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Soil Carbon and Nitrogen
Pools and Fractions

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Soil organic matter (SOM) is important as a major source of most nutrients, especially nitrogen (the one major plant nutrient not supplied by weathering), and as a source of cation exchange capacity, especially in highly weathered soils. SOM also contributes to good soil structure, thus promoting drainage, water-holding capacity, aeration, and root penetration, and provides strong control of soil pH, especially in highly weathered soils. In addition, soil organic matter is a major global C source and sink. Jenkinson et al. (1991) calculated that an increase in global temperature of 0.03 °C/yr could increase CO_2 release from soil by about 1 Gt C/yr over the next 60 years; by comparison, fossil fuel burning currently releases about 5 Gt C/yr.

Soil nitrogen availability limits plant growth in many terrestrial ecosystems, yet increased N deposition due to atmospheric pollution has been implicated in the decline of forest productivity in some heavily industrialized parts of the northern temperate zone (Schulze 1989). The relation between total soil N and nitrogen availability to plants is still unclear, in large part because the nature of soil organic N is only now beginning to be understood.

In this chapter we present standard methods for determination of total soil C and N and measurement of total organic and inorganic soil C. Methods are also presented for separating SOM into biologically meaningful fractions. Field sampling methods and soil standards are discussed in Chapter 2, this volume.

Available Methods

Total Carbon and Nitrogen Analysis

Dry (Dumas) combustion is the most suitable method for routine analysis of total C and N in soil, litter, and plant samples. The Walkley–Black technique, a wet combustion method, is no longer recommended because it can underestimate soil C by 20–30% (Nelson and Sommers 1982) and can give spurious results in highly reduced soils unless precautions are taken (Snyder and Trofymow 1984). The Walkley–Black method is also laborious and produces toxic wastes. Measuring carbon content by measuring mass loss following high-temperature combustion (the loss-on-ignition, [LOI] method) is easily performed but has serious shortcomings. LOI measures volatilization of all material, including the water retained in clay structures at standard ovendrying temperature (105 °C). Allophane, for example, was found to lose 26% of its mass upon heating from 100 to 800 °C; gibbsite lost 54% (Gardner 1986). Nonetheless, because of its low cost, LOI may have a place in the soils lab when large numbers of similar samples must be processed. LOI should always, however, be checked against a dry-combustion method.

Soil Organic Matter Fractionation

Because SOM is so heterogeneous, much effort has focused on methods for isolating biologically meaningful pools ("separates"), which can then be analyzed for composition or biological availability. There are two general approaches: chemical extraction and physical separation. The latter can be based on particle size, particle density, degree of physical protection, or a combination of the three. It is also possible to estimate the size of fast turnover, slow turnover, and inactive SOM pools based on biological assays (see Chapter 13, this volume).

Chemical fractionation relies traditionally on solubility in acid and base, producing fractions termed *humic acid* (base extractable, acid insoluble), *fulvic acid* (base extractable, acid soluble), and *humin* (insoluble in base). Unfortunately, these procedures do not produce separates that differ consistently in their biological properties. Despite many studies, none have documented clear or consistent variation in age or amount of these chemical fractions by soil type or in response to management (Duxbury et al. 1989). Although the procedure continues to give useful results for fractionating dissolved organic C (DOC) from soil solution and streams, as a procedure for soils, it is not recommended here. Two chemical fractionation methods that we do recommend are extraction in cold water and acid hydrolysis (boiling 6N HCl). The former appears to provide a useful measure of microbially degradable C, while acid hydrolysis leaves a residue that gives a consistently old ^{14}C date and can thus be regarded as a slow turnover pool (Paul et al. 1997).

Physical fractionation methods separate the soil into component particles, many of which are aggregates consisting of smaller particles bound together by various agents of different binding strengths (Tisdall 1996). Aggregates can be dispersed with chemicals that remove binding agents or by mechanical action such as shaking or ultrasonic vibration. After dispersion the particles are sorted by density and/

or size. The effectiveness of dispersion techniques increases with the amount of energy applied. It may be convenient to think there is some ultimate dispersion point beyond which no decrease in particle size is possible, but in practice this is unachievable: (1) with enough energy even clay micelles are disrupted, and (2) many of the smallest particles can be highly reactive and reflocculate after dispersal (see Christensen 1992). The problem of achieving and maintaining dispersion is especially critical in soils rich in amorphous constituents such as allophane, ferrihydrite, and Al-OM complexes. Since complete, permanent dispersion is never possible, the effectiveness of a given method for dispersion can only be judged by the usefulness of the resulting data.

Sample pretreatment greatly affects dispersibility and thus the results of any physical fractionation procedure. Air drying soils rich in amorphous constituents often causes aggregation that cannot be reversed even with vigorous sonication (e.g., Sollins 1989). For other soils (e.g., quartz sand soils), fractionation results may be essentially unaffected even by oven drying at 105 °C. For both chemical and physical fractionation methods, the resulting pools are operationally defined (defined only by the method). There is thus no single right answer; the usefulness of the results can only be judged by how biologically meaningful they prove. Biologically meaningful could mean, for example, that the separates (i.e., the fractions after separation) play distinctly different roles in any of the functions listed earlier for soil organic C, differ consistently across landscapes and ecosystems, or change consistently with management or disturbance.

Characterization of the Molecular Structure of SOM

Recent technological advances permit considerable characterization of the molecular structure of SOM. Broad categories are often defined, such as carbohydrates, aromatics, and alkyls, rather than specific compounds. To some extent, lignin-related compounds can be distinguished from other aromatics. These techniques can be used effectively on whole soil samples or on physically or chemically isolated soil fractions. Often they have been used to document changes in composition of the SOM with depth in profile, generally by assuming that the OM in deeper positions is older, as has been shown by ^{14}C dating (Scharpenseel and Schiffmann 1977). Some of the most intriguing results have been obtained by comparing soils of differing age, degree of weathering, or mineralogy. All SOM characterization techniques require expensive equipment and extensive training and thus are not described in detail here.

The oldest of the techniques is high-temperature pyrolysis, which splits molecules into fragments that can then be identified by mass spectrometry or other techniques (e.g., Kögel-Knabner et al. 1992). Because pyrolysis creates quite small fragments, only limited inferences are possible about the original compounds. Nonetheless, this technique has provided some of the best early evidence for the large amount of alkyl compounds in SOM (Theng et al. 1989).

^{13}C nuclear magnetic resonance (NMR) spectrometry, with cross-polarization and magic-angle spinning, quantifies the amount of C in each of several typical molecular positions in solid-phase soil samples and in aqueous SOM extracts (Kinchesh

et al. 1995; Preston 1996). Most spectra can be divided into alkyl (10–45 ppm), O-alkyl (45–110 ppm), aromatic (110–160 ppm), and carbonyl (160–200 ppm) regions (Baldock and Preston 1995). Alkyl C consists of long polymethylene (CH_2) chains (e.g., fatty acids, waxes, and resins), along with varying amounts of shorter polymethylene branches and methyl groups. The O-alkyl region includes C in carbohydrates such as cellulose and hemicelluloses, as well as the oxygenated and methoxyl C in phenylpropane lignin units and some amine C. Aromatics comprise both C, H, and O substituted rings and most lignin C. The carbonyl region includes C in carboxylic, amide, ester, ketone, and aldehyde groups.

^{13}C-NMR has several limitations. First, some C atoms produce similar resonance signals despite quite different positions within organic molecules; thus some peaks are ambiguous. Second, paramagnetic elements such as iron cause interference, in effect raising detection limits for carbon. To some extent this last problem can be offset by increasing counting time, but a better solution has been to find soils that are low in iron yet still suitable for the intended study. Typical of the recent ^{13}C-NMR work on soils are indications that there is sequential conversion of carbohydrate to aromatics and then to alkyls (see Baldock and Preston 1995), a process that may be arrested or at least slowed in soils with abundant free Al (Sollins et al. 1996).

C and N Analysis by Dry Combustion

Most dry-combustion C and N (CN) analyzers oxidize samples at high temperature (approx. 1000 °C), then measure the CO_2 and N gases evolved by infrared gas absorption (IRGA) analysis or gas chromatography (GC). Depending on the individual instrument, the maximum allowable sample size may be as small as 20 μg. The maximum sample size depends on the C concentration, which may require some initial data before a strategy can be chosen. No hard-and-fast rules can be offered for sample size because the precision and accuracy needed for any individual sample depend on the overall sampling and data analysis scheme. Use of small samples, however, always requires careful attention to subsampling and especially to grinding.

Materials

1. CN analyzer
2. Tin sample capsules
3. Microbalance
4. Soil or rock mill (e.g., Spex mixer-mill)
5. Gas pressure gauge

Procedures

High-temperature multiple-sample dry-combustion analyzers are manufactured by several companies including LECO and Carlo-Erba. The Carlo-Erba NA 1500 elemental analyzer is discussed here. The detection limit is 10 ppm, and measurements

are reproducible to better than ±0.1% absolute value. Sample mass needed for analysis may range from 0.5 to 30 μg depending on the nature of the material. Because such a small sample is needed, material must be homogenized thoroughly by grinding several hundred grams of soil to pass a 40- to 60-mesh screen. A typical sample run comprises one or two "bypass" samples of high concentration to condition the columns, two "blanks" consisting of empty tin sample cups, three standards of known C and N composition to calibrate the instrument (EDTA is used commonly), and three to five check standards scattered throughout the sample run. Typically, 39 unknowns can be included in one run of 50 samples. Extra sample trays may be purchased and set up to make consecutive runs more convenient.

Samples are weighed into tin capsules, which are loaded into an autosampler that drops the capsule plus sample into a combustion column maintained at 1020 °C. The sample and container are flash combusted in a temporarily enriched atmosphere of O_2. The combustion products are carried by a carrier gas (helium) past an oxidation catalyst of chromium trioxide kept at 1020 °C inside the combustion column. To ensure complete oxidation, a layer of silver-coated cobalt oxidè is placed at the bottom of the column. This catalyst also retains interfering substances produced during the combustion of halogenated compounds. The combustion products (CO_2, CO, N, NO, and water) pass through a reduction reactor in which hot metallic copper (650 °C) removes excess O_2 and reduces N oxides to N_2. These gases, together with CO_2 and water, are next passed through magnesium perchlorate to remove water, then through a chromatographic column to a thermal conductivity detector. The detector generates an electrical signal proportional to the concentration of N or C present. This signal is graphed on a built-in recorder and ported to a computer, which integrates the area under each curve and converts it to concentrations after each sample is run.

Before the start of each run, pressure should be checked to ensure against gas leaks. Gas flow rates (helium, oxygen, and air) are checked with a stopwatch and set to the correct values. Routine maintenance involves removing the slag (residue from combustion of the tin sample capsules) from the top of the combustion column after 150 samples, then refreshing the top 10 cm of the column with CrO_3. The combustion column and its chemicals can be used for 350–425 samples. The reduction column can be used for up to 900 samples, or until its copper is three-fourths spent as indicated by change to a black color. The moisture trap must be changed every 300–350 samples.

Calculations

Most CN analyzers read out directly in concentration units.

Special Considerations

The need to measure both bulk density and stone content cannot be overstated (see Chapters 1 and 4, this volume). Typically, only the <2 mm soil is analyzed for C and N. We recommend, however, that at least one subsample of the 2–10 mm material be ground and analyzed. If this coarse soil fraction contains significant C and

N, then the fraction should be analyzed in all field samples. The 2–10 mm fraction will usually contain coarse roots or other woody debris. Since these pools may be the focus of work by other investigators at a site (see Chapters 11 and 19, this volume), care must be taken to ensure that sites do not double-count C and N when calculating ecosystem totals on an areal basis.

Volcanic rocks tend to sorb C and N strongly. Thus at volcanic sites we recommend that at least a subsample of the >10 mm fraction be analyzed also, focusing on coarse aggregates (e.g., shot) and the most weathered rocks. Material will need to be weighed wet and a subsample dried (see Chapter 3, this volume).

Carbonates

Soils in arid and semiarid environments often contain large amounts of carbonates, as do soils derived from carbonate parent materials regardless of climate. Carbonates decompose during dry combustion, releasing CO_2 that is counted as soil C during dry-combustion analysis. Carbonates, however, do not play any of the roles listed previously for soil organic C; thus the amounts of carbonate (inorganic) and organic C need to be measured separately.

Most carbonate methods involve treatment with strong mineral acid to convert carbonates to CO_2. The residue is then analyzed to provide an estimate of the organic C, and the inorganic C is estimated by difference. The acid, however, can solubilize organic as well as carbonate C, so the solution must be evaporated and the soil completely dried to fully measure the organic C. H_2SO_4 is not recommended because it is too strong an oxidant, cannot easily be removed by evaporation, and may damage CN analyzers. HCl can be removed by evaporation, but the residual Cl^- likewise damages CN analyzers. Some researchers remove the Cl^- by washing, but this also removes water-soluble organic C.

At present the simplest solution is to use phosphoric acid and measure the CO_2 released with a gas chromatograph or infrared gas analyzer. Alternatively, small samples of soil can be weighed into metal boats (CN-analyzer sample holders), the acid added directly to the boat, and the boat and sample run through a CN analyzer once the released CO_2 has bubbled off and the sample has redried.

Recent work with ^{13}C abundance indicates that grinding is necessary in some soils rich in carbonates to ensure complete carbonate removal (C. Van Kessel, personal communication). The grinding step may be omitted if an initial trial shows that it does not increase the amount of C removed by the acid hydrolysis.

Dissolved Organic Nitrogen (DON)

Dissolved organic nitrogen (DON) is an important component of the soil solution, and in many undisturbed forests it is the main vector for N loss from the soil via leaching (Sollins and McCorison 1981; Lajtha et al. 1995). Precipitation may also contain a significant amount of DON, an input that has largely been ignored by ecologists.

Several methods exist for the analysis of DON and total N, including Kjeldahl digestion. We prefer a persulfate digestion technique because it is simpler, requires less specialized equipment, does not produce a toxic waste that is difficult to dispose, and is more sensitive than the standard Kjeldahl technique. In addition, persulfate N includes DON, nitrate, and ammonium, whereas Kjeldahl N includes only DON and ammonium.

A standard method for total N analysis of seawater is given by D'Elia et al. (1976). This procedure was modified by Ameel et al. (1993) to increase sensitivity in freshwater samples; we recommend the use of the Ameel et al. (1993) procedure, which is outlined here for the analysis of soil solution. Dissolved organic phosphorus (DOP) can be analyzed with the same extract, as described later in this chapter.

This method presumes that soil solution has already been collected, via either extraction (see Chapter 6, this volume) or lysimetry (see Chapter 9, this volume).

Materials

1. 40 mL borosilicate glass screw-top vials with rubber-lined caps.
2. Teflon cap liners (optional)
3. 0.148 M $K_2S_2O_8$; dissolve 20 g $K_2S_2O_8$ in 500 mL deionized water
4. 3 M NaOH; dissolve 12 g NaOH in 100 mL deionized water
5. Standard organic solutions; at least 4 concentrations including 0 μg N/L. For DON, use an organic N standard such as urea or EDTA; for DOP, use an organic P standard such as ATP.
6. Standard NO_3^-–N solutions at the same concentrations as for standard organic solutions, described earlier, including 0 μg N/L
7. Spike solution; a 200 μg N/L^1 standard organic solution
8. A means for measuring NH_4^+–N, NO_3^-–N, and (as needed) orthophosphate in solution samples (see Chapter 7, this volume, for phosphorus)

Procedure

1. Set aside a separate predigestion aliquot for inorganic N analysis (NH_4^+ and NO_3^-)
2. Add 15 mL of a nitrate standard, organic standard, spiked sample, or sample to a 40 mL digestion vial. Spiked samples and organic standards are used to determine digestion efficiency (see the section "Special Considerations," below); add 15 μL of the organic spike solution to the 15 mL of sample in a digestion vial.
3. Add 5 mL of the 0.148 mol/L persulfate solution and 0.25 mL of the 3 mol/L NaOH solution to each digestion vial, and seal the vials.
4. Autoclave at 121 °C and 17 psi for 55 minutes.
5. Cool, then add 0.25 mL of the 3 mol/L NaOH solution.
6. Analyze samples for NO_3^-–N using the nitrate standards also brought through the digestion procedure. If measuring DOP, also analyze for orthophosphate.
7. Analyze the predigestion samples for NO_3^-–N and NH_4^+–N.

Calculations

$$\text{DON } (\mu\text{g N/L}) = (\text{Persulfate N} / \text{Digestion Efficiency}) - \text{Inorganic N}$$

where

Persulfate N = NO_3^-–N in solution after persulfate digestion (μg NO_3–N/L); based on nitrate standards also brought through the digestion procedure.

Digestion efficiency (*DE*) can be calculated as either

(1) *DE* = (NO_3^-–N in spiked sample − NO_3^-–N in unspiked sample) / (0.200 μg/L organic-N spike); or

(2) *DE* = (NO_3^-–N in organic standard) / (NO_3^-–N in equivalent nitrate standard). Use an equivalent formula if analyzing for phosphorus. For any given sample, use the average digestion efficiency for all spiked samples within that run.

Inorganic N = NO_3^-–N + NH_4^+–N in solution prior to digestion (μg N/L); based on separate inorganic N analysis of the predigestion aliquot set aside in step 1.

Special Considerations

1. To determine digestion efficiency, 10% of all samples from each run should be analyzed with the spike solution. Alternately, compare the organic standards with the inorganic (NO_3^-–N) standards to determine efficiency (see the section "Calculations," above). Digestion efficiencies will normally be >90% but will vary among sample runs. If efficiency drops below 80%, suspect a bad batch of persulfate and discard results. Accuracy may be measured using a quality control check standard from a commercial supplier or with the organic standards.
2. All standards for colorimetric analysis are brought through the digestion procedure. Solutions are analyzed for nitrate and orthophosphate colorimetrically.
3. Seawater samples or samples high in Mg should be run according to the D'Elia et al. (1976) procedure to remove the $Mg(OH)_2$ precipitate that can form.
4. The Ameel et al. (1993) procedure assumes that there is no evaporation of liquid from the tubes; this should be verified, and if evaporation is detected, then vials must be brought to a standard volume with deionized water before analysis.

Water-Extractable Carbon

Materials

1. 200–300 mL plastic bottle
2. Centrifuge

3. 0.2 μm acid-washed polycarbonate filters
4. DOC analyzer

Procedure

1. Shake 50 g field-moist soil overnight in 150 mL distilled water (dH_2O) (200–300 mL plastic bottle).
2. Centrifuge.
3. Filter the supernatant through a 0.2 μm acid-washed polycarbonate membrane (chosen to remove virtually all organisms except viruses).
4. Analyze the solution for DOC with any of several DOC analyzers (e.g., Dohrmann, Shimadzu).

Calculations

Most CN analyzers read out directly in concentration units.

HCl—Insoluble Organic Carbon

Refluxing with 6N HCl yields a chemically resistant residue that gives much older ^{14}C dates than the bulk soil C (Paul et al. 1997; Follett et al. 1997). The acid hydrolysis solubilizes amino compounds, pectins, and most cellulose, and also releases C as CO_2 by decarboxylation. Acid hydrolysis does not degrade aromatics present in lignin or soil humic structures. Soils often contain lignin derived from recent plant residues. Acid hydrolysis, therefore, does not provide complete separation between old and new C, so it is important to remove identifiable plant residues before analysis. The amount of soil C remaining as non-acid-hydrolyzable C is related to the clay content and type of parent materials (Paul et al. 1998), and can be used to define a resistant SOM pool (see "Long-Term Respiration Potential (SOM Pool Sizes)" in Chapter 13, this volume).

Materials

1. 250 mL round-bottomed flasks with heating mantles or Kjeldahl digestion tubes with block digester
2. Reflux condensers for flasks or digestion tubes
3. Vacuum filtration unit with Whatman no. 50 or other hardened fine-pore filters or centrifuge
4. Soil or rock mill
5. NaCl solution (1.2 g/mL)

Procedure

1. Remove identifiable plant materials by flotation in NaCl (1.2 g/mL), then by hand-picking under a 20× microscope.
2. Grind soil to 100-mesh.

3. Remove any inorganic carbonates as described earlier.
4. Place soil and HCl in 250 mL round-bottomed flasks fitted with heating mantles or in Kjeldahl digestion tubes heated in a digestion block. Reflux condensers fitted to the digestion flasks or tubes with ground-glass joints provide the best reflux. The acid:soil ratio should be 10:1. Up to 100 mL acid can be accommodated in flasks. Kjeldahl tubes should not be filled above the level of the block (10–20 mL).
5. Heat the mixture overnight in the temperature-controlled digestion block, allowing the acid to reflux. Swirl the tubes occasionally to wash down soil that collects on the tube walls. After hydrolysis, rinse the reflux condensers with distilled water.
6. Isolate the residue by vacuum filtration through preweighed Whatman no. 50 or other hardened fine-pore filters, then wash with water and dry. Alternatively, isolate the residue by repeated centrifugation and washing.
7. Analyze for total C or specific C isotopes.

Special Considerations

Note that use of hardened filter papers permits the residue to be scraped off nearly quantitatively. If the residue is to be ^{14}C-dated, however, we recommend glass fiber filters even though recovery will not be as complete.

Density Fractionation

Density fractionation takes advantage of the fact that the density of soil particles reflects differences in the ratio of organic materials, which are light, to mineral materials, most of which are heavy (see Gregorich and Janzen 1996). The lightest particles comprise mainly organic debris—fragments of dead roots and leaves. In many soils, however, amorphous mineral materials adsorb on the surfaces of the debris, increasing its density. Secondary minerals (e.g., clays) have reactive surfaces that accumulate thick coatings of organic materials. Because these organic coatings are light, the secondary mineral particles tend to be of intermediate density. In addition, secondary mineral particles, along with their organic coatings, often form the components of aggregates. Such aggregates are also of intermediate density. Primary mineral particles are generally the heaviest, both because the minerals themselves may be heavy and because the surfaces of such particles tend to be unreactive and thus tend not to accumulate thick organic layers.

Soil particles span a continuum of densities, with the majority falling in the range of 1–2 g/cm^3. Many soils have bimodal density distributions with a trough around 1.5–1.8 g/cm^3. Thus 1.6 to 1.7 is often a convenient cutoff for separating a light and heavy fraction (Ladd and Amato 1980; Spycher et al. 1983). This cutoff is not applicable to all soils: some may not show a bimodal distribution or may include light rocks such as pumice in the light fraction (Sollins et al. 1983). Each group of soils should be checked initially by sequential separation at a series of densities from about 1.2–1.9 g/cm^3. The resulting light and heavy fractions should ideally be an-

alyzed for ash content (muffle furnace), C content, and C:N ratio. The density above which ash content of the light fraction increases markedly or C content decreases, or at which the difference in C:N ratio of light and heavy fraction peaks, will be optimal for separating a biologically meaningful light fraction.

The simplest method for density fractionation is to disperse soil in a heavy liquid, then aspirate the floating material from the surface of the liquid (Strickland and Sollins 1987). Several heavy liquids are popular, including Si suspension (Meijboom et al. 1995), sucrose, NaI, and Na polytungstate (NaPT). NaPT is the generally preferred flotation medium (Baldock et al. 1990). It is much less reactive than NaI and thus solubilizes much less C. It is also much less viscous than sucrose solutions of the same density and does not introduce readily metabolizable C into the system. The Si suspension is not recommended because it does not allow separation at densities much greater than 1.37 g/cm^3.

Sample preparation and dispersion procedures are critical and must be described carefully if work is to be reproducible. Dispersive energy that is applied should be expressed as joules/mL, calculated based on wattage of the instrument, volume of the suspension, and time sonicated. Applied dispersive energy has been measured directly as pressure exerted downward in the liquid (as measured with an electronic balance) and heat buildup in the liquid (Christensen 1992). Note that C-containing surfactants should not be used because they sorb onto mineral surfaces and inflate C values.

Materials

1. 10 mL volumetric pipet
2. Sodium polytungstate [$Na_6(H_2W_{12}O_{40})\cdot H_2O$]
3. 400 mL tall-form (Berzelius) beakers
4. Ultrasonic probe
5. Mixer (e.g., Hamilton Beach bench mixer, Scovil soil mixer, or Sorvall Omni Mixer)
6. Buchner flasks and funnels (1 L)
7. Whatman no. 50 or other hardened fine-pore filters
8. Vacuum source

Procedure

1. Soil samples may be stored field moist at 4 °C until needed. Subsample to determine moisture content. Fractionation should be done in triplicate.
2. Prepare the NaPT solution by adding about 1050 g/L dH_2O. Remove a 10 mL aliquot with a volumetric pipet and weigh (a 1.7 g/cm^3 solution should weigh 17.0 g). Add NaPT or dH_2O as necessary to adjust the density to the desired value.
3. Suspend 40 g field-moist soil in 200 mL of the NaPT solution. Disperse soil with mixer (1800 rpm for 0.5 minute). Rinse any soil adhering to the post and blades with NaPT solution.
4. Allow samples to settle for 48 hours at room temperature. The suspended light

fraction is then aspirated through a Tygon hose (1.0 cm i.d.) attached to a 1 L Buchner flask and a strong vacuum source. Care must be taken to avoid disturbing the heavy fraction (sediment) because several centimeters of NaPT will be aspirated with the light fraction into the Buchner flask, decreasing the headspace of the density solution. A second Buchner flask may be connected to the first to avoid loss of light fraction and to protect the vacuum source from moisture and organic particles. Wash the captured light fraction into a separate container and store at 4 °C.

5. Add NaPT solution to the heavy fraction to bring the suspension back to the original volume, and disperse the heavy fraction again (as described earlier). Allow the suspension to sit for 48 hours. Aspirate the new light fraction and combine with the previously collected light fraction.
6. Pour the combined light fraction onto Whatman no. 50 filters (2.7 μm retention), vacuum filter, and rinse the light fraction three times with 100 mL dH_2O. Set aside the rinse solution if desired for DOC or DON analysis.
7. Remove NaPT from the heavy fraction by adding at least 200 mL dH_2O and mixing thoroughly. In many soils the suspended particles may be allowed to settle and the clear supernatant aspirated into a Buchner flask, taking care not to disturb the heavy fraction. In sandy soils it may be possible to wash the heavy fraction onto filter paper and rinse. In clay-rich soils, the suspension should be rinsed into a centrifuge tube, spun down, and the supernatant aspirated. For all soils, the rinse solutions should be saved and analyzed appropriately if C or N recovery is to be gauged. The rinse procedure is repeated at least three times, more often for soils with high clay content.
8. Wash the heavy and light fractions into preweighed tins with dH_2O, dry at 105 °C, and weigh. Material may be ground and analyzed for total C and N as described earlier.

Calculations

Most CN analyzers read out directly in concentration units.

Special Considerations

The use of tall, narrow beakers is strongly recommended: it allows the soil:solution ratio to be maximized, saving considerably on the cost of the NaPT, prevents splashing during mixing, and maximizes the vertical separation between the floating light fraction and settled heavy fraction. The use of a mechanical mixer, rather than an ultrasonicator, is based on the finding that, in many soils, sonication yields essentially no light-fraction material beyond that released by mechanical mixing. This assertion may not hold for all soils and should be checked for each group of soils before a method is finalized.

The procedure can be modified to allow sequential separation at a series of densities. An initial separation may be made at 1.0 g/cm^3 using dH_2O. The resulting heavy fraction can then be resuspended in NaPT at a higher density, and the process repeated.

Although NaPT is expensive, a procedure to recycle used NaPT has recently been developed (J. Six and J. Jastrow, personal communication, 1997). The used NaPT, adjusted to about 1.4 g/cm^3, is passed through a column (6 cm i.d.) packed, from top to bottom, with 3 cm glass wool, 25 cm activated charcoal, 1 cm glass wool, 7.5 cm cation exchange resin (sodium-form), 1 cm glass wool, 7 cm activated charcoal, and 3 cm glass wool. The column should be kept moist and rinsed with 1 L dH_2O for every 2 L of NaPT.

Golchin et al. (1994) distinguished between a free and protected or occluded light fraction. The free light fraction is that obtained after soil is dispersed by gentle inversion only, without mechanical mixing or sonication. The resulting heavy fraction is then resuspended in the same density medium and dispersed by sonication. The additional light fraction recoverable after sonication is termed protected or occluded. ^{13}C-NMR revealed compositional differences between the free and occluded light fractions.

Coarse Particulate Organic Matter

Size fractionation assumes that smaller particles, because of their larger specific surface area, are more reactive chemically and biologically. As discussed in detail in Chapter 4, this volume, particle size separation relies on the decrease in sedimentation rate with increasing particle size. Particle shape and density also affect sedimentation rate; thus the clay size fraction will inevitably contain light fraction material that is larger than the upper size cutoff for clay. Procedures for solubilizing organic matter and amorphous mineral coatings are inappropriate as pretreatments when separating organic matter pools because they will alter the distribution of the organic matter among the pools.

Many methods have been proposed that use particle size separation to isolate soil organic matter pools. The usefulness of these procedures has been much debated, and the one recommended here is separation of sand-size organic matter (coarse particulate organic matter [CPOM]). In grassland and cultivated soils, this pool responds strongly to changes in management and vegetation (Cambardella and Elliott 1992, 1993).

The sieving procedure described here is equivalent to the sedimentation procedure described in Chapter 4, this volume, for separating coarse particulate inorganic matter (CPIM) from bulk soil. Use the sedimentation procedure if also performing particle size (texture) analysis.

Materials

1. 5 g/L Na hexametaphosphate
2. Reciprocating shaker
3. 53 μm (250-mesh) sieve
4. 50 mL plastic bottles
5. Al drying pans
6. Soil or rock mill

Procedure

1. Hand pick and discard identifiable plant material.
2. Weigh 10 g air-dry soil (previously sieved to pass a 2 mm screen) into a 50 mL bottle.
3. Add 30 mL Na hexametaphosphate solution.
4. Shake on a reciprocating shaker for 18 hours.
5. Pass the suspension through a 53 μm sieve, rinse the retained material several times with DW, transfer it quantitatively to a preweighed aluminum pan, and oven-dry.
6. The CPOM is finely ground and analyzed for C and N by dry combustion as described above.

Calculations

Most researchers will want to express CPOM-C on a whole soil basis. For CPOM-N, substitute N for C in the following calculations.

For the Seiving Procedure

$$\text{CPOM-C} = C_s \times W_s \times 10$$

where

CPOM-C = coarse particulate organic matter C (g C/kg soil)
C_s = %C of sand fraction
W_S = dry mass of sand fraction (g fraction/g soil); CPOM mass is assumed to be insignificant relative to the mass of the sand fraction

For the Sedimentation Procedure

$$\text{CPOM-C} = [C_{ts} - (C_f \times W_f)] \times 10$$

where

C_{ts} = total soil C (%)
C_f = silt + clay fraction C (%)
W_f = mass of silt + clay fraction (g fraction/g soil)

Special Considerations

The CPOM fraction may contain large amounts of mineral grains. In many soils such grains will contain relatively little C and N. Lacking C and N, they will dilute C and N concentrations but will not affect C:N ratios. In some soils, however, especially those of volcanic origin, the sand grains will contain large amounts of adsorbed SOM and fine aggregates containing both adsorbed and occluded SOM. In such soils a density fractionation step should be added to separate true CPOM (wide

C:N ratio) from the OM adsorbed on mineral particles and occluded within aggregates (narrow C:N ratio).

The CPOM method includes drying at 50 °C, which can cause irreversible aggregation in soils that do not normally dry, especially those rich in amorphous weathering products or in Fe and Al (hydr)oxides (e.g., Sollins 1989). For such soils the procedure will need to be modified to use field-moist soil.

The extent to which light fraction and CPOM are equivalent remains unresolved. Some CPOM particles are heavy (>1.8 g/cm^3) and would thus not be included in a light fraction (Gregorich and Janzen 1996). The other issue is whether a significant amount of the light fraction consists of particles finer than sand size (<50 μm). Barrios et al. (1996) compared density and size fractions in a Kenyan Alfisol but performed density fractionation only on the whole soil and on the sandsize fraction. Thus there is no way to determine what proportion of the light fraction was in silt- and clay-sized particles. Turchenek and Oades (1978) report that, for one soil after sonication, coarse clay (0.4–2 μm) that was light (<1.8 g/cm^3) accounted for 12.1% of total soil C.

Young and Spycher (1979) report the only data of which we are aware on density fractionation after particle size fractionation of unsonicated soil. They worked with seven soils of greatly differing mineralogy. Generalizations are difficult because they used different density cutoffs in each soil, but for the three in which their density cutoff was ≤ 1.8 g/cm^3, 11–22% of total SOM was in clay-sized light fraction material. Although the proportion this represents of total light fraction material in all size fractions was not reported, it seems clear that, in these three soils, CPOM excludes a major portion of the light fraction material, and thus CPOM and light fraction are not equivalent.

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STANDARD SOIL METHODS

FOR

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