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13 Potentially Mineralizable Nitrogen as an Indicator of Biologically Active Soil Nitrogen

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Almost all of the N in surface soils is present in the form of organic compounds that cannot be used directly by plants and also are not susceptible to loss through leaching. The amount of N converted from organic to mineral forms (mineralization) on an annual basis varies, depending on the past management history, annual climatic variation and inherent soil properties (Sprent, 1987; Paul & Clark, 1989). This capacity of the soil to supply plant-available N is an important indicator of soil quality and many chemical and biological methods have been developed in an effort to provide a simple, reliable indicator of potentially mineralizable N (Keeney, 1982; Bundy & Meisinger, 1994). In this chapter, we discuss the use of N mineralization potential as an indicator of soil quality and the advantages and disadvantages of the various methods available. We then recommend and describe two biologically-based laboratory methods of determining N mineralization potential.

USE OF N MINERALIZATION POTENTIAL AS AN INDICATOR OF SOIL QUALITY

Nitrogen mineralization potential is useful in conjunction with total N or soil C as an indicator of soil organic matter quality. Soil organic matter (SOM) is

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composed of a continuum of readily decomposable and resistant components. The accessibility of these various SOM fractions to mineralization varies, ranging from extremely labile microbial biomass, labile nonliving organic residues, to chemically recalcitrant and physically protected pools (Paul, 1984; Parton et al., 1987). We are using the term *active soil N* to refer to the biologically dynamic, labile organic N fractions that can be mineralized in the course of one growing season (Duxbury et al., 1991). While some soil properties, such as cation-exchange capacity are dependent on the more stable, humic organic matter fractions, many desirable soil properties are linked to biological activity. Properties such as aggregate stability and N availability are often highly correlated with indicators of biological activity such as microbial biomass, but are frequently poorly correlated with total organic C and N (Tisdall & Oades, 1982; Kay, 1990; Roberson et al., 1991). Soils that have been managed differently can have similar levels of total soil N but very different N mineralization potentials, indicating differences in SOM quality. A recent study comparing soils receiving inputs of organic residues in place of mineral fertilizers concluded that even after 10 yr of management with organic residues, qualitative rather than quantitative differences in SOM were more prominent (Wander et al., 1994). Thus, indicators that are sensitive to SOM quality and that reflect the size of the labile SOM pools (i.e., microbial biomass, N mineralization potential and soil respiration) are needed in order to fully assess overall soil quality.

COMPARISON OF METHODS FOR ESTIMATING POTENTIALLY MINERALIZABLE NITROGEN

Numerous chemical and biological methods have been developed for determination of N mineralization potential. Chemical methods for estimating potentially mineralizable N range from fairly mild treatments such as extraction of N with concentrated salt solutions, to drastic treatments that use acids to partially hydrolyze organic N compounds. Although these chemically-based techniques are usually more rapid and convenient than biological methods, they do not simulate the activities of soil microorganisms nor do they selectively release the fraction of soil organic N that is made available for plant growth by microbial activity (Bremner, 1965). Thus chemical methods are entirely empirical and limited by the degree to which they correlate with reliable biological measurements of soil N availability, e.g., N uptake, crop yield or mineralizable N (Stanford, 1982). Several reviews are available that discuss the pros and cons of specific chemical methods for potentially mineralizable N (Keeney, 1982; Bundy & Meisinger, 1994; Rice & Havlin, 1994).

Biological methods for estimating potentially mineralizable N involve measuring the amount of mineral N released in the soil by microbial activity during incubation. These procedures are usually more time consuming and tedious than chemical methods, but generally are considered to provide a more realistic assessment of the potential ability of soils to provide N for plant growth, since they rely on the normal biological mineralization processes (Keeney, 1982); however, laboratory procedures cannot simulate field conditions. First, N mineraliza-

tion in the field is strongly influenced by climate- and management-induced changes in soil temperature and soil moisture, and by the availability of organic N substrates (Duxbury & Nkambule, 1994). Secondly, actively growing roots also effect mineralization and can either increase or decrease the mineralization rate of SOM (Helal & Sauerbeck, 1986; Liljeroth et al., 1990; 1994). The effect roots have on mineralization of native SOM depends on crop species and soil environmental conditions such as N availability (Liljeroth et al., 1994). Thus, while laboratory incubations offer better estimates of mineralizable N than chemical procedures, they are sometimes poorly correlated with N mineralization dynamics in the field; however, insensitivity to environmental conditions is a positive attribute for a soil quality indicator, particularly if a wide array of soils are compared across climatic zones and crop rotations.

A range of incubation procedures have been published involving short-term (7–28 d) or long-term (30–200 d) incubations under either aerobic or anaerobic (waterlogged) conditions (see Keeney, 1982 or Bundy & Meisinger, 1994 for extensive reviews of these published procedures). All of these incubation procedures measure *net* mineralization potential that is the mineralized N remaining after microbial immobilization. Recently, Duxbury et al. (1991) developed an incubation method based on pulse labeling with ^{15}N followed by aerobic incubation to estimate the size of the biologically active N pool. This method relies on microbial immobilization–mineralization to distribute ^{15}N to all biologically active pools. Therefore this method reflects microbial activity as well as biologically active N in the nonliving organic pools. In situ incubation methods have been developed to measure net N mineralization under field conditions (Hart & Firestone, 1988). These in situ procedures can provide excellent information on the temporal dynamics of N mineralization.

Aerobic Incubation

Standford and Smith (1972) proposed a 30-wk aerobic incubation method to estimate soil N mineralization potential. The soil is incubated under optimum conditions and inorganic N is removed at various times during the incubation by leaching with a dilute Ca salt solution. Cumulative mineralized N after 30 wk of incubation is used to calculate potentially mineralizable N (N_o) by assuming the mineralization of labile organic N follows first order kinetics. It has been suggested that long-term aerobic incubations (12–30 wk) may be better indicators of total mineralization potential than short-term incubations (14–28 d) because active nonbiomass N and stabilized, labile organic N pools are mineralized in addition to the most active microbial biomass pool (Stanford & Smith, 1972); however, while the contribution of microbial biomass N to total mineralizable N during these long-term incubations is sometimes minimal (Juma & Paul, 1984) others have found that a major portion of the N mineralized is derived from the microbial biomass (Bonde et al., 1988; Harris, 1993). Bonde et al. (1988) reported that the proportion of mineralized N derived from the microbial biomass during long-term incubations depends on management history and the initial size of the microbial biomass N pool. Harris (1993) found that up to 60% of the N mineralized after 200 d originated from the microbial biomass in soils with a history

of leguminous residue inputs. Paustian and Bonde (1987) and Duxbury and Nkambule (1994) review the pros and cons of long-term aerobic incubations in detail.

Short-term aerobic incubations quantify a portion of the total potentially mineralizable N pool and reflect the relative N- supplying capacities of soil under specific environmental conditions (Stanford & Smith, 1972; Stanford et al., 1974). Net mineralizable N from short-term incubations has been related to ryegrass (*Lolium perenne* L.) uptake in the greenhouse (Keeney & Bremner, 1966, 1967), ryegrass and barley (*Hordeum vulgare* L.) N uptake in the greenhouse and in the field (Jenkinson, 1968), oat (*Avena sativa* L.) N uptake in the greenhouse (Stanford & Legg, 1968), and maize (*Zea mays* L.) yield response to N in the field (Walmsley & Forde, 1976). Typically, as with long-term incubations, correlation with field data is often less satisfactory than correlation with greenhouse data. Short-term production of net mineralizable N during aerobic laboratory incubation also has been used successfully by ecologists to quantify biologically active soil organic N pools (Woods et al., 1982; Schimel et al., 1985; Schimel, 1986; Wood et al., 1990; Davidson et al., 1991; Cambardella & Elliott, 1994; Cambardella & Kanwar, 1995).

Anaerobic Incubation

Aerobic incubation procedures provide optimal temperature, moisture, and aeration conditions for the microbial population responsible for mineralization of soil organic N under most field conditions. The major disadvantage of aerobic procedures is the difficulty in maintaining optimal soil water content during incubation. A second disadvantage is the need to measure both NH_4^+ and NO_3^- concentrations following incubation. An anaerobic incubation procedure was first proposed by Waring and Bremner (1964) as a simpler, more rapid alternative to aerobic incubations. Conducting the incubation under water-logged conditions eliminates the need for establishing and maintaining a standard soil water content during the incubation. A further advantage is that since nitrification is prevented, all N mineralized during the incubation will be in the form of NH_4^+. Since mineralization is occurring under anaerobic conditions, immobilization may be reduced compared with aerobic conditions because of the reduced energetic efficiency (C is not completely oxidized to CO_2). This difference in energetic efficiency does not fundamentally change the mineralization of N.

As with other laboratory incubations, many questions remain about the source of the mineralized N produced during anaerobic incubations. Some have suggested that the anaerobic incubation method may serve as a substitute for microbial biomass determinations since it apparently involves killing and mineralizing the obligate aerobes, a process somewhat analogous to the fumigation–incubation method of microbial biomass determinations (Doran et al., 1987; Myrold, 1987). Myrold (1987) labeled the resident microbial biomass in several forest soils with ^{15}N and then determined net mineralized N using anaerobic incubation and microbial biomass using chloroform fumigation–incubation. He found a highly significant correlation between both the amount and the proportion of ^{15}N released by the two methods and concluded that the anaerobic

Table 13–1. Relationship between microbial biomass and anaerobic N-mineralization potential under differing fertility input regimes. Pearson correlations and *P* values are given.

Fertility management	*n*	Nmin† vs. MBN‡	MBN vs. MBC§	Source
Inorganic N 1992	36	NS¶	0.68, $P = 0.0001$	Doran, unpublished#
1993	33	NS	0.57, $P = 0.0003$	
Cover crop residues, varying C/N ratios	31	0.91, $P = 0.0001$	0.95, $P = 0.0001$	Sarrantonio, unpublished#
Inorganic N	42	−0.35, $P = 0.02$	NS	Gunapala & Scow,††
Cover crop residues, manure	52	0.52, $P = 0.0001$	0.52, $P = 0.0001$	(in press)

† Anaerobic net N-mineralization potential.
‡ Microbial biomass N.
§ Microbial biomass C.
¶ NS = $P > 0.05$.
# Fumigation–incubation method.
†† Fumigation–extraction method.

incubation measures N released primarily from the microbial biomass. This kind of detailed isotope experiment has not yet been done in other soil types or ecosystems such as grasslands or agricultural systems. Soil microbial dynamics in agricultural systems differ from those in natural ecosystems because management interventions disrupt C turnover equilibrium resulting in boom/bust cycles of microbial activity (Drinkwater et al., 1995b; Wyland et al., 1995; Gunapala & Scow, 1997). A number of studies have compared anaerobic net N mineralization and fumigation–incubation microbial biomass N and C determinations in agricultural soils with inconsistent results. Sometimes the correlation is very strong, consistent with the hypothesis that both techniques are measuring N mineralized from the microbial biomass N pool, however in many cases there appears to be no relationship between the two (Table 13–1). Thus, while anaerobic N mineralization potential is probably a good indicator of the potential for soil to deliver N, it does not necessarily reflect microbial biomass N levels and cannot be considered a dependable substitute for the fumigation–incubation method. A probable explanation for these discrepancies is that other properties that are not being measured, such as the composition of the microbial community and its metabolic status (i.e., dormant vs. active, C-limited vs. N-limited) or composition of the organic substrate being mineralized are differentially affecting these measurements.

Sample Handling and Cautions

Sample handling presents the greatest challenge in terms of standardization of these incubation procedures. Quantification of mineralized N using incubation methods can be affected by: (i) soil sampling procedures; (ii) soil drying; (iii) grinding or sieving the soil and the mesh size used; and (iv) length of storage time and temperature of storage. Samples collected shortly after management practices that stimulate microbial activity, i.e., tillage or residue additions, will have greater N mineralization potentials (Drinkwater et al., 1995a,b; Wyland et al., 1995). Likewise, seasonal effects on microbial biomass will effect N mineralization

potentials (Patra et al., 1990). Thus, the timing of sample collection is an important consideration (Dick et al., 1996, this publication).

Sieving soil is somewhat analogous to tillage and increases N mineralization potential, particularly in soils from no-till systems, by exposing protected labile organic matter (Ross et al., 1985). Sieving soils prior to incubation has the advantage of increasing homogeneity by mixing composite samples and ensuring removal of large pieces of undecomposed organic debris such as roots. If the main goal is to use N mineralization potential as an indicator of SOM quality, then sieving soils to improve standardization is probably desirable; however, if the goal is to estimate the potential for N release in the field, then incubations with sieved soils from untilled systems will usually result in overestimations of N mineralization (Duxbury & Nkambule, 1994).

There is an on-going debate about the pros and cons of conducting biological incubations on field-moist vs. air-dried soils. Air-drying has been shown to increase the amount of mineral N produced compared with field-moist soil and this increase is positively correlated with the length of time the air-dry sample is stored prior to incubation (Keeney & Bremner, 1966; Stanford & Legg, 1968); however, in some cases, estimates of available N obtained from aerobic laboratory incubations of air-dry soil have been found to be better correlated with crop response than aerobic incubations of field-moist soil (Keeney & Bremner, 1966). Perhaps the greatest problem with using air-dried soils in these incubations is that the impact of air drying and rewetting on SOM accessibility and the soil biota will vary, depending on soil texture and climate and possibly management history.

Dry–wet cycles can influence N mineralization through aggregate disruption and the release of soluble C from the microbial biomass (Kemper & Rosenau, 1986; Kieft et al., 1987). One option is to minimize moisture variability by collecting samples at field capacity, either following irrigation or rainfall. This may not be feasible for some rainfed systems especially in semiarid and arid areas. In these situations, since surface soils become air-dry in the field at some point during the growing season, it seems appropriate to recommend air-drying prior to incubation for routine laboratory analysis in order to standardize the incubations. Soil samples (field-moist or air-dry) should be stored at 4°C prior to incubation (Chaudhry & Cornfield, 1971). Since mineralization potential is strongly controlled by soil moisture at the time of sampling, we encourage researchers to adopt the pretreatment that is most appropriate to the dominant climate–soil–cropping scenario of a given region.

Interpretation

The interpretation of net N mineralization potential must be done in conjunction with other soil measurements such as organic N and C and mineral N pools. In general, short-term incubations are useful for estimating labile soil N but may not reflect differences in the active soil N from the larger, more stable pools (Duxbury & Nkambule, 1994; Wander et al. 1994). Greater mineralization potentials have been found in a variety of cropping systems under organic man-

agement (Doran et al., 1987; Drinkwater et al., 1995a; Gunapala & Scow, 1997) reflecting the increased role of decomposers in determining N availability in systems based on organic soil amendments. The ratio of N mineralized to total organic N can serve as a sensitive indicator of SOM quality differences. The proportion of total soil N mineralized in short-term, anaerobic incubations was more than two-fold greater in organically compared with conventionally-managed soils (1.2 vs. 0.5%) indicating significant qualitative differences in the SOM (Drinkwater et al., 1995a).

Taken together, microbial biomass N, N mineralization potentials and mineral N pools can serve as indicators on the status of N dynamics in the soil. Large mineralization potentials in conjunction with high concentrations of mineral N, especially during times of reduced crop uptake, could indicate susceptibility to N losses through leaching. Organically managed soils are sometimes characterized by higher levels of microbial activity and potentially mineralizable N, in conjunction with lower instantaneous mineral N pools compared with soils receiving conventional mineral fertilizers (Drinkwater et al., 1995a). The combination of low instantaneous mineral N pools and enhanced microbial activity in the organic soils are indicative of a more tightly coupled N cycle (Sprent, 1987; Jackson et al., 1989; Jenkinson & Parry, 1989), with higher turnover rates of mineral N pools than in conventional soils.

RECOMMENDED AEROBIC INCUBATION PROCEDURE

During the aerobic incubation, results are affected by soil water content, temperature, and pH, primarily through the effects on microbial activity. Optimal soil conditions for aerobic incubation are given as soil water content at 60% water-filled pore space (WFPS, Linn & Doran, 1984), soil temperature between 30 and 35°C, and soil pH between 6.6 and 8.0 (Paul & Clark, 1989). Adjustment of soil water content requires preliminary analysis to determine the amount of water required for incubation. Keeney and Bremner (1967) suggest mixing 10 g of air-dried soil with 30 g of 30- to 60-mesh acid-washed quartz sand and then moistening the mixture with 6 mL of water. The resulting water content is between 40 and 60% WFPS. We have found it almost impossible to get the soil–sand uniformly mixed so H_2O contents vary among the small microcosms of heterogeneous soil–sand mixtures during incubation (Cambardella & Reeder, unpublished data).

Schimel et al. (1985) suggested an alternative approach that eliminates some of the problems associated with repeated leachings that are used during long-term incubations. Subsamples are extracted with 2.0 *M* KCl at 10, 20, and 60 d. Cumulative mineralized N ($NH_4^+ + NO_2^- + NO_3^-$) is calculated after subtraction of initial soil inorganic N. Leaving samples sealed for long periods may result in low atmospheric O_2 levels and high CO_2 that restrict mineralization. Modifications of both the Keeney and Bremner (1967) method and the method of Schimel et al. (1985) by Cambardella (1994) have produced the following method for aerobic determination of mineralizable N in soil.

Soil Sample Preparation

1. Field-moist soil is passed through 2- to 8-mm sieve. Large pieces of organic material and rocks are removed. The mesh size that is practical for sieving field-moist soil will depend on soil type and water content. Always use the same sieve size and report results based on the sieve size used, e.g., mineralizable N (<2 mm).
2. Soil water content is determined gravimetrically while the soil is stored at 4°C until incubation.
3. Soil baseline mineral N [$(NO_2^- + NO_3^-) + NH_4^+$] is determined using a colorimetric method (Keeney & Nelson, 1982) after a 30 min extraction in 2 *M* KCl (1:5 soil/solution ratio).

Laboratory Incubation

4. 10-g subsamples of sieved soil are weighed into each of four scintillation vials for incubations.
5. Soil is tamped down, where possible, to a uniform bulk density of 1.0 g cm^{-3}. Soil is adjusted to 60% WFPS with deionized or distilled water. The volume of water needed to obtain 60% WFPS is calculated using bulk density and initial water content (see Lowery et a., 1996, this publication).
6. The vials are placed in a quart Mason jar (about 950 mL) to which a small amount of water has been added at the bottom to maintain 100% relative humidity inside the sealed Mason jar. (Four empty jars containing everything but soil are run as blanks if mineralizable C is being quantified along with mineralizable N). The mason jars with the four vials are incubated at 30°C.
7. One vial is removed from each Mason jar at time zero plus 1 wk and extracted with 2 *M* KCl (1:5 soil/solution ratio). Again, mineral N is measured using a colorimetric method (Keeney & Nelson, 1982). Baseline mineral N is subtracted to give net cumulative mineral N.
8. One vial is removed at time zero plus 2 wk, plus 3 wk, and plus 4 wk and mineral N assessed as in Step 7.
9. Mineralizable N is reported as net cumulative mineral N after 4 wk incubation.
10. *Mineralizable C also can be assessed by using NaOH base traps in the Mason jars to trap CO_2–C. Mineralizable C is quantified by back-titration using HCl after addition of $BaCl_2$ to precipitate the CO_3–C. See Rice et al., 1996, this publication on soil biomass C determinations.

* Samples are removed each week for 4 wk in order to ascertain that the slope of the net cumulative mineral N curve vs. time (in weeks) remains constant over the 4 wk incubation. Most often, if the slope is different at all, the slope for the first 1 or 2 wk of the incubation will be different than for the last 2 wk. If this occurs, mineralizable N is reported as net cumulative mineral N at 4 wk minus net cumulative mineral N at 1 or 2 wk. In practice, however, the slope is generally the

same across the 4 wk. If possible duplicate or triplicate samples are recommended.

RECOMMENDED ANAEROBIC INCUBATION PROCEDURE

The recommended anaerobic method for N mineralization potential is based on the method of Waring and Bremner (1964). The anaerobic incubation method determines net potentially mineralizable N with a 7 d anaerobic incubation at a standard temperature, usually between 25 and 37°C. After the incubation, NH_4^+ is extracted with 2 *M* KCl and analysis of NH_4^+ concentration with a Lachat Flow Injection system. The amounts of NO_3^- and NH_4^+ originally in the soil are determined by a 2 *M* KCl extraction on separate soil samples prior to incubation. Potentially mineralizable N is calculated by subtracting the initial amount of NH_4^+ in the soil from the amount of NH_4^+ released during the incubation. It is essential that soil samples remain completely anaerobic during incubation to eliminate possible nitrification–denitrification reactions at the soil-water interface that would lead to low results (Keeney, 1982). For this reason, in the recommended procedure described below, incubation containers are purged with N_2 and sealed with rubber stoppers and electrical tape prior to incubation. We recommend sieving soils and starting the incubations–extractions out in the field if possible, or immediately upon return to the laboratory. Samples should be transported to the laboratory on ice. This approach is most appropriate when accurate determinations of mineral N pools in conjunction with N mineralization potentials are a priority.

We have recommended two sizes of soil samples and incubation containers. The smaller sample version is appropriate for most soils, particularly if a large number of incubations will be performed. The larger sample size (80 g field moist soil in a 500 mL plastic centrifuge bottle) is recommended for soils that have recently received fresh residues.

The main advantage of the centrifuge tube method is that it avoids the need to transfer samples from one container to another and then to filter funnels. If samples are to be filtered rather than centrifuged, an alternate method using 60 mL syringes as incubation–extraction vessels accomplishes the same thing and might be more convenient (Lober & Reeder, 1993).

Reagents

2.0 *M* KCl: 149 g L^{-1}
2.67 *M* KCl: 199 g L^{-1}

Materials

Plastic, disposable 50 mL polypropylene centrifuge tubes (25/tray) work well as incubator containers for most applications and usually last for two field seasons before they begin to leak. The incubation tubes are sealed with no. 6 rubber stoppers and electrical tape. For larger samples, 500 mL plastic centrifuge bottles can be used. For sieving the moist soils, we recommend the aluminum

seed sieves, 2 to 6 mm (depending on soil type), from Seedsburo (New Jersey). Traditional mesh soil sieves clog easily when soils are moist if the clay content is high. A centrifuge, or a filtering system is needed to remove soil from the extract. Extracts can be stored in 20-mL scintillation vials with polypropylene caps (caps without liners are needed). A cylinder of N gas is needed to purge the incubations.

Procedure

Prior to collecting soil samples:

1. Prepare solutions.
2. Rinse all labware twice with deionized or distilled H_2O.
3. Prepare two sets of sample containers: one set for initial (T_0) extraction and one for anaerobic incubation. Add 2 *M* KCl to T_0 containers and deionized H_2O to containers for incubations. For 50 mL centrifuge tubes: Add 40 mL 2 *M* KCl to T_0 tubes and 10 mL deionized H_2O to incubations using repipettes or graduated cylinders. Get a tare weight for each tube (tube + liquid + cap).

   For 500 mL bottles: Add 400 mL 2 *M* KCl to T_0 bottles and 100 mL deionized H_2O to incubations. These don't need to be preweighed because the soil is weighed into them.

The day of sampling:

4. Field moist soil should be sieved as soon after sampling as possible and measured into extraction–incubation containers immediately after sieving. Sieving should remove large organic debris, roots, and stones.

   For 50 mL centrifuge tubes: Add about 8 g of moist soil (equal to a volume change of 5–6 mL to each tube. Weigh again with caps on as before to determine how much soil was added.

   For 500 mL bottles: Add exactly 80 g of moist soil.
5. Place extractions (KCl) on the shaker for 1 h.
6. Remove KCl extractions from shaker and stand upright so the soil can begin to settle. Swirl so that the solution cleans off any soil that has accumulated in the top of the centrifuge tube. Make sure the inside of the tops are clean before placing the tubes in the centrifuge.

   For 50 mL centrifuge tubes: Centrifuge for 10 min at 400 to 500 × *g*. The amount of time and gravitational force needed to clear the extracts by centrifugation may vary with soil type so adjust as needed. Collect supernatant from below the surface (avoid floating organic debris) using a pipetman and put into labeled scintillation vials. These extracts also can be filtered.

   For 500 mL bottles: Set up filter paper and funnels with tall 100 mL beakers or wide-mouthed 250 mL flasks.
7. Store all extracts in the freezer until NO_3^- and NH_4^+ can be analyzed.
8. While the KCl extractions are shaking, the incubations can be purged with N_2 and sealed with rubber stoppers and electrical tape. A Pasteur pipette is connected to the tank with flexible tubing. During purging,

the tip of the Pasteur pipette should be just above the water level, and should not be submerged. Purge for 45 to 60 s, withdrawing the pipette tip as you put the stopper in place (or cover the bottle opening with the cap). It is important to completely seal the tubes. Usually two pieces of tape are needed, one over the top of the stopper and the other is wrapped around the tube–stopper seal. For the large samples, bottle caps should be secured tightly. Place sealed incubation vessels in 37°C incubator for exactly 7 d.

9. Determine soil water content gravimetrically by weighing sieved, field moist soil subsamples and placing them in the drying oven.

After one week:

1. Remove samples from incubator and add 30 mL of 2.67 *M* KCl to each tube.
2. Replace caps tightly and loosen the soil in the bottom of the tube or bottle by shaking or thumping tubes on the counter. Go to Steps 5 to 7 above to extract NH_4^+. Incubations are analyzed for NH_4^+ only.

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