# 13

Robertson, G. P., J. M. Blair, P. M. Groffman, D. Harris, E. Holland, K. Nadelhoffer, and D. Wedin. 1999. Soil carbon and nitrogen availability: nitrogen mineralization, nitrification, and soil respiration potentials in G. P. Robertson, C. S. Bledsoe, D. C. Coleman, and P. Sollins, eds. Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York.

# Soil Carbon and Nitrogen Availability

## *Nitrogen Mineralization, Nitrification, and Soil Respiration Potentials*

G. Philip Robertson
David Wedin
Peter M. Groffman
John M. Blair
Elisabeth A. Holland
Knute J. Nadelhoffer
David Harris

A soil's capacity to transform organic nitrogen in soil organic matter to inorganic nitrogen—its nitrogen mineralization potential—is often used as an index of the nitrogen available to plants in terrestrial ecosystems. It is perhaps the most common and best means available to assess nitrogen fertility (e.g., Keeney 1980; Binkley and Hart 1989; Palm et al. 1993), related as it is to both the size of the labile soil organic matter (SOM) pool and the activity of the organisms responsible for its oxidation. Mineralization potentials (the net production of inorganic nitrogen under standard conditions) are superior to inorganic soil nitrogen concentrations (pool size) as an indicator of site fertility simply because the supply rate of a limiting nutrient is more important to its availability than is its instantaneous concentration. Most mineralization assays are designed to exclude plant uptake and leaching, and most also ignore immobilization (microbial uptake) and denitrification, so net mineralization potentials provide a good general index of the capacity of a soil to make nitrogen available to plants via the soil solution.

Operationally, N mineralization usually refers to the net increase in both ammonium ($NH_4^+$) and nitrate ($NO_3^-$) in soil, since any nitrate formed must first have been ammonium. While other forms of inorganic nitrogen are also produced during mineralization assays (e.g., $NO_2^-$, $N_2O$, and $NO_x$), in most soils their appearance is highly transient and pools are quickly converted to another form ($NO_2^-$), or their fluxes are inconsequential relative to increases in the $NH_4^+$ and $NO_3^-$ pools ($N_2O$, $NO_x$).





Net nitrification refers specifically to the conversion of ammonium to nitrate by nitrifiers, bacteria that oxidize ammonium to nitrite and then nitrate. Nitrification assays also usually measure the net flux, excluding the nitrate that may be immobilized into microbial biomass (e.g., Davidson et al. 1991) or denitrified to nitrogen gas (e.g., Robertson and Tiedje 1985) during the course of the assay.

While both net N mineralization and net nitrification assays have their limitations as measures of nitrogen availability, they can nevertheless provide substantial insight into soil fertility and ecosystem function at many sites, and they are used widely. Large differences among sites or among experimental treatments, for example, imply large differences in plant-available nitrogen, as well as large differences in the potential loss of nitrogen from an ecosystem. Nitrate, for example, is more readily lost from most ecosystems than is ammonium, so high potential nitrification rates at a site can indicate a higher likelihood of nitrogen loss, all else being equal. It is harder to interpret small differences in mineralization or nitrification among sites or treatments because of artifacts intrinsic to the assays—microbial immobilization may be higher in an incubation than in situ, for example—but relative differences can still provide insight into ecosystem-level processes among sites.

Soil carbon availability is another component of soil fertility that can provide important information about ecosystem status. As for nitrogen availability, carbon availability also can be assessed by measuring a soil's ability to transform organic carbon to an inorganic form—in this case $CO_2$. A soil's capacity to oxidize fixed carbon to $CO_2$ under optimal moisture and temperature conditions—its respiration potential—can be a useful index of carbon availability in most ecosystems. The long-term release of $CO_2$ from SOM under optimal conditions can, in fact, be used mathematically to indicate the functional pools of SOM commonly referred to as active and passive fraction SOM (e.g., Juma and Paul 1981; Hess and Schmidt 1995). Respiration potentials can thus also provide valuable insight into the potentials for carbon storage at a site (e.g., Paul et al. 1998).

## Available Protocols

There are many different ways to assess nitrogen and carbon mineralization rates in soil; no single method enjoys universal acceptance, reflecting the complexity of factors that can affect rates of carbon and nitrogen turnover and the diverse nature of the compromises required to produce a reasonable estimate for a specific site. Chief among these compromises is the level of soil disturbance permitted. All available methods require some degree of soil disturbance, and this disturbance can artificially depress or, more commonly, accelerate mineralization rates. A second compromise is the environmental conditions under which mineralization is assessed. It is, for example, a straightforward matter to duplicate the effects of field temperature conditions on mineralization rates but very difficult to duplicate the effects of in situ soil moisture. Thus it is important to recognize that all common mineralization protocols are indices that can be sensitive to assay conditions; consequently, temporal and cross-site comparisons demand that close attention be paid to reproducing comparable disturbance and soil moisture/temperature regimes across experimental units.

Common to all net nitrogen mineralization assays is an incubation period over which accumulated inorganic nitrogen is used to calculate the rate of nitrogen mineralization during that period. As noted earlier, this approach represents the net balance between gross mineralization and microbial immobilization and in vitro losses of nitrogen. The gross rate of mineralization may in fact be more than an order of magnitude higher than net mineralization (Hart et al. 1994); the net rate thus represents the *minimum* amount of nitrogen available for either plant uptake or inorganic nitrogen loss. During incubation a portion of the organic nitrogen mineralized to ammonium will be oxidized to nitrate by nitrifiers; net mineralization is thus calculated as the sum of ammonium plus nitrate nitrogen at the end of the incubation interval. Nitrification itself can be assayed by considering only the net rate of nitrate increase during incubation and can be expressed as a proportion of net mineralization, ($NO_3^-$-N/[$NH_4^+$−N + $NO_3^-$−N]).

The variety of techniques available for measuring nitrogen mineralization and nitrification can be classed into three main groupings: (1) in situ incubations of enclosed soils, in which inorganic nitrogen accumulation is measured at the end of a 2–6 week incubation period; (2) laboratory incubations under standard moisture and temperature conditions in which inorganic nitrogen accumulation is monitored at 7–30 day intervals for up to a year or more; and (3) isotopic incubations during which changes in a $^{15}N$-labeled inorganic nitrogen pool is measured over the course of a 1–3 day incubation. These techniques all isolate a quantity of soil from its environment during the incubation period; this isolation provides the opportunity for monitoring inorganic N accumulation in the absence of processes that might otherwise affect inorganic N pools. These processes include primarily plant uptake, leaching, and atmospheric N deposition. Other processes that might affect these pools—notably denitrification and $NH_3$ volatilization—are measured separately, are assumed to be minimal or constant among sites, or are treated like immobilization—implicit factors that effectively reduce net nitrogen mineralization rates.

There are many possible permutations of procedures within the three major groups. These include the amount of soil disturbance (intact cores versus sieved soils), incubation temperatures (in situ temperature fluctuations versus a constant temperature such as 25 °C), incubation moisture (field moisture at the time of sampling versus some proportion of water-filled pore space or field capacity), and—for laboratory incubations—aeration status (ranging from added sand to increase aeration to anaerobic slurries to eliminate oxygen altogether). In the procedures outlined here we suggest specific protocols for each of these three major assay types.

The assays described here should be chosen for use based on experimental objectives and available resources. If one is comparing long-term treatments and can sample only once, then long-term laboratory incubations will provide a reasonable basis for insightful comparisons. If one is attempting to capture differences among sites or treatments with respect to shorter-term in situ dynamics, then the 28 day field incubation is appropriate. Detailed comparisons of specific processes among sites or treatments will require the $^{15}N$ isotope dilution approach.

Other methods for assessing nitrogen availability have been used to great advantage in many types of ecosystems. These include ion exchange resin bags inside intact cores (e.g., DiStefano and Gholz 1986; Zou et al. 1992), anaerobic slurries



(Waring and Bremner 1964; Keeney 1980), and various enzyme assays (Tabatabai 1994; Schmidt and Belser 1994). Bundy and Meisinger (1994), Hart et al. (1994), and Binkley and Hart (1989) provide excellent reviews of various techniques and their advantages and limitations.

There are also a variety of techniques available for measuring biologically available carbon. While the total pool of soil carbon can be adequately evaluated by chemical means (see Chapter 5, this volume), available carbon—that portion of the total pool actually available to the microbial community—is best assayed biologically. We recommend the laboratory incubation described later because of its simplicity and power (see "Potential Carbon Availability—Respiration Potentials" in this chapter). Measurements of $CO_2$ flux in the field (see Chapter 10, this volume) can also indicate potential differences in C availability among sites (e.g., Paul et al. 1998), but interpretation can be complicated by the inclusion of in situ root respiration. Likewise, enriched carbon-isotope tracer techniques are complicated by difficulties associated with labeling specific carbon pools. An additional value of the incubation technique described in this chapter is that C availability can be assessed for the same samples (and at the same time) as N availability.

## Potential Nitrogen Mineralization—Field Incubations

Field or in situ incubations of intact soil cores provide estimates of net nitrogen mineralization at temperatures typical for a given site without unduly disturbing soil structure. In the technique described here (a modification of methods proposed by Adams and Attiwill 1982; Raison et al. 1987; and Hart et al. 1994), a set of soil cores enclosed in PVC sleeves are loosely capped to minimize moisture changes but not gas exchange, and cores are then allowed to incubate for several weeks in situ. Adjacent cores are taken to provide an estimate of initial nitrate and ammonium levels. At the end of the incubation period the field-incubated cores are removed and analyzed for accumulated nitrate and ammonium. The difference between final and initial levels of total inorganic N (ammonium + nitrate) is the rate of net nitrogen mineralization, best expressed on both a gravimetric (mg $N \cdot kg^{-1} \cdot d^{-1}$) basis for within-site comparisons and on an areal (g $N \cdot m^{-2} \cdot d^{-1}$) basis to normalize for differences in soil bulk density among different sites. The net nitrification rate is the amount of nitrate nitrogen accumulated over this period. The relative net nitrification rate—the proportion of net mineralized nitrogen that is nitrified—can be expressed on a percentage basis as noted earlier.

Monthly incubations can provide information on seasonal patterns of nitrogen mineralization and, if moisture at the time of sampling is typical for the site over the remainder of each incubation period, can provide a rough estimate of the annual rates of net nitrogen mineralization and net nitrification for a site. If moisture at sampling significantly differs from the site's moisture dynamics over the incubation interval, the method will over- or underestimate actual net nitrogen mineralization rates, but rates may still be valuable for nearby treatment comparisons if moisture dynamics are similar among treatments. For cross-ecosystem comparisons, however, it is extremely important to design sampling intervals that capture local mois-

ture, temperature, and vegetation dynamics. Otherwise a standardized laboratory approach may be more appropriate.

Mineralization rates tend to exhibit high spatial variability in the field. As for many soil properties (see Chapter 1, this volume), net mineralization rates are usually lognormally distributed, and a stratified sampling strategy may be justified. Even so, it is not uncommon for net rates to span an order of magnitude over even a several-meter area in a site that visually appears to be homogeneous. Replication thus becomes very important and is often critical for quantifying significant differences among sites.

How many samples are sufficient? The answer to this question will be site-specific. Rarely are fewer than six cores adequate, and many sites may require a dozen or more to accurately quantify a mean flux. An initial intensive sampling will help to identify the variability present and thus the most efficient sampling strategy for subsequent efforts (see also Chapter 1, this volume).

### Materials

1. At least six thin-walled cylinders (schedule 40 PVC or steel) per site, 24 cm long × ≥5 cm inside diameter (of a sufficient length to penetrate the organic and A horizons and of a sufficient diameter to minimize compaction; see the section "Special Considerations," below), sharpened on one end with a file or bench grinder
2. Loose-fitting caps for each cylinder
3. 1 mol/L potassium chloride solution and other supplies sufficient for the triplicate extraction of initial soil samples equal to the number of cylinders (see Chapter 5, this volume). The same amount of this solution will be needed for the final extraction.
4. Paper bags or soil cans for determination of soil moisture as described in Chapter 3, this volume
5. Other materials and supplies as required for $NH_4^+$ and $NO_3^-$ analyses of KCl soil extracts

### Procedure

1. Hand-drive with a rubber mallet all but 2 cm of each cylinder into the soil in a pattern consistent with the area's vegetation cover (see Chapter 1, this volume); for many sites this pattern will be random. Include the O-horizon layer if present. Cover the top of the cylinder with a loose-fitting cap.
2. Remove a soil sample taken in an equivalent manner (same diameter, depth) from within 20–30 cm of each core and transport back to the laboratory for immediate extraction and inorganic-N analysis. These provide initial N values for each in situ core. If extraction will not take place within 6 hours, put the cores in an ice-cooled container after collecting and extract within 24 hours.
3. In the laboratory, weigh, sieve, and extract in triplicate the initial soil samples, then analyze for inorganic-N as described in Chapter 5, this volume. Analyze a 50 g subsample for gravimetric moisture as described in Chapter 3, this volume.
4. At the end of 28 days (4 weeks), remove the field-incubated cores and composite, weigh, sieve, extract in triplicate, and analyze for inorganic-N as for the initial cores. Also analyze a 50 g subsample for gravimetric moisture as described earlier. Discard any cores disturbed by animals.



### Calculations

The following equations assume $NO_3^-$–N and $NH_4^+$–N units that have already been converted to both a gravimetric basis (mg N/kg soil) and an areal basis (g N/m$^2$) using formulas presented in Chapter 6, this volume. It is sometimes useful to include rates expressed on another basis such as SOM (μg N·kg SOM$^{-1}$· d$^{-1}$). Always report soil bulk density values together with turnover rates to allow others to interconvert. A typical mineralization range for a forest or grassland soil sampled during a growing-season period with adequate moisture is 0.1–1.0 g N·m$^{-2}$·d$^{-1}$ or 0.5–5.0 mg N·kg$^{-1}$·d$^{-1}$ (Robertson 1982b); low moisture can readily reduce these values by an order of magnitude or more.

*Net N Mineralization*

$$N_{mineralized} = [(Nitrate_f + Ammonium_f) - (Nitrate_0 + Ammonium_0)]/T_{days}$$

where

$N_{mineralized}$ = net N mineralization rate, expressed as mg N·kg$^{-1}$·d$^{-1}$ or g N·m$^{-2}$·d$^{-1}$
$Nitrate_f$ = final nitrate concentration, expressed as mg $NO_3^-$–N/g soil or g $NO_3^-$–N/m$^2$
$Ammonium_f$ = final ammonium concentration, expressed as mg $NH_4^+$–N/g soil or g $NH_4^+$–N/m$^2$
$Nitrate_0$ = initial nitrate concentration, as mg $NO_3^-$–N/g soil or g $NO_3^-$–N/m$^2$
$Ammonium_0$ = initial ammonium concentration, as mg $NH_4^+$–N/g soil or g $NH_4^+$–N/m$^2$
$T_{days}$ = incubation time, in days

*Net Nitrification*

$$N_{nitrified} = (Nitrate_f - Nitrate_0)/T_{days}$$

where

$N_{nitrified}$ = net nitrification rate, expressed as mg $NO_3^-$–N·kg$^{-1}$·d$^{-1}$ or g $NO_3^-$–N·m$^{-2}$·d$^{-1}$

*Relative Nitrification*

$$\%\ \text{Nitrified} = 100 \times N_{nitrified}/N_{mineralized}$$

### Special Considerations

Specific sites may require different soil core dimensions than those described earlier (5 cm inner diameter × 24 cm long). Soils with shallow A horizons may be sampled with shorter cores, and A horizons deeper than 20 cm will require longer cores. Ideally one should analyze soil nitrogen mineralization at least once at multiple depths and then use a core length that captures most of the activity present. Fine-textured soils may compact with cores only 5 cm in diameter, in which case a larger diameter will be necessary. Compaction should be kept to 5% (e.g., 1 cm per 20 cm length). Compacted or rocky soils (e.g., desert soils and some agricultural soils) may require steel rather than PVC cylinders, and in some cases it may be necessary to substitute polyethylene bags for cores, in which case due care should be taken to further minimize soil structural disturbance when sampling.

Many sites may require more than six pairs of cores to adequately characterize nitrogen mineralization within an area. If not already available, it will be important to perform an initial sampling to assess the sample intensity necessary to adequately capture within-site variability (see Chapter 1, this volume).

## Potential Nitrogen Mineralization—Laboratory Incubations

Often it is useful to assess the nitrogen mineralization potential of a soil independently of short-term environmental conditions such as temperature and moisture. Such measurements of a soil's potential to mineralize organic nitrogen, usually made under constant moisture and temperature conditions, provide a different means for comparisons of nitrogen availability across systems and treatments.

A variety of approaches are available for measuring potential nitrogen mineralization in incubated soils, ranging from microlysimeter methods (e.g., Stanford and Smith 1972; Robertson 1982a; Nadelhoffer 1990) to simple static incubations of soil in jars or bags. We recommend a static incubation approach because of its simplicity and because with this approach it is easy to assess potentially available carbon at the same time by using incubation vessels that can be tightly sealed (see the section "Potentially Available Carbon," below). Additionally, this approach is suitable for indices of both short-term (28 day) and long-term (252 day) turnover.

### Materials

1. Six 125 mL flasks or equivalent per soil sample location
2. Access to a 25 °C incubator or constant-temperature room
3. Paper bags or soil cans for moisture determinations
4. Inorganic N extraction material as per the field incubation technique
5. Access to $NO_3^-$ and $NH_4^+$ analysis as per the field incubation technique

### Procedure

1. Collect soil from each area to be sampled (experimental replicate or subsite of a larger area) and composite by area, weigh, and sieve (4 mm). As for field incubations, the sample pattern should be consistent with the area's vegetation cover, and the sample number consistent with within-site variability; samples should include the O-horizon.
2. Determine the moisture content of each sample as per Chapter 3, this volume.
3. Adjust the moisture content of >100 g of each sieved soil to approximately 60% water-filled pore space (determined as per Chapter 3, this volume); be certain the moisture-adjusted soil is well mixed.
4. Weigh 10 g of each soil composite into each of the six flasks.
5. Set aside three flasks for immediate inorganic N ($NO_3^-$–N and $NH_4^+$–N) analysis.
6. Cap the remaining flasks loosely or cover with polyethylene film (permeable to $O_2$ and $CO_2$ but not to $H_2O_v$) and place in a humidified, darkened, 25 °C incubator or constant-temperature room.
7. Extract the soil in each of the initial flasks in KCl and analyze for inorganic N as described in Chapter 6, this volume.
8. Check for water loss periodically by weighing a subset of flasks at the outset and reweighing at intervals. Replace evaporated soil moisture as needed for each soil. After 28 days, extract soil as in step 7.



### Calculations

Convert nitrate and ammonium values to both an areal basis (g $N/m^{-2}$) and a gravimetric basis (mg $N/kg^{-1}$) using formulas provided in Chapter 6, this volume. Use the formulas for field incubations (earlier) to determine net nitrogen mineralization potential, potential net nitrification, and relative nitrification.

### Special Considerations

See the field incubation procedure for special considerations related to field sampling. To use this method for long-term N mineralization assays, use a sufficient number of flasks per soil composite to allow three extractions per incubation interval; use the same intervals as for long-term respiration potentials, discussed later.

## Potential Carbon Availability—Respiration Potentials

Potentially available carbon is best assayed in aerobic incubations and, as for potential nitrogen mineralization, under environmental conditions that are near optimal. The $CO_2$ produced over the incubation interval is used as an index of the amount of carbon available to microbes. Microbial growth over this interval (C-assimilation) is presumed to be a constant or insignificant alternate sink for metabolized carbon, or is assumed to be similar among experimental areas. Short-term incubations provide information on immediately available carbon. Long-term incubations provide information on those carbon pools that turn over more slowly, and thus provide a means for partitioning SOM into the functionally distinct pools that are an important component of most of today's major soil carbon and nitrogen mod-

els (e.g., Juma and Paul 1981; Molina et al. 1983; Jenkinson et al. 1987; Parton et al. 1987; Paustian et al. 1992). Both short- and long-term incubations can provide valuable insight into carbon and nitrogen cycling in a given ecosystem.

## Materials

1. Three 125 mL Erlenmeyer flasks (e.g., Corning no. 5020), small canning jars, or equivalent sealable containers per soil composite or site. For flasks, cap with large butyl rubber septa (e.g., Aldrich Chemical Co., Milwaukee, WI; part no. Z10, 145–1 for Corning no. 5020 flasks); for canning jars, lids should be new, boiled before use, and fitted with a rubber septa (e.g., the cap from a disposable blood collection tube) that is inserted snugly into a predrilled hole in the jar lid.
2. Access to a 25 °C incubator or constant-temperature room
3. Paper bags or soil cans for moisture determinations
4. Access to an infrared gas absorption (IRGA) analyzer or gas chromatograph for $CO_2$ analysis

## Procedure

1. Collect soil from each area to be sampled (experimental replicate or subsite of a larger area) and composite by area, weigh, and sieve (4 mm preferred). As for field incubations, the sample pattern should be consistent with the area's vegetation cover, and the sample number consistent with within-site variability; samples should include the O-horizon.
2. Determine the moisture content of the sample as per Chapter 3, this volume.
3. Adjust the moisture content of >100 g of sieved soil to approximately 60% water-filled pore space (determined as per Chapter 4, this volume); be certain the moisture-adjusted soil is well mixed.
4. Weigh 10 g of each composite into each incubation jar.
5. For short-term incubations, sample flasks after 7 days. For long-term incubations, sample flasks at intervals of 7, 14, 28, 42, 63, 84, 105, 140, 196, and 252 days (1, 2, 4, 6, 9, 12, 15, 20, 28, and 36 weeks). At each sample time, vent each flask with a stream of humidified air sufficient to fully aerate the flask headspace and soil macropores (humidify the airstream by bubbling it through a water-filled flask). Recap the flask and at intervals over the next 2–3 hours remove at least three 1 mL headspace samples for $CO_2$ analysis as per Chapter 10, this volume. The rate of production over this 2–3 hour period (the slope of a linear regression of concentration versus time) represents the respiration potential over that time interval.

## Calculations

*Short-term Respiration Potential (C Mineralization Rate)*

The following calculations assume that $CO_2$ flux values will be reported in parts per million by volume ($ppm_v$) per minute or $\mu L \cdot L^{-1}$ $CO_2 \cdot min^{-1}$ (gas standards are usually purchased in $ppm_v$ concentrations). Total $CO_2$–C in an enclosed soil volume includes $CO_2$–C in the headspace (including soil pore space) plus the $CO_2$–C dissolved in soil water. Normally increases in the $CO_2$–C dissolved in soil water over the incubation interval constitute a minor part of the $CO_2$ flux and can be ignored. For wet soils (e.g., bog soils or sediments), however, $CO_2$–C dissolved in water should be included in the calculation and can be calculated based on headspace $CO_2$ concentration, water content, pH, and a temperature-dependent equilibrium constant termed the *Bunsen coefficient* (see Tiedje 1982).



A. Convert concentrations of $CO_2$ to mass units and correct for incubation conditions through the application of the Ideal Gas Law:

$$C_m = (C_v \times M \times P)/(R \times T)$$

where

$C_m$ = μg $CO_2$–C/L headspace
$C_v$ = $ppm_v$ $CO_2$ or μL $CO_2$/L headspace
$M$ = molecular weight of $CO_2$–C (12 μg/μmol)
$P$ = barometric pressure (in atmospheres), e.g., 1 atm
$R$ = universal gas constant (0.0820575 L atm·°K·mole)
$T$ = incubation temperature, in °K (°K = °C + 273.15)

B. Calculate $CO_2$–C flux for the incubation period:

$$F = C_{rate} \times V/A \text{ or } W$$

where

$F$ = C mineralization rate, expressed as μg $CO_2$–C·$cm^{-2}$·$d^{-1}$ or μg $CO_2$–C·g $soil^{-1}$·$d^{-1}$
$C_{rate}$ = change in $CO_2$ concentration over the incubation period, expressed as μg $CO_2$–C·L $headspace^{-1}$·$d^{-1}$, calculated by regressing $C_m$ versus incubation time (d)
$V$ = Headspace volume of flask (L), calculated as total flask volume less soil volume; soil volume can be calculated from mass and bulk density (BD) (e.g., mL soil volume = g soil × [1/BD])
$A$ = surface area represented by soil in flask, based on soil bulk density values (see Chapter 4, this volume)
$W$ = dry mass equivalent of soil in flask (g)

*Long-term Respiration Potential (SOM Pool Sizes)*

Soil organic matter (SOM) pool sizes can be inferred from a graph of long-term carbon mineralization versus time with a two-pool model. The C mineralization rate formula noted earlier should be used to calculate a separate carbon mineralization rate for each incubation interval; these rates are then graphed as per Figure 13.1 and fit to the following model using a nonlinear regression procedure such as that available in SAS (SAS Institute 1985).

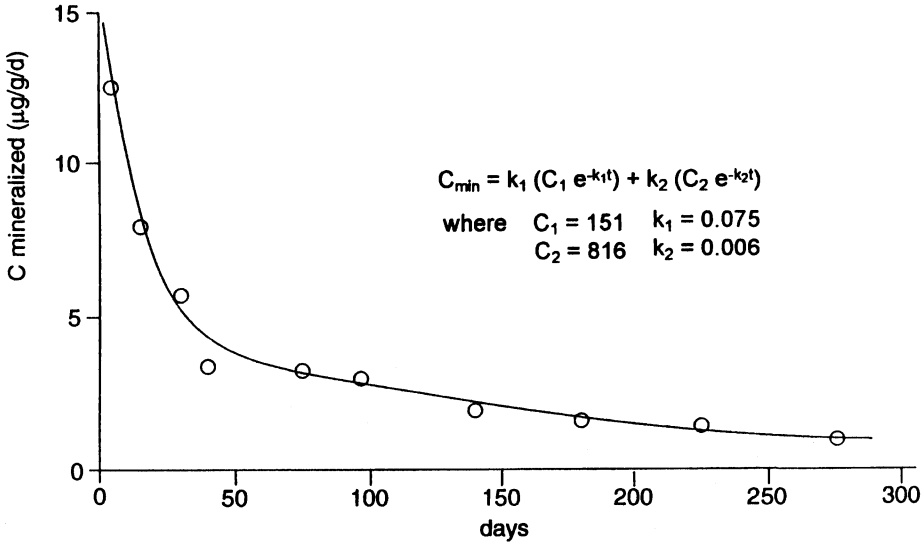


Figure 13.1. Long-term carbon mineralization in a cultivated soil from the KBS LTER site. The solid line is fit with the two-pool nonlinear model as noted in the figure and described in the text ($r^2 = 0.99$).

$$\text{Mineralized C} = k_1(C_1 e^{-k_1 t}) + k_2(C_2 e^{-k_2 t})$$

where

$C_1$ = carbon content of the active pool
$k_1$ = rate constant for the active pool
$C_2$ = carbon content of the intermediate pool
$k_2$ = rate constant for the intermediate pool
$e$ = the base of the natural logarithms

The third, resistant pool can be very roughly estimated as total C (as measured chemically; see Chapter 5, this volume) less $C_1$ and $C_2$. A more accurate estimate of the resistant pool can be made by using acid hydrolysis to define its size (Paul et al. 1998). In this case the intermediate pool ($C_2$) can then be estimated by difference ($C_2$ = Total C − $C_3$ − $C_1$), leaving only three parameters to be estimated by regression. See "HCl-Insoluble Organic Carbon" in Chapter 5 (this volume) to measure the resistant pool by acid hydrolysis.

### Special Considerations

If an infrared gas absorption (IRGA) analyzer or gas chromatograph is unavailable for $CO_2$ determinations, a vial of 1.0 mL of 2.0 mol/L NaOH can be used to trap $CO_2$ over a 24 hour incubation period. $CO_2$ captured by the vial can then be determined via titration with 0.1 N HCl as described in Chapter 15, this volume.



### $^{15}N$ Isotope Dilution

Where it is important to understand gross rates of nitrogen mineralization or to quantify nitrogen turnover on a relatively short time scale (e.g., following a rain event or other transient disturbances), we recommend the use of $^{15}N$ isotope dilution techniques as described by Hart et al. (1994). This technique successfully avoids problems of other tracer techniques such as those associated with changes in the size of N-substrate pools (Davidson et al. 1991). The technique is especially useful for exploring links with C cycling and for providing a more accurate picture of the nature and extent of soil N cycling.

In the isotope-dilution technique, a product pool (either $NH_4^+$ or $NO_3^-$) is labeled, and the rate of dilution of one or the other of these now-enriched pools with unlabeled $NH_4^+$ or $NO_3^-$ is taken to indicate the rates of gross nitrogen mineralization or nitrification, respectively. The calculations for these rates—which take into account the simultaneous production and consumption of inorganic-N—were worked out in the early 1950s (Kirkham and Bartholomew 1954).

The specific procedure recommended for LTER sites follows that presented in Hart et al. (1994:990–999). Because the procedure described therein is specific and because this is not a technique that will be commonly used at all sites, we provide no further details here. We do, however, note that a number of laboratories have found spatial variability within individual soil cores to be a major impediment to the interpretation of results using the Hart et al. (1994) protocol, a problem that is commonly reduced by mixing the soils instead of using intact cores. Results should be expressed in the same units as for other nitrogen mineralization processes, i.e., g $N \cdot m^{-2} \cdot d^{-1}$ and mg $N \cdot kg\ soil^{-1} \cdot d^{-1}$.

*Acknowledgments* We thank a number of individuals for their helpful discussions and comments on earlier versions of this manuscript, including E. A. Paul, D. D. Myrold, G. Ponciroli, and two thorough, anonymous reviewers. Financial support was provided in part by the KBS LTER project and the Michigan Agricultural Experiment Station.

References

Adams, M. A., and P. M. Attiwill. 1982. Nitrogen mineralization and nitrate reduction in forests soil. *Soil Biology and Biochemistry* 14:197–202.

Binkley, D. 1984. Ion-exchange resin bags for assessing soil N availability: the importance of ion concentration, water regime, and microbial competition. *Soil Science Society of America Journal* 48:1181–1184.

Binkley, D., and S. C. Hart. 1989. The components of nitrogen availability assessments in forest soils. *Advances in Soil Science* 10:57–112.

Bundy, L. G., and J. J. Meisinger. 1994. Nitrogen availability indices. Pages 951–984 *in* R. W. Weaver, J. S. Angle, P. J. Bottomley, D. F. Bezdicek, M. S. Smith, M. A. Tabatabai, and A. G. Wollum, editors, *Methods of Soil Analysis. Part 2, Microbiological and Biochemical Properties.* Soil Science Society of America, Madison, Wisconsin, USA.

Davidson, E. A., S. C. Hart, and M. K. Firestone. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. *Ecology* 73:1148–1156.

Davidson, E. A., S. C. Hart, C. A. Shanks, and M. K. Firestone. 1991. Measuring gross nitrogen mineralization, immobilization, and nitrification by $^{15}N$ isotopic pool dilution in intact soil cores. *Journal of Soil Science* 42:335–349.

DiStefano, J. F., and H. L. Gholz. 1986. A proposed use of ion exchange resins to measure nitrogen mineralization and nitrification in intact soil cores. *Communications in Soil Science and Plant Analysis* 17:989–998.

Firestone, M. K. 1982. Biological denitrification. Pages 289–326 *in* F. J. Stevenson, editor, *Nitrogen in Agricultural Soils.* American Society of Agronomy, Madison, Wisconsin, USA.

Hart, S. C., and M. K. Firestone. 1989. Evaluation of three *in situ* soil nitrogen availability assays. *Canadian Journal of Forest Research* 19:185–191.

Hart, S. C., J. M. Stark, E. A. Davidson, and M. K. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. Pages 985–1018 *in* R. W. Weaver, J. S. Angle, P. J. Bottomley, D. F. Bezdicek, M. S. Smith, M. A. Tabatabai, and A. G. Wollum, editors, *Methods of Soil Analysis. Part 2, Microbiological and Biochemical Properties.* Soil Science Society of America, Madison, Wisconsin, USA.

Hess, T. F., and S. K. Schmidt. 1995. Improved procedure for obtaining statistically valid parameter estimates from soil respiration data. *Soil Biology and Biochemistry* 27:1–7.

Horwath, W. R., and E. A. Paul. 1994. Microbial biomass. Pages 753–774 *in* R. W. Weaver, J. S. Angle, P. J. Bottomley, D. F. Bezdicek, M. S. Smith, M. A. Tabatabai, and A. G. Wollum, editors, *Methods of Soil Analysis. Part 2, Microbiological and Biochemical Properties.* Soil Science Society of America, Madison, Wisconsin, USA.

Jenkinson, D. S., P. B. S. Hart, J. H. Rayner, and L. C. Parry. 1987. Modeling the turnover of organic matter in long-term experiments at Rothamsted. *INTECOL Bulletin* 15:1–8.

Juma, N. G., and E. A. Paul. 1981. Use of tracers and computer simulation techniques to assess mineralization and immobilization of soil nitrogen. Pages 145–154 *in* M. J. Frissel and D. A. vanVeen, editors, *Simulation of Nitrogen Behavior in Soil-Plant Systems.* PUDOC, Wageningen, Netherlands.

Keeney, D. R. 1980. Prediction of soil nitrogen availability in forest ecosystems: a literature review. *Forest Science* 26:159–171.

Kirkham, D., and W. V. Bartholomew. 1954. Equations for following nutrient transformations in soil, utilizing trace data. *Proceedings of the Soil Science Society of America* 18:33–34.

Molina, J. A. E., C. E. Clapp, M. J. Shaffer, F. W. Chichester, and W. E. Larson. 1983. NCSOIL, a model of nitrogen and carbon transformations in soil: description, calibration and behavior. *Soil Science Society of America Journal* 47:85–91.

Myrold, D. D., and J. M. Tiedje. 1986. Simultaneous estimation of several nitrogen cycle rates using 15N: theory and application. *Soil Biology and Biochemistry* 18:559–568.

Nadelhoffer, K. J. 1990. A microlysimeter for measuring nitrogen mineralization and microbial respiration in aerobic soil incubations. *Soil Science Society of America Journal* 54:411–415.

Palm, C., G. P. Robertson, and P. M. Vitousek. 1993. Nitrogen availability. Pages 158–163 *in* J. M. Anderson and J. S. I. Ingram, editors, *Tropical Soil Biology and Fertility: A Handbook of Methods. 2d edition.* CAB International, Oxford, UK.

Parton, W. J., D. S. Schimel, C. V. Cole, and D. S. Ojima. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal* 51:1173–1179.

Paul, E. A., H. P. Collins, D. Harris, U. Schulthess, and G. P. Robertson. 1998. The influence of biological management inputs on carbon mineralization in ecosystems. *Applied Soil Ecology* 3:1–13.



Paustian, K., W. J. Parton, and J. Persson. 1992. Modeling soil organic matter in organic-amended and nitrogen-fertilized long-term plots. *Soil Science Society of America Journal* 56:476–488.

Raison, R., M. Connell, and P. Khanna. 1987. Methodology for studying fluxes of soil mineral-N *in situ. Soil Biology and Biochemistry* 19:521–530.

Robertson, G. P. 1982a. Factors regulating nitrification in primary and secondary succession. *Ecology* 63:1561–1573.

Robertson, G. P. 1982b. Nitrification in forested ecosystems. *Philosophical Transactions of the Royal Society of London* 296:445–457.

Robertson, G. P., and J. M. Tiedje. 1985. Denitrification and nitrous oxide production in successional and old growth Michigan forests. *Soil Science Society of America Journal* 48:383–389.

SAS-Institute. 1985. *SAS User's Guide: Statistics, Version 5.* SAS Institute, Cary, North Carolina, USA.

Schmidt, E. L., and L. W. Belser. 1994. Autotrophic nitrifying bacteria. Pages 159–177 *in* R. W. Weaver, J. S. Angle, P. J. Bottomley, D. F. Bezdicek, M. S. Smith, M. A. Tabatabai, and A. G. Wollum, editors, *Methods of Soil Analysis. Part 2, Microbiological and Biochemical Properties.* Soil Science Society of America, Madison, Wisconsin, USA.

Stanford, G., and S. J. Smith. 1972. Nitrogen mineralization potentials of soils. *Soil Science Society of America Journal* 36:465–472.

Tabatabai, M. A. 1994. Soil enzymes. Pages 775–826 *in* R. W. Weaver, J. S. Angle, P. J. Bottomley, D. F. Bezdicek, M. S. Smith, M. A. Tabatabai, and A. G. Wollum, editors, *Methods of Soil Analysis. Part 2, Microbiological and Biochemical Properties.* Soil Science Society of America, Madison, Wisconsin, USA.

Tiedje, J. M. 1982. Denitrification. Pages 1011–1026 *in* A. L. Page, editor, *Methods of Soil Analysis. Part 2, Chemical and Microbiological Properties.* American Society of Agronomy, Madison, Wisconsin, USA.

Waring, S. A., and J. M. Bremner. 1964. Effect of soil mesh-size on the estimation of mineralizable nitrogen. *Nature* 202:1141.

Zou, X., D. W. Valentine, R. J. J. Stanford, and D. Binkley. 1992. Resin-core and buried-bag estimates of nitrogen transformations in Costa Rica lowland forests. *Plant and Soil* 139:275–283.