

Scope and Application

The Total Kjeldahl Nitrogen (TKN) method is based on the wet oxidation of soil organic matter using sulfuric acid and digestion catalyst and conversion of organic nitrogen to ammonium nitrogen. Ammonium is determined using spectrophotometric, diffusion-conductivity or distillation techniques. The method is readily adapted to manual or automated techniques. The procedure does not quantitatively digest nitrogen from heterocyclic compounds (bound in a carbon ring), oxidized compounds such as nitrate, or ammonium from within mineral lattice structures. The method has a detection limit of approximately 0.020% N and is generally reproducible within ± 1

Equipment

1. Analytical balance: 250 g capacity minimum ± 0.1 mg.
2. Acid fume hood.
3. Volumetric digestion tubes, 75 mL and digestion heating block (400 °C).
3. Repipette dispenser, calibrated to 3.0 ± 0.2 mL.
4. Spectrophotometer, diffusion-conductivity instrument or distillation apparatus.

Reagents

1. Deionized water, ASTM Type I grade.
2. Sulfuric acid, concentrated - reagent grade.
3. Digestion Catalyst (K_2SO_4 , $CuSO_4$, and SeO : ratio 100:10:1), Kjel-tab.
4. Standard Calibration solutions (NH_4-N). Prepare six working standards of ammonium, concentration range $0.1 - 40$ mg L^{-1} , made from 1000 mg L^{-1} ammonium nitrogen standard solution and diluted to volume with 12 % sulfuric acid (v/v).

Procedure

1. Weight 1.000 ± 0.005 g of air dry soil to pass 10 mesh sieve (< 2.0 mm) into a 75.0 mL volumetric digestion tube (See Comment #1). Include a method blank.
2. Add digestion catalyst, (200 mg of mixed catalyst or Kjel-tab) and 3.0 mL of concentrated sulfuric acid (See Comment #2 and #3). Note: it is essential that all dry material be completely moistened by acid and well mixed to insure complete digestion.
3. Place tubes on a digestion block at $150^\circ C$ and after thirty (30) minutes raise to $350^\circ C$ for two (2) hours or until samples are completely digested. At completion, mineral soils will be whitish-gray and organic soils blue-green in color.
4. Remove samples from block and place under fume hood for 5-10 minutes. Add 10-20 mL of deionized water using a wash bottle to each tube to prevent hardening and crystal formation. Dilute digest to volume with deionized water, cap, invert three times, and allow digest to clear.
5. Determine digest ammonium concentration using the spectrophotometric, diffusion-conductivity instruments or distillation techniques using standard calibration solutions (See Comment #4 and #5). The ammonium nitrogen content of the digest solution can be determined with a rapid flow analyzer (Technicon Method No. 334-74A/A) or an flow injection analyzer (FIA). This determination can also be made using the Kjeldahl distillation method. Adjust and operate instruments in accordance with manufacturer's instructions. Determine ammonium concentration of a method blank, unknown samples and record ammonium concentration as mg L^{-1} of NH_4-N in soil digest.

Calculations

Report total Kjeldahl nitrogen results to the nearest 0.001% as:

$$\% \text{ N} = \frac{(\text{mg L}^{-1} \text{NH}_4\text{-N in digest} - \text{method blank}) \times (0.075) \times (100)}{(\text{Sample size mg})} \quad \text{S-8.10-1]$$

Comments

1. Use 250 mg of soil if sample is greater than approximately 10% organic matter.
2. Check repipette dispenser delivery volume, recalibrate using an analytical balance.
3. When adding reagents to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable lab gloves, and shoes). Always handle reagent and digestion labware in hoods capable of high air flow, 100 cfm.
4. The Kjeldahl method outline by Bremmer and Mulvaney (1982) is modified eliminating the water from the digestion step.
5. Kjeldahl soil acid digest is classified as hazardous waste and must be disposed of in a suitable manner.

Literature

Bremmer, J.M. and C. S. Sulvaney. 1982. Total nitrogen p.595-624. *In*: A.L. Page et al. (eds.) Methods of soil analysis, part 2. Agron. Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Carlson, R.M. 1978. Automated separation and conductometric determination of ammonia and dissolved carbon dioxide. *Anal. Chem.* 48: 1528-1531.

Horneck, D.A., J.M. Hart, K. Topper and B. Koespell. 1989. Methods of soil analysis used in the soil testing laboratory at Oregon State University. *Ag. Expt. Station SM 89:4.*

Scope and Application

This method quantifies the amount of oxidizable soil carbon as determined by reaction with $\text{Cr}_2\text{O}_7^{2-}$ and sulfuric acid. The remaining unreacted dichromate is titrated with FeSO_4 using Ortho-phenanthroline as an indicator and organic carbon calculated by difference. The method is based upon that described by Mebius (1960) and is an estimate since not all the organic carbon present is oxidized. Soil organic matter values are used to estimate potential nitrogen mineralization, for pesticide management and for crop production management. Chromium disposal costs have forced many laboratories to consider Loss on Ignition (LOI, see Method 9.20) as a means for estimating soil organic matter content. The method detection limit is approximately 0.10% and is generally reproducible to within $\pm 8\%$.

Equipment

1. Analytical Balance: 100 g capacity, resolution ± 0.001 g.
2. Erlenmeyer flask 125 mL and 250 mL beaker.
3. Buchner funnel 11 cm.
4. Whatman No. 42 filter paper 11 cm, or equivalent highly retentive filter paper.
5. Repipette dispenser(s), calibrated to 5.0 ± 0.1 , 10.0 ± 0.2 and 15.0 ± 0.2 mL.
6. 50 mL burette with graduations of 0.1 mL.
7. Magnetic stir plate and microsize teflon coated magnetic stir bar.

Reagents

1. Deionized water, ASTM Type I grade.
2. Potassium dichromate solution, 1.0 N solution: Dissolve 49.04 g of $\text{K}_2\text{Cr}_2\text{O}_7$ (dried at 105°C) in deionized water and dilute to 1000 mL.
3. Ferrous sulfate heptahydrate solution, 0.5 N: Dissolve 140 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 500 mL of deionized water, add 15.0 mL of concentrated 16 N H_2SO_4 and dilute to 1000 mL. (See Comment #1).
4. Concentrated sulfuric acid solution (16 N).
5. Ortho-phenanthroline-ferrous complex solution, 0.025 M. Dissolve 3.71 g of O-phenanthroline monohydrate and 1.74 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in deionized water and dilute to 250 mL. Store in plastic bottle (See Comment #2).

Procedure

1. Weigh 0.500 ± 0.005 g of air dry soil pulverized to pass 40 mesh sieve (< 0.42 mm) (See Comment #3) into 125 mL erlenmeyer flask. Include a blank solution.
2. Using repipette dispenser add 5.0 mL of 1.0 N $\text{K}_2\text{Cr}_2\text{O}_7$ solution to the flask containing soil. Include a blank flask (See Comment #4).
3. Using a repipette add rapidly 10.0 mL of concentrated H_2SO_4 acid, directing the stream of liquid into the center of soil suspension. Immediately swirl for one (1) minute, cool on a heat resistant surface for thirty (30) minutes and add 100 mL of deionized water.
4. Filter the suspension into 250 mL beaker and refilter if filtrate is cloudy (See Comment #5).

5. Add 0.30 mL of Ortho-phenanthroline-ferrous complex 0.025 M indicator solution. Titrate the solution with 0.50 N ferrous sulfate from 25 mL buret (See Comment # 6). As the endpoint is approached, the solution takes on a greenish cast and changes to dark blue green. An additional 0.20 mL of indicator may be used to sharpen the endpoint. At this point, add the ferrous sulfate drop by drop until the color changes sharply from blue to orange red (maroon in reflected light) and record amount (mL) of ferrous sulfate solution used (See Comment # 7).

Calculations

$$\text{Normality (N) FeSO}_4 = \frac{\text{mL K}_2\text{Cr}_2\text{O}_7 \times \text{N K}_2\text{Cr}_2\text{O}_7}{\text{mL FeSO}_4}$$

$$\text{meq FeSO}_4 = \text{mL FeSO}_4 \times \text{N (FeSO}_4)$$

$$\text{Organic Carbon (\%)} = \frac{((\text{meq K}_2\text{Cr}_2\text{O}_7 - \text{meq FeSO}_4) \times 0.003 \times 100)}{\text{sample dry weight} \times 1.33 - \text{blank}}$$

$$\text{Organic Carbon (\%)} = \frac{((5 - \text{meq FeSO}_4) \times 0.399) - \text{blank}}{\text{sample dry weight}} \quad (\text{See Comment \#8})$$

$$\text{Organic Matter (\%)} = 1.72 \times \text{Organic Carbon \%}$$

Comments

1. Allow solution to cool to room temperature before diluting to standard volume. Store in pyrex bottle.
2. N-phenylanthranilic acid can be substituted as an indicator: dissolve 0.100 g of N-phenylanthranilic acid and 0.107 g of Na₂CO₃ in deionized water and dilute to 100 mL. The endpoint proceeds rapidly from violet to gray to bright green.
3. For soils containing greater than 8 mg of organic carbon reduces sample size. Soils should be pulverized to pass 30 mesh sieve (0.5 mm).
4. When adding reagents to vessels always wear protective clothing (i.e. eye protection, lab coat, disposable lab gloves, and shoes). Always handle reagent and digestion labware in hoods capable of high air flow, 100 cfm.
5. The filtrate is classified as a hazardous waste and must be disposed of in a suitable manner.
6. Flush buret with 0.5 N ferrous sulfate solution before titration, as it is light sensitive.
7. If more than 75% of the dichromate is reduced, repeat with smaller sample size. Samples containing large amounts of manganese or carbonates may give erroneous results and require pretreatment with 0.1 N HCl to remove.
8. The value of 1.33 represents an average correction factor since the dichromate does not completely oxidize all soil organic carbon. The value may be replaced by a more suitable value found through experimentation. Multiply organic carbon value by 1.72 to calculate organic matter (%).

Literature

Mebius, L.J. 1960. A rapid method for the determination of organic carbon in soil. *Anal. Chim. Acta.* 22:120-124.

Janitzky, P. 1986. Cation exchange capacity, p. 34. *In*: M.J. Singer and P. Janitzky (ed.) *Field and laboratory procedures used in a soil chromosequence study*. U.S. Geological Survey Bulletin 1648.

Nelson, D.W. and L.E. Sommers. 1982. Total carbon, organic carbon and organic matter, p. 539-594. *In*: A.L. Page et al. (eds.) *Methods of soil analysis, part 2*. Agron. Monogr. 9. 2nd ed. ASA and SSSA, Madison, WI.

Nelson, D.W. and L.E. Sommers. 1975. A rapid and accurate procedure for estimation of organic carbon. *Soil Proc. Indiana Acad. Sci.* 84:456-462.

Schulte, E.E. 1988. Recommended soil organic matter tests, p. 29-31. *In*: W.C. Dahnke (ed.) *Recommended chemical soil test procedures for the North Central Region*. North Dakota Agricultural Experiment Station Bulletin 499 (revised).

Scope and Application

This method semiquantifies the amount of oxidizable organic matter as determined by the gravimetric weight change associated with high temperature oxidation of soil organic matter in a muffle furnace. The method is based on one described by Storer (1984) and is an estimate. As a result of chromium hazardous waste disposal costs associated with Walkley-Black method (S-9.00), many laboratories have chosen to switch to loss on ignition as a means for estimating soil organic matter content. The LOI method is poorly correlated with the Walkley-Black method for soils containing less than 3% organic matter. Soil organic matter values are used to estimate potential nitrogen mineralization, pesticide management and for crop production management. The method is generally reproducible within $\pm 20\%$.

Equipment

1. Analytical balance: 100 g capacity, resolution ± 0.001 g.
2. High temperature, crucibles 20 cc capacity.
3. Drying oven, 105 °C.
4. Dessicator, containing desiccating agent.
5. Muffle furnace capable of heating to 360 °C.

Reagents

1. Calcium carbonate, reagent grade (See Comment #1).

Procedure

1. Weigh 10.0 ± 1.0 g of air dry soil pulverized to pass 10 mesh sieve (< 2.0 mm) into a preweighed crucible (record to the nearest ± 0.001 g). Prepare a crucible containing calcium carbonate (See Comment #1).
2. Place in drying oven for two (2) hours at 105 °C. Place in dessicator for one (1) hour.
3. Record crucible + soil as Initial wt to the nearest ± 0.001 g.
4. Heat in muffle furnace to 360 °C for two (2) hours (after temperature reaches 360 °C).
5. Place in drying oven at 105 °C for one (1) hour and place in dessicator for one (1) hour.
6. Record crucible + soil as Final wt sample weight to the nearest ± 0.001 g.

Calculations

$$\text{LOI \%} = \frac{(\text{Initial wt at } 105^\circ\text{C} - \text{Final wt. at } 105^\circ\text{C}) \times 100}{(\text{Initial wt at } 105^\circ\text{C} - \text{crucible wt})} \quad \text{u. S -9.20-1]$$

Estimation of organic matter by LOI is done by regression analysis with organic matter. Select fifty soils covering a range in organic matter expected, determine organic matter based on Walkley-Black method (Method S-9.10) and LOI value. Use this equation to convert LOI values to estimated percent organic matter (See Comment #2).

Comments

1. Calcium carbonate is included as a method standard to evaluate potential loss of carbonates of alkali metals. If appreciable losses (>0.05% weight change) are noted check temperature calibration of the muffle furnace.
2. The regression slope for organic matter for the LOI method on the Walkley-Black method ranges from 0.68 to 1.04 for soils reported in the literature. Regression intercept values range from -0.04 to -0.36, (Schultee and Hopkins, 1996).

Literature

Schulte, E.E. 1988. Recommended soil organic matter tests, p. 29-31. *In*: W.C. Dahnke (ed.) Recommended chemical soil test procedures for the North Central Region. North Dakota Agricultural Experiment Station Bulletin 499 (revised).

Schulte, E.E. and B.G. Hopkins. 1996. Estimation of soil organic matter by weight Loss-On-Ignition. P. 21-32. *In*: Soil Organic matter: Analysis and Interpretation. (ed.) F.R. Magdoff, M.A. Tabatabai and E.A. Hanlon, Jr. Special publication No. 46. Soil Sci. Soc. Amer. Madison, WI.

Storer, D.A. 1984. A simple high sample volume ashing procedure for determining soil organic matter. *Commun. Soil Sci. Plant Anal.* 15: 759-772.

Combustion Method

Scope and Application

This method quantitatively determines the amount of organic carbon in soil materials by combustion of the sample in an O₂ environment using an automated resistance furnace and with subsequent quantification of nitrogen by thermal conductivity detector and CO₂ using an infrared or conductivity detector. For very low nitrogen analysis (< 0.05%) specific instruments are available with chemiluminescence detectors. Soils with a pH > 7.4 (method S-2.10) and containing inorganic carbon (carbonates), organic carbon is determined by the difference between total carbon by combustion minus the quantity of inorganic carbon as determined by Method S-13.10 or S-13.20. The method for specific instruments requires that soils be pulverized to pass 60 mesh sieve to ensure homogeneity. It is based on the method originally described by Dumas whereby soil samples encased in tin (Sn) foil, are ignited in a furnace in excess of 1000°C, in a helium and oxygen environment in a quartz combustion tube. The combustion gas is passed through a catalyst (instrument manufacturer dependent) to complete conversion of CO to CO₂, scrubbed of moisture and CO₂ determined by an infrared detector and for nitrogen by thermal conductivity detector. Specific instruments provide for the simultaneous determination of H or S. Total nitrogen and organic carbon is used to assess nutrient mineralization, water infiltration, soil structure and absorption or deactivation of agricultural chemicals. The method has a detection limit of 0.03% N and 0.02% TOC (dry sample basis instrument dependent) and is generally reproducible to within ± 7.0% for nitrogen and ± 5.0% for organic carbon..

Equipment

1. Analytical balance: 250 g capacity, resolution ± 0.1 mg.
2. Total Nitrogen Analyzer: Leco CHN-1000, CNS-2000, Elementar, Carl-Erba, Perkin-Elmer or Elementar, with a resistance furnace with infrared and/or thermal conductivity detector and operating supplies.
3. Tin foil encapsulating capsules (See Comment #1).
4. Desiccator, containing a desiccating agent.

Reagents

1. Compressed Oxygen, 99.99% purity.
2. Helium carrier gas 99.99% purity.
3. Total Organic Carbon calibration standards: EDTA, 9.57% ± 0.05% N; sulfanilic acid (C₆H₇NO₃S) 41.6% C; Leco part number 502-203 soil, 2.75% ± 0.09 % C; and Leco part number 502-062 soil, 0.85% ± 0.05% C.

Procedure

1. Determine the soil moisture content (See comment #2).
2. Weigh of air dry soil (quantity is instrument dependent) pulverized to pass a 30 mesh sieve (< 250 μm) (See Comments #3 and #4 #5) and place in into a tarred tin foil container, encapsulate, close and record sample weight to the nearest 0.1 mg.
3. Initialize the instrument following manufacturer's suggested protocol. Conduct a system leak check on combustion system. Perform blank stabilization test, analyze consecutive blanks until the blanks stabilize at a constant value (See Comment #6).
4. Adjust and operate the instrument according to manufacturer instructions using calibration standards. Enter sample dry matter content and analyze unknown sample for total nitrogen. Report results to the nearest 0.001% carbon (See Comment #7).

Calculations

Report total nitrogen results to the nearest 0.001%

Report total organic carbon results to the nearest 0.01%

Comments

1. Tin (Sn) foil capsules is utilized as combustion catalyst. Capsules can be obtained from the manufacturer's, after market vendors, or fabricated from sheets of tin foil material.
2. Samples limited in material, should be dried over phosphorus pentoxide or magnesium perchlorate for forty-eight (48) hours and analyzed with no correction for moisture content or reported on as received basis.
3. Sample particulate material must be ground to pass a 30 mesh sieve ($< 600\mu\text{m}$) for macroanalysis instruments which require sample sizes in excess of 250 mg (i.e. LECO, CHN-2000 and Elementar) in order to assure adequate sample homogeneity. For instruments utilizing a sample sizes 5-10 mg (Carlo-Erba and Perkin-Elmer) samples must be ground to pass 100 or 140 (106-150 μm) mesh sieve.
4. Soils containing free carbonates must be analyzed for free calcium carbonate according to Methods S-13.10 or S-13.20 for the determination of inorganic carbon. Total organic carbon is calculated by subtracting the inorganic carbon from the total carbon value determined by the combustion instrument.
5. Sample weight may be entered into instrument software using a balance interface.
6. All soil calibration samples should be: (1) checked for homogeneity; and (2) nitrogen and organic carbon content verified using standard addition techniques using two chemical standards such as EDTA and sulfanilic acid. A quality control certified reference sample (NIST SRM 2704, 3.348% \pm 0.10% C) is available from the National Institute of Standards and technology, see appendix B.
7. To convert total soil organic carbon (%C) to soil organic matter (SOM by Walkely-Black method, S-9.10), multiply %C by 1.724 to estimate soil organic matter. The conversion factor ranges from 1.6 to 2.5 dependent on the soil and cropping system management.

Literature

Nelson, D.W. and L.E. Sommers. 1996. Total carbon, organic carbon and organic matter. p. 961-1010. *In*: J.M. Bartel et al. (ed.) Methods of soil analysis: Part 3 Chemical methods. (3rd.ed.) ASA and SSSA, Madison, WI. Book series no. 5

McGeehan, S.L. and D.V. Naylor. 1988. Automated instrumental analysis of carbon and nitrogen in plant and soil samples. Commun. in Soil Sci. Plant Anal. 19:493-50-5.

Sheldrick, B.H. 1986. Test of the LECO CHN-600 Determinator for soil carbon and nitrogen analysis. Can. J. Soil Sci. 66:543-545

Schepers, J.S. D.D. Francis, and M.T. Thompson. 1989. Automated total nitrogen of soil and plants samples. Commun. in Soil Sci. Plant Anal. 20:949-959.

Tiessen, H., J.A. Bettany, and J.W.B. Stewart 1981. An improved method of the determination of carbon in soils and soil extracts by dry combustion. *Commun. Soil Sci. Plant Anal.* 12(3): 211-218.

Yeomans, J.C. and J.M. Bremner. 1991. Carbon and nitrogen analysis of soils by automated combustion techniques. *Commun. in Soil Sci. Plant Anal.* 22:843-850.