Thermal stability of soil carbon pools: inferences on soil nature and evolution

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Abstract

The quantification of soil carbon pools is a pressing topic both for the agriculture productivity and to evaluate the Greenhouse Gases (GHG) sequestration potential, therefore a rapid and precise analytical protocol for carbon speciation is needed. Temperature-dependent differentiation of soil carbon in compliance with the DIN (German Institute for Standardization) 19539 standard has been applied for the first time on 24 soil samples from the Po River Plain (Italy), with the aim of investigate their thermal behavior in the 50-900°C interval. The results invariably show the existence of three soil carbon pools having different thermal stabilities, namely, thermally labile organic carbon (TOC400), residual oxidizable carbon (ROC) and total inorganic carbon (TIC900), in the intervals of 300-400°C, 510-600°C and 700-900°C, respectively. Significant relationships have been observed between the above mentioned organic and inorganic carbon pools and the associated isotopic composition: 1) inverse correlation between TOC400/ROC and $\delta^{13}C$ links thermal stability and soil organic matter (SOM) composition; 2) direct correlation between carbonate breakdown temperature and $\delta^{13}C$ denotes the mineralogical association of the inorganic pool. The results give clues regarding the nature and evolution of soil carbon pools.

Keywords: soil carbon, SOM dynamics, SOM stabilisation, DIN 19539, thermal speciation, $^{13}C/^{12}C$ isotopic ratio

Introduction

The study of the soil carbon (and nitrogen) cycle is paramount to evaluate agricultural productivity and to estimate the role of soils as GHG sources/sinks. In this light, it is of primary importance to identify a rapid methodology for correct soil carbon (and nitrogen) speciation.

Recent analytical developments demonstrated that thermal analysis techniques are suitable for the rapid and precise determination of the distinct carbon pools in soil samples [1-5].

As thoroughly described in the Supplementary Information, the main advantage of the thermal approach with respect to the conventional methods (e.g., [4-5]) for carbon speciation is the ability to conduct direct analysis without preliminary sample chemical treatments, which often lead to variable and unpredictable losses of carbon (and nitrogen) fractions [6-8]. On this basis, Natali and Bianchini [9,10] and Natali et al. [11] set up a thermally based separation (TBS) methodology specifically designed for the elemental and isotopic analysis of distinct soil carbon pools (total organic carbon-TOC, total inorganic carbon-TIC) using an elemental analyzer (EA) coupled with an isotope ratio mass spectrometer (IRMS) analytical system. Notably, the application of TBS to a set of agricultural soils from the Po river Plain in northern Italy (Supplementary Information) highlighted the precise relationships between the nature and evolution of soil organic matter (SOM) and the related pedogenetic environment [12].

In this new study, the same soil sample set was further investigated by the use of a new analytical device for the measurement of C and N concentrations under step-heating conditions, in compliance with the German Institute for Standardization-DIN 19539 standard [13], which is progressively catching as termochemical approach for the analysis of complex environmental matrices.

This- analytical device allows the separation and analysis of two oxidisable soil carbon pools having different thermal stabilities (thermally labile organic carbon, TOC400, stripped out at temperatures below 400°C; residual oxidisable carbon, ROC, at temperatures between 400 and 600°C) and one non-oxidisable carbon pool (TIC 900) derived by the thermal breakdown of carbonate minerals at temperatures between 600 and 900°C (see analytical details in the Supplementary Information). The results of these two methodologies have been integrated and critically discussed to shed light on the significance of different soil carbon pools characterised by different thermal stabilities and will provide new insights into the relationship between the thermal and biological stability of SOM (see [3]).

Results

The new analyses, carried out by the use of an Elementar SoliTOC cube are reported as thermochemical diagrams (Fig. 1). These diagrams show the release of carbon (and nitrogen) from soil at increasing temperatures; this release is related to the thermal destabilisation of organic and inorganic phases. The investigated samples show three carbon fractions characterised by distinct thermal stabilities (Fig 1a). These fractions are well separated and are recorded in the temperature intervals of 300-400°C, 510-600°C and 700-900°C; according to the DIN 19539 standard, the fractions are referred to as TOC400, ROC and TIC900, respectively. Thermochemical diagrams show that nitrogen is exclusively associated with TOC400 and ROC, suggesting the organic nature of both fractions (Fig 1b). The complete set of results is presented in Supplementary Table 1, which includes the new analyses of TOC400, ROC, TIC900 and TN, as well as those previously obtained by TBS [11,12]. Summarising:

- TOC400 varied between 0.86 and 1.94 wt% in the topsoils and between 0.11 and 1.13 wt% in the subsoils, with the exception of samples from a peaty site (38) characterised by TOC400 values of 4.21 wt% at the surface and 12.33 wt% at depth.
- ROC varied between 0.28 and 0.72 wt% in the topsoils and between 0.12 and 1.14 in the subsoils, excluding the topsoil of the abovementioned peaty site (sample 38A), which showed a ROC value of 1.60 wt%.
- TIC900 varied between 0.13 and 2.25 wt% in the topsoils and between 0.05 and 2.36 in the subsoils.

The average standard deviation (SD), based on replicate analyses on three representative samples and a soil standard (Bodenstandard Nr.2, HEKA tech GmbH Analysentechnik, Wegberg, Germany) was 0.05 for TN, 0.08 wt% for TC, 0.07 wt% for TIC, 0.07 for TOC, 0.18 wt% for TOC400 and 0.16 wt% for ROC.

The resulting total carbon ($TC_{DIN\ 19539}$) varied between 1.43 and 4.68 wt% in the topsoils and between 0.94 and 3.70 wt% in the subsoils, with the exception of the organic-rich sample (38), which exhibited $TC_{DIN\ 19539}$ values of 6.79 wt% at the surface and 12.71 wt% at depth. The total nitrogen content ($TN_{DIN\ 19539}$) varied between 0.14 and 0.29 wt% in the topsoils and between 0.01 and 0.21 in the subsoils, with the exception of samples from site 38 showing $TN_{DIN\ 19539}$ values of 0.58 wt% at the surface and 0.80 wt% at depth.

The TC/TN ratio was distinctly higher in the subsoils (31.3-303.3, average of 78.9) with respect to topsoils (8.9-28.9, average of 18.7), with the exception of a subset of organic-rich deep samples (8B, 38B and 41B) that showed TC/TN ratios comparable to those of topsoils (11.1, 16.2 and 9.0, respectively).

The TOC400/ROC ratio was generally lower in the subsoils (0.9-2.6 average of 1.9) with respect to topsoils (2.5-3.8, average of 3.0), with the exception of the deep sample 38B, which showed an extremely high value (40.2).

The TOC (TOC400+ROC)/TIC ratio was often below 1 (average of 0.53) in subsoils and varied between 0.06 to 2.24, with the exception of samples 38B e 41B that showed extremely high values (33.2 and 178, respectively). In topsoils, TOC/TIC ratio is comparatively higher (average of 2.52) and ranged between 0.51 and 10.43.

Discussion

The comparison of the new data obtained at step-heating conditions by the SoliTOC cube with those obtained by TBS [11,12] through EA-IRMS analyses on the same samples shows very good agreement (Supplementary Table 1, Supplementary Figure 1). In particular, we observed significant correlations between the total carbon and nitrogen obtained with the two methodologies; the correlations are characterised by a very high distribution coefficient (r^2>0.99), a slope from 1.05 to 1.07 and an intercept between 0.00 and -0.08. A similar relationship involves the total organic carbon measured as the sum of TOC400 and ROC ($TOC_{DIN\ 19539}$) and the TOC measured by TBS (TOC_{TBS}); this relationship is characterised by r^2 = 1.00, a slope of 1.07 and an intercept of 0.07. The total inorganic carbon measured in compliance with the DIN 19539 standard (TIC900) is also in perfect agreement with that obtained by TBS (TIC_{TBS}), showing a distribution coefficient r^2 = 0.98, a slope of 0.99 and an intercept of 0.02.

It must be emphasised that the DIN 19539 standard allows a thermal stability index of the soil organic matter to be obtained through the TOC400/ROC ratio, which is an important parameter intimately related to its composition. A systematic difference is observed between the TOC400/ROC ratios in the topsoils and the subsoils, the former being characterised by higher values (TOC400/ROC from 2.5 to 3.7) with respect to the latter (TOC400/ROC from 0.9 to 2.6), with the exception of the abovementioned peaty sample (38B) characterised by the highest value (TOC400/ROC = 40.2). Notably, a significant inverse relationship (r^2 = 0.64) is observed between the $\log_{10}$ (TOC400/ROC) and the carbon isotopic composition of the soil organic matter ($\delta^{13}C_{TOC}$ ‰), as measured by the TBS (Fig. 2). The inverse relationship between these parameters does not significantly decrease (r^2 = 0.57) excluding the extreme peaty sample. The topsoils were characterised by comparatively higher TOC400/ROC ratios and generally more negative $\delta^{13}C_{TOC}$ values (average of -24.7 ‰) with respect to the subsoils that showed comparatively low TOC400/ROC ratios and less negative $\delta^{13}C_{TOC}$ values (average of -22.5 ‰), excluding the organic-rich sample (38B, $\delta^{13}C_{TOC}$ -28.7 ‰).

Regarding the inorganic fraction, we observed a wide temperature range (ca. 200°C) associated with carbonate breakdown, which suggests a significant variability in the mineralogical composition of the investigated samples. Notably, we recorded a significant (r^2 = 0.72) direct relationship between the carbonate breakdown temperature and the isotopic composition of the inorganic fraction ($\delta^{13}C_{TIC}$ ‰), possibly related to the variable presence of secondary carbonates having low temperature stability [10,14]. This also suggests that primary carbonates are dominated by calcite, and not by dolomite or other soil carbonate minerals having lower breakdown temperature [15].

The inverse relationship between the TOC400/ROC ratio and the isotopic composition of the soil organic matter suggests that the thermally labile carbon fractions are invariably characterised by more negative isotopic compositions, whereas the more refractory organic compounds display less negative carbon isotopic compositions. In particular, the comparatively high TOC400/ROC ratio and very negative $\delta^{13}C_{TOC}$ values that characterise topsoils are indicative of “fresh” or –untransformed– organic matter. Conversely, the relatively low TOC400/ROC ratios associated with the less negative $\delta^{13}C_{TOC}$ values that characterise the subsoils are indicative of organic matter affected by a transformation mediated by biological activity. The ^{13}C enrichment that characterises the organic fraction of the subsoils with respect to that of topsoils is a commonly-observed phenomenon along soil profiles (e.g., [16]). ^{13}C enrichment can be the result of several processes, such as the preferential stabilisation of ^{13}C enriched (polysaccharides and amino acids) compounds and the preferential decomposition of ^{13}C depleted (lipids and lignin) compounds, or it could result from SOM decomposition by microbial activity [17 and references therein]. Similar effects have been observed by Lopez-Capel et al. [18], who reported a progressive homogenisation of the isotopic composition of coexisting SOM compounds towards ^{13}C enriched values as a result of fungal degradation. This finding is in agreement with recent research, which found that thermally labile aliphatic compounds (destabilised at 300-350 °C) are ca. 3 ‰ ^{13}C depleted with respect to the more refractory aromatic compounds that decompose at higher temperatures (400-450 °C) [18-21]. The observed inverse relationship between TOC400/ROC ratio and the $\delta^{13}C_{TOC}$ value suggests therefore a link between the thermal stability index and the SOM composition, which is in turn related to its origin (nature of the original vegetal detritus) and evolution (transformation/decomposition by microbial activity).

Regarding the inorganic carbon, the straightforward relationship between the breakdown temperature of carbonates and the associated isotopic compositions could be related to the variable contribution of authigenic/pedogenic minerals that appear to be thermally more labile than detrital –primary– carbonates.

Conclusions

This work highlights for the first time the potential of a new analytical approach developed for the precise, rapid and cost-effective determination of soil carbon pools. It demonstrates that the application of the DIN 19539 standard to soil samples is an effective technique that fulfils these compelling requirements. Moreover, the results highlight that the DIN 19539 standard provides additional tools to define the soil organic matter thermal indexes that are related to the soil composition, which in turn is linked the nature of the original vegetal detritus, the transformation mediated by micro and macroorganisms. This statement is based on a significant inverse correlation between TOC400/ROC and $\delta^{13}C_{TOC}$, which gives insights on a link between thermal stability and of soil organic matter (SOM) composition.

Direct correlation between carbonate breakdown temperature and $\delta^{13}C_{TIC}$ gives insights on the mineralogical association of the inorganic pool, thus completing the understating of the soil carbon pools. Therefore, this analytical approach represents a promising tool for unravelling the possible relationships between thermal and biological stabilities in soil matrices.

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References

[1] Plante, A.F., Fernández, J.M., Haddix, M.L., Steinweg, J.M., Conant, R.T. 2011. Biological, chemical and thermal indices of soil organic matter stability in four grassland soils. *Soil Biology and Biochemistry*, **43**, 1051–1058.

[2] Peltre, C., Fernández, J.M., Craine, J.M., Plante, A.F. 2013. Relationships between bio- logical and thermal indices of soil organic matter stability differ with soil organic carbon level. *Soil Science Society of America Journal,* **77,** 2020–2028.

[3] Hou, Y., Chen, Y., Chen X., He, K., Zhu, B. 2019. Changes in soil organic matter stability with depth in two alpine ecosystems T on the Tibetan Plateau. *Geoderma*, **351**, 153-162.

[4] Vuong, T.X., Heitkamp, F., Jungkunst, H.F., Reimer, A., Gerold, G. 2013. Simultaneous measurement of soil organic and inorganic carbon: evaluation of a thermal gradient analysis. *Journal of Soils and Sediments*, **13**, 1133-1140.

[5] Vuong, T.X. 2015. Highly resolved thermal analysis as a tool for simultaneous quantification of total carbon, organic carbon, inorganic carbon and soil organic carbon fractions in landscapes. *PhD, thesis, University Göttingen, Germany*.

[4] Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science*, **63**, 251–263.

[5] Schumacher, B. A. 2002. Methods for the Determination of Total Organic Carbon (TOC) in Soils and Sediments. Ecological Risk Assessment Support Center Office of Research and Development US. Environmental Protection Agency, Las Vegas, NV, USA.

[6] Serrano, O., Serrano, L., Mateo, M.A., Colombini, I., Chelazzi, L., Gagnarli, E. & Fallaci, M. 2008. Acid washing effect on elemental and isotopic composition of whole beach arthropods: implications for food web studies using stable isotopes. *Acta Oecologica,* **34**, 89–96.

[7] Brodie, C.R., Leng, M.J., Casford, J.S.L., Kendrick, C.P., Lloyd, J.M., Yongqiang, Z. & Bird, M.I. 2011. Evidence for bias in C and N concentrations and $\delta^{13}C$ composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods. *Chemical Geology*, **282**, 67–83.

[8] Schlacher, T.A. & Connolly, R.M. 2014. Effects of acid treatment on carbon and nitrogen stable isotope ratios in ecological samples: A review and synthesis. *Methods in Ecology and Evolution*, **5**, 541–550.

[9] Natali, C. & Bianchini, G. 2014. Understanding the carbon isotopic signature in complex environmental matrices. *EQA-International Journal of Environmental Quality*, **14**, 19-30.

[10] Natali, C. & Bianchini, G. 2015. Thermally based isotopic speciation of carbon in complex matrices: a tool for environmental investigation. *Environmental Science and Pollution Research*, 22, 12162–12173.

[11] Natali, C., Bianchini, G. & Vittori Antisari L. 2018a. Thermal separation coupled with elemental and isotopic analysis: A method for soil carbon characterisation. *Catena*, **164**, 150-157.

[12] Natali, C., Bianchini, G., Vittori Antisari, L., Natale, M. & Tessari, U. 2018b. Carbon and nitrogen pools in Padanian soils (Italy): origin and dynamics of Soil Organic Matter. *Chemie der Erde* **78**, 490-499.

[13] DIN Standards Committee Water Practice 2015. Investigation of solids—Temperature-dependent differentiation of total carbon (TOC400, ROC, TIC900). Beuth, Berlin, Germany.

[14] Rovira, P. & Vallejo, V.R. 2008. Changes in δ^{13}C composition of soil carbonates driven by organic matter decomposition in a Mediterranean climate: A field incubation experiment. *Geoderma*, **144**, 517–534.

[15] Cuthbert, F. & Rowland, R. 1947. Differential thermal analysis of some carbonate minerals. *American Mineralogist*, **32**, 111–116.

[16] Natelhoffer, K.J. & Fry, B. 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. *Soil Science Society of America Journal*, **52**, 1633–1640.

[17] Rumpel, C. & Kögel-Knabner, I. 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant and Soil*, **338**, 143–158.

[18] Lopez-Capel, E., Abbott, G.D., Thomas, K.M. & Manning, D.A.C. 2006. Coupling of thermal analysis with quadrupole mass spectrometry and isotope ratio mass spectrometry for simultaneous determination of evolved gases and their carbon isotopic composition. *Journal of Analytical and Applied Pyrolysis*, **75**, 82–89.

[19] Manning, D.A.C., Lopez-Capel, E. & Barker, S. 2005. Seeing soil carbon: use of thermal analysis in the characterization of soil C reservoirs of differing stability. *Mineralogical Magazine*, **69**, 425-435.

[20] De la Rosa, J.M., Lopez-Capel, E., Gonzalez-Vila, F.J., Gonzalez-Perez, J.A. & Manning, D.A.C. 2008. Direct detection of black carbon in soils by Py-GC/MS, ^{13}C NMR spectroscopy and thermogravimetric techniques. *Soil Science Society of America Journal*, **72**, 258-267.

[21] Araya, S.M., Fogel, L.M. & Berhe, A.A. 2017. Thermal alteration of soil organic matter properties: a systematic study to infer response of Sierra Nevada climosequence soils to forest fires. *Soil*, **3**, 31-44.

FIGURE CAPTIONS

Figure 1 – Diagrams showing the step-heating (T °C expressed by the red line) extraction of C (detected as CO_2; a) and N (detected as NO_x; b) carried out by Elementar SoliTOC Cube elemental analyser, in compliance with the DIN 19539 standard, for the investigated soils.

Figure 2 – Binary diagram showing the logarithmic relationship between the TOC400/ROC ratio and $\delta^{13}C_{TOC}$ (‰) for the investigated soils. The inset reports the sample distribution without the extreme organic-rich sample. See text for further details.

SUPPLEMENTARY FIGURE CAPTIONS

Supplementary Figure 1 – Linear relationships between TN, TC, TOC and TIC (wt%) obtained by elemental analyser (Elementar SoliTOC Cube) in compliance with the DIN 19539 standard and by TBS [11] using an elemental analyser (Elementar Vario Micro Cube) coupled with an Isotope Ratio Mass Spectrometer (Isoprime 100). Dashed lines represent the 1:1 ratio for all variables.

SUPPLEMENTARY TABLE CAPTIONS

Supplementary Table 1 – Thermal separation of carbon pools according to TBS by EA-IRMS (Elementar Vario Micro Cube- Isoprime 100) and to DIN 19539 by EA (Elementar SoliTOC Cube) for the investigated soils. TN (wt%) measured by the two methodologies is also reported for all samples.