution molecular data with regard to component analysis of organic matter. However, some ambiguity exists in pyrolyzed samples of original organic matter in relation to their representativeness.

NMR Spectroscopy for SOM estimation

NMR spectroscopic technique aids in the determination of atomic and molecular structure of the soil samples, the samples being de-mineralized accurately to evade the instrumental (Alexis *et al.*, 2012). The employment of external magnetic fields possessing different strengths by different NMR

spectrometer result in the distribution of the nuclei spins among different energy levels. Solid-state NMR spectroscopic method has proven an acceptable and influential analytical method for characterization of soil organic and for the determination of lability of soil organic matter fractions. ^{13}C NMR spectroscopic technique has been employed for the characterisation of humic acids after forest fires (Chen *et al.*, 2020), for the characterisation of humic acids in bio-solid amended soils, for categorization of humic substances in per-humid montane forest soils, for characterisation of humic substances isolated from post-fire forest soils and for description of soil organic matter in virgin and *Alnus* forest. However, the reduced cross polarization efficiency for mobile constituents and non-protonated carbons along with the broad and overlapping of organic matter spectra pose a major challenge in future.

CONCLUSIONS

The recent advancements in estimating the soil organic matter promise a high precision along with the less time requirement for sample preparation and their subsequent analysis. The conventional laboratory estimation methods mark well accepted uncertainties and limitations, being laborious and time consuming and additionally complexed by the variability of organic carbon content along the profile both spatially and temporally. Therefore, the development of more rapid, accurate, robust, precise and cost-effective methodology with the requisition of no or minimal sample pre-treatment, minimizing the uncertainties and enhancing the organic carbon estimates and fluxes, is a crucial and dire need. The introduction of recent advancements is thus expected to empower a healthier quantification and validation of organic matter changes both temporally and spatially so as to address the issues of global climate change and terrestrial carbon management. Despite, of possessing myriads of advantages, a notable number of instrumental, methodological and procedural challenges might be faced that need to be figured out in present and future research.

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