Calibration of Loss On Ignition Method for Soil Organic Carbon and Nitrogen Calculation Using a C/N Analyzer

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1 Introduction

Soil organic carbon (SOC) and nitrogen (N) are essential in agricultural sustainability and carbon sequestration. The carbon-to-nitrogen (C/N) ratio serves as a fundamental indicator of soil organic matter quality, decomposition rates, and nutrient cycling processes. In sandy coastal agricultural soils, such as those in the Niigata region of Japan, understanding C/N ratios is important due to soil texture, drainage characteristics, and proximity to marine environments.

In our previous approach, we employed the Loss On Ignition (LOI) method to quantify soil organic carbon (SOC), utilizing the Van Bemmelen constant, which estimates SOC as 58% of Soil Organic Matter (SOM). However, this method is characterized by limited accuracy. Consequently, we analyzed 24 samples (4 replicates of 6 treatments) using both the LOI method and a C/N analyzer. The samples were collected at a soil depth of 15-20 cm.

2 Objectives

The objectives of this study are to: (1) calculate and evaluate C/N ratios in sandy coastal agricultural soils from Niigata, Japan; (2) assess the need for inorganic carbon measurement based on observed C/N values; (3) compare field and laboratory measurement methods; and (4) calibrate the Loss on Ignition method and derive the optimal model equation for calculating SOC and nitrogen within the dataset.

3 Methods

3.1 Experimental design

The study was conducted on sandy coastal agricultural soils at Niigata University. The experimental design included six treatments with four replicates each (n=24 total samples), representing different agricultural management practices:

CT+C: Conventional tillage with chemical fertilizer

CT+M: Conventional tillage with organic mulch (crop residues, dry chipped weed)

CT+O: Conventional tillage with organic fertilizer (compost: mix chicken and cow manure, and rice husk)

RT+C: Reduced tillage with chemical fertilizer

RT+M: Reduced tillage with organic mulch

RT+O: Reduced tillage with organic fertilizer

3.2 Carbon and Nitrogen measurement

We used the Loss on Ignition method to quantify Soil Organic Matter (SOM) by assessing the weight difference following the calcination of soil samples at 750 °C. This weight loss was subsequently employed to calculate Soil Organic Carbon (SOC) as 58% of SOM.

Furthermore, we used the C/N analyzer to determine the carbon and nitrogen content in the same samples by combusting them at elevated temperatures (950–1,000 °C), converting all carbon to CO_2 and nitrogen to N_2 gases. These resultant gases are separated and quantified using detectors, such as thermal conductivity detectors (TCD) and infrared (IR) sensors. The measured gas concentrations were then used by the device to automatically calculate the percentage of carbon and nitrogen in the original sample.

We employed the results from the C/N analyzer to conduct a regression analysis of SOC and nitrogen and evaluated the coefficients of determination (R^2).

3.3 Statistical analysis

Statistical analysis included:

Descriptive statistics for all measured parameters

Analysis of variance to assess treatment effects

Linear regression analysis comparing field vs. laboratory measurements

Correlation analysis between LOI and laboratory SOC measurements

4 Results and discussions

4.1 Soil carbon and nitrogen concentrations

Table 1 presents the calculated C/N ratios for all treatments and replicates. Laboratory SOC concentrations ranged from 0.22% to 1.10%, while nitrogen concentrations varied from 0.03% to 0.11%. The resulting C/N ratios showed variation, ranging from 3.31 to 36.67.

Table 1. Soil organic carbon, nitrogen concentrations, and C/N ratios by treatment and replicate.

Treatment	Replicate	SOC Lab (%)	N Lab (%)	C/N Ratio
CT+C	1	0.22	0.03	7.33
CT+C	2	0.22	0.07	3.35
CT+C	3	0.34	0.05	6.39
CT+C	4	0.29	0.08	3.81
CT+M	1	0.75	0.03	21.57

CT+M	2	0.47	0.04	12.28
CT+M	3	0.51	0.04	13.04
CT+M	4	0.55	0.05	12.00
CT+O	1	0.78	0.08	9.48
CT+O	2	0.59	0.07	8.31
CT+O	3	1.05	0.06	17.53
CT+O	4	0.85	0.06	14.13
RT+C	1	0.30	0.05	5.69
RT+C	2	0.28	0.08	3.31
RT+C	3	0.62	0.11	5.69
RT+C	4	0.40	0.11	3.54
RT+M	1	0.57	0.06	9.14
RT+M	2	0.79	0.03	26.20
RT+M	3	0.40	0.05	7.72
RT+M	4	0.43	0.03	13.63
RT+O	1	0.99	0.04	20.96
RT+O	2	0.83	0.05	16.58
RT+O	3	1.10	0.05	21.68

RT+O	4	1.10	0.03	36.67

4.2 Treatment effects on C/N ratios

Figure 1 shows the mean values and standard deviations for SOC, N, and C/N ratios across treatments. Significant differences were observed among treatments, with RT+O showing the highest mean C/N ratio (23.97 ± 8.76), followed by CT+M (14.72 ± 4.59) and RT+M (14.17 ± 8.41). The lowest C/N ratios were observed in RT+C (4.56 ± 1.31) and CT+C (5.22 ± 1.94).

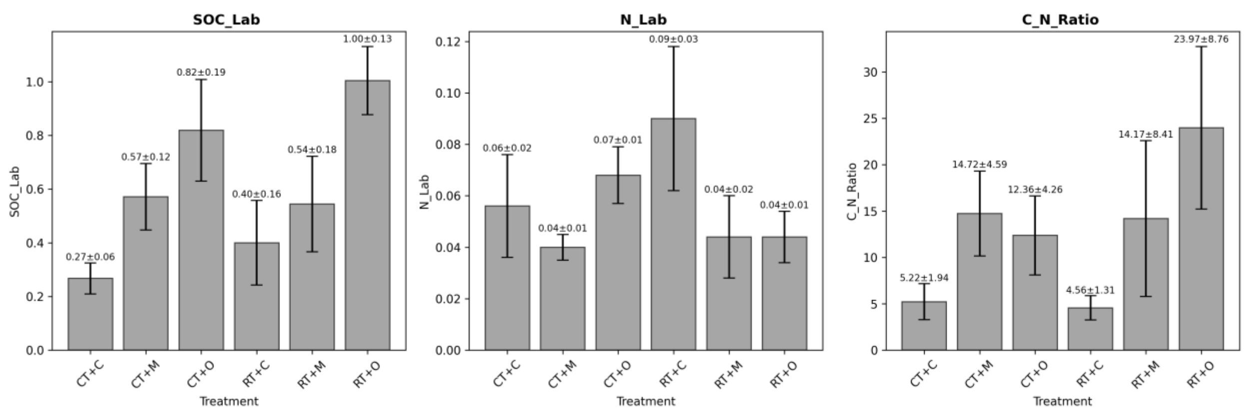


Figure 1. Mean laboratory soil organic carbon (SOC), nitrogen (N), and C/N ratio among conventional-tillage (CT) and reduced-tillage (RT) treatments with chemical fertilizer (C), organic mulch (M), and organic fertilizer (O) (±1 SD)

Figure 2 shows the distribution of individual data points and highlights the variability within treatments. Outliers were observed in RT+O and CT+M treatments, particularly for C/N ratios exceeding 25. This might be due to contaminations during preparation of the samples before analysis (sieving process).

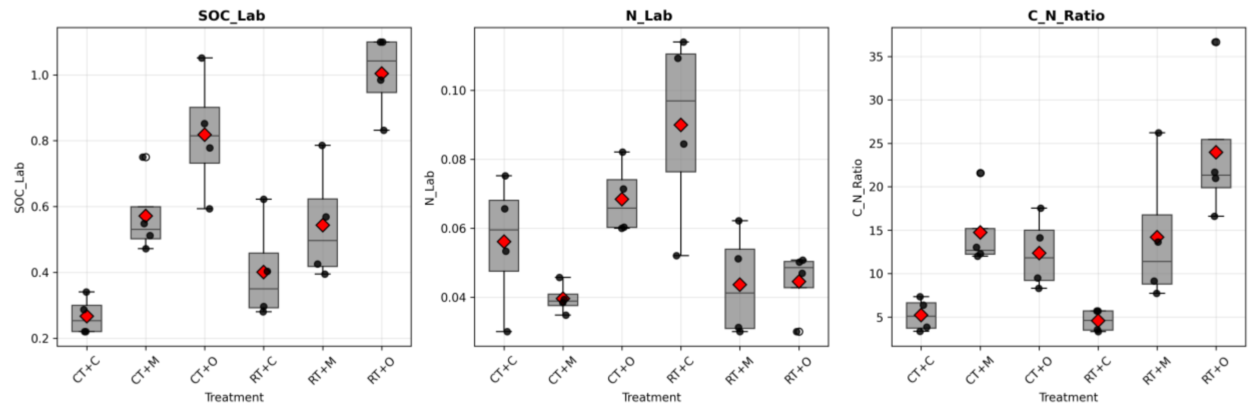


Figure 2. Distribution of laboratory soil organic carbon (SOC_Lab), nitrogen (N_Lab), and C/N ratio among conventional-tillage (CT) and reduced-tillage (RT) treatments with chemical fertilizer (C), organic mulch (M), and organic fertilizer (O), showing individual data points, box boundaries (quartiles), medians, and mean values (red diamonds).

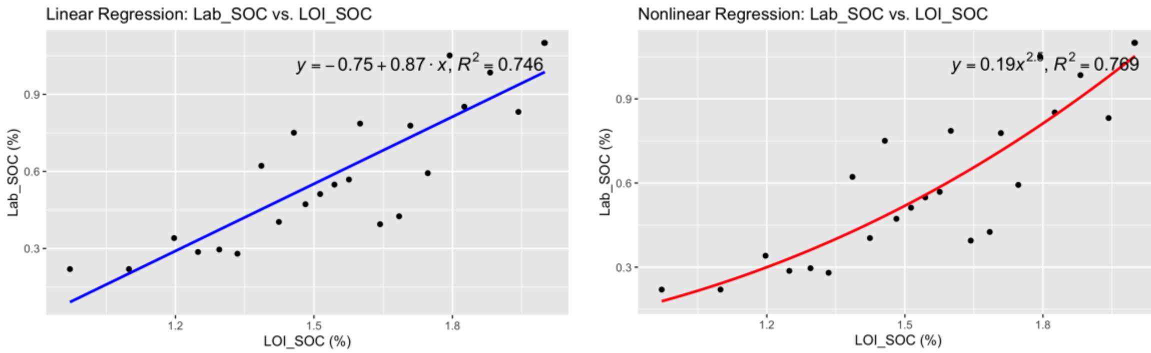
The overall mean C/N ratio is 12.50 ± 8.34, with a median of 10.74. Several samples showed C/N ratios exceeding 20, with the highest value reaching 36.67 in the RT+O treatment. These elevated ratios imply investigation for potential inorganic carbon interference.

4.3 Comparison between field and laboratory measurement

4.3.1 Soil Organic Carbon

In the linear regression model, the coefficient of determination (R^2) indicated that about 75% of the variation in Lab_SOC (%) could be attributed to LOI_SOC (%) (Figure 1.A). A slope of 0.87 demonstrates a strong positive correlation. However, the intercept is negative at −0.75. When this model equation is applied to calculate SOC within the dataset, it results in negative Lab_SOC values at very low LOI_SOC. In contrast, the nonlinear model exhibited a higher R^2 value of 77% compared to the linear model (Figure 1.B). This model not only provides a superior fit for explaining the variation in Lab_SOC but also avoids producing negative Lab_SOC values during the calculation process.

(A) (B)



R^2 LOI_SOC ~ Lab_SOC: 0.75

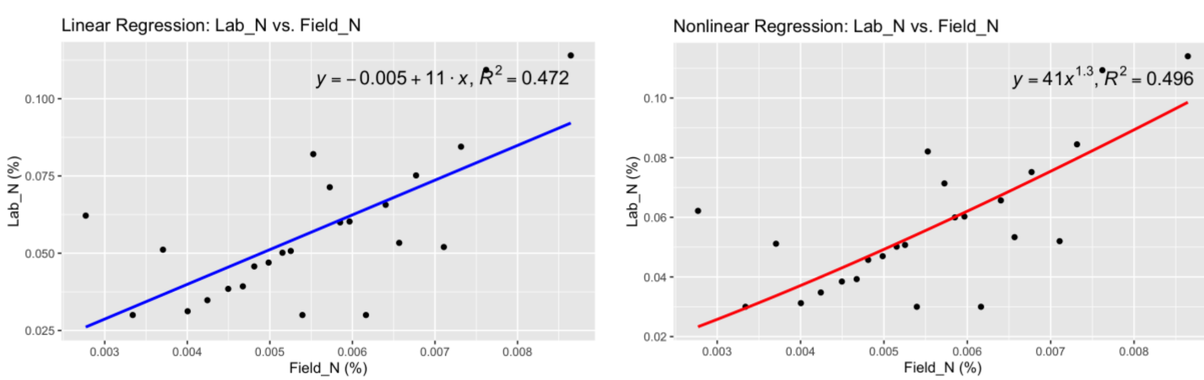
Correlation LOI_SOC ~ Lab_SOC: 0.86

Figure 1. Regression analysis. (A) Linear relationship and (B) nonlinear relationship between SOC as determined by the Loss on Ignition method and SOC as measured using a C/N Analyzer

4.3.2 Soil Nitrogen Content

In the linear model, approximately 47% of the variation in Lab_N (%) was accounted for by its linear association with Field_N (%) (Figure 2.A). Although this relationship is positive, 53% of the variability in Lab_N remains unexplained by Field_N. In contrast, the nonlinear model accounted for approximately 50% of the variation in Lab_N (%), which was better (Figure 2.B).

(A) (B)



R^2 Field_N ~ Lab_N: 0.4721418

Correlation Field_N ~ Lab_N: 0.6871258

Figure 2. Regression analysis. (A) Linear correlation and (B) nonlinear correlation between nitrogen levels measured in the field and those quantified using a C/N Analyzer

5 Discussion

5.1 C/N ratio distribution and critical values

According to the literature, healthy agricultural soils have C/N ratios between 8-15, with values below 8 indicating rapid nitrogen mineralization and values above 20 suggesting nitrogen immobilization or potential analytical interference (Brady and Weil 2008; Stevenson 1994; Piccolo and Drosos 2025). The high C/N ratios observed in some samples, are concerning for several reasons:

1. Inorganic Carbon Interference: In coastal environments, the presence of carbonate minerals ($CaCO_3$) from marine sources can inflate apparent organic carbon measurements when using elemental analyzers without acid pretreatment (Chatterjee et al. 2009; Schumacher 2002; McNally et al. 2025).

2. Methodological Considerations: The literature emphasizes the importance of distinguishing between organic and inorganic carbon fractions, particularly in coastal and calcareous soils (Bisutti et al. 2004; Ng et al. 2025). Standard protocols recommend acid fumigation or treatment to remove carbonates before elemental analysis to ensure accurate SOC quantification.

5.2 Carbon sequestration and stability implications

The range of C/N ratios observed has important implications for carbon sequestration assessment. Literature indicates that C/N ratios influence organic matter stability, with moderate ratios (10–15) associated with optimal conditions for long-term carbon storage (Manzoni et al. 2008; "(PDF) Stoichiometry of Litter Decomposition under the Effects of Climate Change and Nutrient Enrichment" 2025). The extremely high ratios observed in some samples, if confirmed to represent organic carbon, would suggest poor nitrogen availability and potential limitations for microbial processing and carbon stabilization.

However, if these high ratios result from inorganic carbon interference, the actual organic carbon stocks may be overestimated, leading to incorrect assessments of soil carbon sequestration potential. This has significant implications for carbon credit calculations and soil management recommendations.

6 Conclusion

The results suggest that organic amendments under reduced tillage systems may enhance soil organic matter accumulation, but accurate quantification requires correction for potential inorganic carbon interference. The variability in C/N ratios necessitate careful interpretation and additional analytical procedures.

7 References

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