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TOC-L Total Organic Carbon Analyzer

Application News

Measurement of Soil Microbial Biomass Carbon and Nitrogen

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User Benefits

- Total organic carbon (TOC) and total nitrogen (TN) content can be measured in soil extracts simultaneously and rapidly.
- Utilizing the instrument's automatic dilution function can mitigate the effects of soil extract salts on catalysts and combustion tubes.
- Multiple samples can be measured automatically by using an ASI-L autosampler.

■ Introduction

Organic carbons in soil are decomposed by microbial activity and released into the atmosphere as carbon dioxide (CO_2). Numerous research institutions are currently focusing on soil carbon sequestration and the role of microbial activity in the decomposition of organic carbon within the soil. The microbial biomass carbon (MBC) content in soil serves as an indicator of microbial activity and soil carbon dynamics, making it a basic research focus within soil microbiology.

The chloroform fumigation-extraction method stands out among various methods for quantifying soil biomass due to its high reliability and reproducibility, with minimal influence from soil properties and microbial types. MBC information can be obtained by using a Shimadzu TOC-L total organic carbon analyzer to measure solutions prepared by chloroform fumigation-extraction and correcting for the extraction rate from soil. Additionally, integrating a TNM-L total nitrogen unit with the TOC-L analyzer enables the concurrent measurement of soil microbial biomass nitrogen (MBN).

This article describes an example of measuring MBC and MBN in soil using the chloroform fumigation-extraction method with a Shimadzu TOC-L total organic carbon analyzer.

■ Sample Preparation

As shown in Table 1, topsoil and subsoil (soil at different depths from the soil surface) were collected from two distinct locations.

For each soil type, a portion was set aside for chloroform fumigation treatment. Those subsamples were exposed to chloroform vapors for 24 hours in a vacuum desiccator. That process ruptures the microbial cell walls, enhancing the extractability of biomass components. Afterward, the residual chloroform was removed.

Following that, biomass was extracted from both the fumigated (F) and non-fumigated (NF) subsamples using a 0.5 M potassium sulfate solution (equivalent to 87 g/L). Since the presence of high salt concentrations in samples can accelerate the degradation of catalysts and combustion tubes, the extracts were diluted fivefold with pure water to prepare the measurement solutions. Note that the TOC-L analyzer is equipped with an automatic dilution function that significantly streamlines that dilution process.

Table 1 Soil Sample Preparation

Samples	Chloroform Fumigation Treatment (F: fumigated; NF: non-fumigated)
Location A Topsoil	F
	NF
Location A Subsoil	F
	NF
Location B Topsoil	F
	NF
Location B Subsoil	F
	NF

■ Analytical Conditions

A system consisting of the TOC-L analyzer combined with a TNM-L unit was employed for the analysis (Fig. 1). The measurement conditions are listed in Table 2.

For TOC measurement, the non-purgeable organic carbon (NPOC) method was applied. That entails initially acidifying the sample to transform all inorganic carbon (IC) into dissolved CO_2, which is subsequently expelled from the sample via sparging. Then the remaining total carbon (TC) is measured to determine the TOC value. TN was also measured in parallel with the TOC measurement.

To calibrate the analyzer, a two-point calibration curve was created for the TC measurements using potassium hydrogen phthalate solutions at concentrations of 0 and 50 mgC/L. Similarly, for TN measurements, potassium nitrate solutions at concentrations of 0 and 10 mgN/L were used. The calibration curve origin points were shifted to adjust for any TOC and TN that may be inherent in the pure water used to prepare the standard solutions.

MBC and MBN values for each soil sample were calculated as the difference between measurements of the fumigated and non-fumigated subsamples.

Fig. 1 System Consisting of the TOC-L Total Organic Carbon Analyzer and the TNM-L Total Nitrogen Unit
(A) ASI-L Autosampler, (B) TNM-L Total Nitrogen Unit,
(C) TOC-L Total Organic Carbon Analyzer

Table 2 Measurement Conditions

Instruments	TOC-L_{CPH} + TNM-L
Catalyst	TOC/TN catalyst
Measured Quantities	NPOC (TOC by acidification and sparging)
	TN
Injection Volume	40 µL
Dilution Ratio	5 times
Option	ASI-L Autosampler
Calibration Curves	TC: 2-point calibration curve with 0 and 50 mgC/L of potassium hydrogen phthalate aqueous solution
	TN: 2-point calibration curve with 0 and 10 mgN/L of potassium nitrate aqueous solution

■ Measurement Results

The TOC concentrations in the soil extracts and the calculated MBC values are indicated in Table 3. Corresponding TN and MBN values are listed in Table 4. To represent the concentrations in the undiluted extracts, the measured TOC and TN values have been adjusted by fivefold dilution. The conversion factors, k_{EC} for carbon and k_{EN} for nitrogen were set at 0.45 and at 0.54, respectively, based on widely accepted values from literature[2,3].

Table 3 TOC Measurement Results

Samples	TOC_F*1 [mgC/L]	TOC_{NF}*2 [mgC/L]	MBC*3 [mgC/L]
Location A Topsoil	126	48.4	172
Location A Subsoil	74.6	50.3	54.0
Location B Topsoil	148	48.5	221
Location B Subsoil	80.9	48.9	71.0

Table 4 TN Measurement Results

Samples	TN_F*1 [mgN/L]	TN_{NF}*2 [mgN/L]	MBN*3 [mgN/L]
Location A Topsoil	21.4	10.7	19.9
Location A Subsoil	5.31	3.23	3.84
Location B Topsoil	30.3	15.4	27.5
Location B Subsoil	6.21	3.78	4.50

*1 TOC_F and TN_F: TOC or TN of fumigated samples
*2 TOC_{NF} and TN_{NF}: TOC or TN of non-fumigated samples
*3 $MBC = (TOC_F - TOC_{NF})/k_{EC}$ $MBN = (TN_F - TN_{NF})/k_{EN}$

MBC and MBN concentrations were higher in the topsoil than in the subsoil from both locations. This trend is consistent with the prevailing understanding that topsoil exhibits higher microbial activity.

The data exemplifying these measurements are indicated in Fig. 2 and 3, where both demonstrate good repeatability.

The findings confirm that the combined use of the TOC-L total organic carbon analyzer and the TNM-L total nitrogen unit is effective for quantifying MBC and MBN in soil samples.

■ Conclusion

This article has verified that the system combining a TOC-L total organic carbon analyzer and a TNM-L total nitrogen unit is well-suited for the determination of soil MBC and MBN levels. Utilizing the ASI-L autosampler enables the automated measurement of large numbers of samples, thereby significantly boosting analytical productivity.

For soil extracts with high salt concentrations, the automatic dilution feature can reduce catalyst and combustion tube degradation caused by salts without extra manual effort. Furthermore, the use of Shimadzu's high-salt sample combustion tube kit, a specialized combustion tube kit designed for high-salt samples, is instrumental in prolonging catalyst life.

In conclusion, the TOC-L plus TNM-L system is expected to be a valuable tool for the measurement of soil MBC and MBN values.

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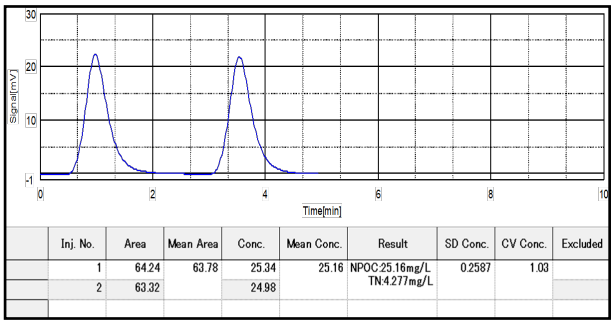
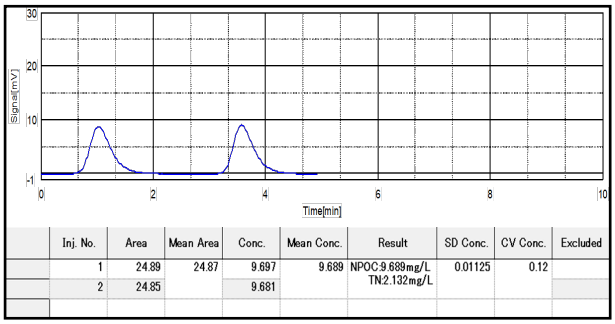


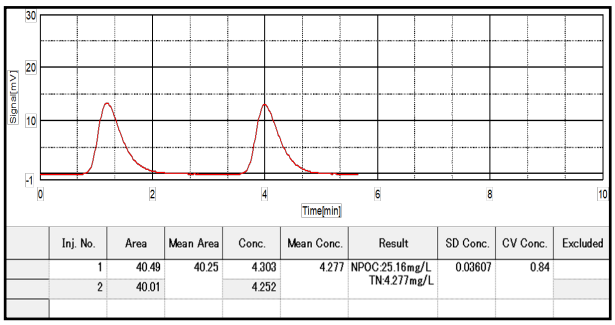
Inj. No.	Area	Mean Area	Conc.	Mean Conc.	Result	SD Conc.	CV Conc.	Excluded
1	64.24	63.78	25.34	25.16	NPOC:25.16mg/L TN:4.277mg/L	0.2587	1.03	
2	63.32		24.98					

(a) TOC in Fumigated (F) Topsoil at Location A



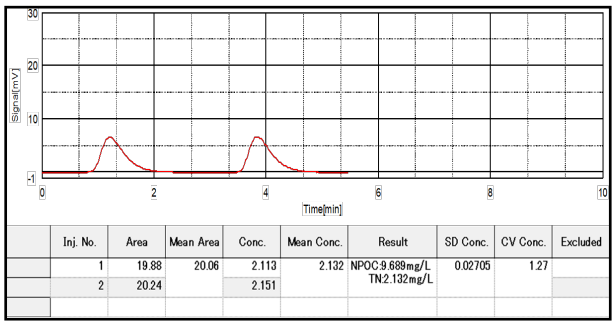
Inj. No.	Area	Mean Area	Conc.	Mean Conc.	Result	SD Conc.	CV Conc.	Excluded
1	24.89	24.87	9.697	9.689	NPOC:9.689mg/L TN:2.132mg/L	0.01125	0.12	
2	24.85		9.681					

(b) TOC in Non-Fumigated (NF) Topsoil at Location A
Fig. 2 TOC Measurement Data from Topsoil at Location A



Inj. No.	Area	Mean Area	Conc.	Mean Conc.	Result	SD Conc.	CV Conc.	Excluded
1	40.49	40.25	4.303	4.277	NPOC:25.16mg/L TN:4.277mg/L	0.03607	0.84	
2	40.01		4.252					

(a) TN in Fumigated (F) Topsoil at Location A



Inj. No.	Area	Mean Area	Conc.	Mean Conc.	Result	SD Conc.	CV Conc.	Excluded
1	19.88	20.06	2.113	2.132	NPOC:9.689mg/L TN:2.132mg/L	0.02705	1.27	
2	20.24		2.151					

(b) TN in Non-Fumigated (NF) Topsoil at Location A
Fig. 3 TN Measurement Data from Topsoil at Location A

References

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3) Rainer Georg Joergensen, Torsten Mueller (1996), The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EN} value, Soil Biology and Biochemistry, 28(1), 33-37.

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