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Comparison of Available Soil Nitrogen Assays in Control and Burned Forested Sites

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ABSTRACT

The existence of several different methods for measuring net N mineralization and nitrification rates and indexing N availability has raised questions about the comparability of these methods. We compared in situ covered cores, in situ buried bags, aerobic laboratory incubations, and tension lysimetry on control and treated plots of a prescribed burn experiment in the southern Appalachians. Environmental influences were examined with soil moisture and temperature measurements. All methods detected significant differences in net N mineralization rates between treated and control plots; $P = 0.04$, 0.007 , 0.001 , and 0.07 for covered cores, buried bags, lab incubations, and lysimeters, respectively. Mean rates of N mineralization during the growing season were similar for the three soil incubation methods. The laboratory incubation of soil from treated plots produced significantly greater potential nitrification rates than the in situ methods. The four methods were not well correlated; this indicates the need for caution when comparing N transformation data derived from different methods. We conclude that the in situ covered core method is superior because it best incorporates site-specific soil temperature and moisture changes into N transformation measurements.

NITROGEN often limits forest growth and productivity (Keeney, 1980). The importance of N in site productivity and its responsiveness to disturbance has prompted the design of several methods to assess potential soil N availability. Measurement of N mineralization potentials in controlled environment incubations began in the 1940s and has been widely used ever since (Harmen and Lindenbergh, 1949). These methods allow determination of potential N availability; however, they are carried out in ideal environmental conditions and may overestimate site processes. Eno (1960) introduced the in situ buried bag incubation technique. While this method incorporates in situ soil temperature variations into estimates of net N mineralization and nitrification, soil moisture remains unchanged during the incubation period. Adams and Attiwill (1986) developed the in situ covered core method for soil N mineralization measurements. Their system uses perforated PVC tubes covered with petri dishes, which allows soil moisture as well as temperature to equilibrate with the surrounding soil environment. Soil solution collection using tension porous cup lysimeters and ion exchange resin bags have also been used to estimate N availability (Hansen and Harris, 1975; Binkley and Matson, 1983; Montagnini et al., 1986).

Binkley and Hart (1989) extensively reviewed the literature to identify the strengths and weaknesses of most methods for determining N availability. There have also been numerous studies comparing two or three methods (Binkley and Matson, 1983; Montagnini et al., 1986; Adams and Attiwill, 1986; Raison et al., 1987; Binkley et al., 1986; Carlyle and Malcolm, 1986; Fyles et al.,

1990; Hart and Firestone, 1989; Hill and Shackleton, 1989). However, these comparisons were limited by the number of methods tested or by the sites on which they were tested.

This study compared four methods of determining N availability: measurement of net N mineralization (NH_4^+ -N plus NO_3^- -N production) and net nitrification (NO_3^- -N production) using in situ covered cores, in situ buried bags, and aerobic laboratory incubations and soil solution concentrations of NH_4^+ -N and NO_3^- -N collected using tension lysimetry. All measurements took place concurrently during the growing season on treated and control plots in a fell and burn forest regeneration experiment. These plots provided a wide range of N transformation rates and environmental conditions. The objective was to compare the methods to: (i) test the hypothesis that all methods discriminate between treatment effects and that soil incubation data are interchangeable, and (ii) examine the relationship of soil incubation methods with lysimeter N, extractable soil N, and soil temperature and moisture.

MATERIALS AND METHODS

Site Description

We selected two sites from a fell and burn treatment experiment already underway in the southern Appalachians (Swift et al., 1993). The sites, Jacob Branch East (JE) and Jacob Branch West (JW), are located in the Wayah Ranger District of the Nantahala National Forest in the southern Blue Ridge Mountains of western North Carolina. Both sites have a south to southwesterly aspect and are occupied by the Cowee-Evard soil series complex. The soils are classified as fine-loamy, mixed-oxidic, mesic Typic Hapludults, but they show signs of disturbance with shallow or mixed A horizons. Soil sampling was restricted to the top 10 cm, a depth that corresponds to the A, AB, and B horizons or a mixture of all three horizons. The sites are characterized by a pine-hardwood overstory and a dense mountain laurel (*Kalmia latifolia* L.) understorey that inhibits regeneration of the overstorey species. The combination of drought, oak (*Quercus* sp.) decline, and the southern pine beetle has degraded the sites (Vose and Swank, 1993). The silvicultural prescription is designed to regenerate a mixed pine-hardwood forest. The first step was to fell all woody vegetation and allow it to cure; second, burn the site under environmental conditions appropriate for a high-intensity, low-severity fire; and third, plant the site with white pine (*Pinus strobus* L.) on wide spacing (4 by 4 m).

Sampling Design

In summer 1989, Swift et al. (1993) established nine 15 by 33 m plots on each of the two sites; five plots were located on the treatment areas and four control plots were on adjacent undisturbed areas. Trees and shrubs were cut in June and July 1990, and the sites were burned in September 1990. The methods comparison experiment was performed the first grow-

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Abbreviations: PVC, polyvinyl chloride; JE, Jacob Branch East; JW, Jacob Branch West; TDR, time domain reflectometry.

ing season after site treatment (1991) during three concurrent 28-d periods in May, July, and September.

On each plot ($n = 18$), two transects were established on the 33-m plot axis that corresponded with the topographic contour. Transects were 1 m apart and began at a randomly selected plot corner. Paired in situ incubations were carried out, one on each transect. All soil samples were collected using PVC cores 4.3 cm in diameter and 15 cm long. Cores were driven into the soil to a depth of 10 cm, 25 cm apart. At $t = 0$, two cores of soil were collected, one from each transect. The soil was cooled, returned to the laboratory, and stored at 4°C until processed within 48 h. The two soil samples were composited by plot and sieved to <6 mm. Five grams of fresh soil were shaken for 1 h in 2 M KC1 at a 1:4 soil/extractant ratio and then centrifuged at 3715 \times g (6000 rpm) for 15 min. The NO_3^- -N and NH_4^+ -N concentrations in KC1 solutions were determined on an autoanalyzer (Technicon Instruments Corp., Tarryton, NY) using the hydrazine sulfate reduction (U.S. Environmental Protection Agency, 1983) and alkaline phenol (Technicon Instruments Corp., 1971) methods, respectively. The total weight of soil (<6 mm) in the two cores was measured for bulk density determination. Soil N content and rates of N transformation are reported on a 105°C oven-dry weight basis. The $t = 0$ NO_3^- -N and NH_4^+ -N concentrations were used for all soil incubation methods.

In Situ Covered Core Incubation

We used the covered core in situ incubation method of Adams and Attiwill (1986) and Adams et al. (1989) modified by using unperforated PVC cores. At $t = 0$, two PVC soil cores, one on each transect, were capped with PVC pipe caps and left in the field. After 28 d, the cores were collected and processed as described above ($t = 1$). The modification was intended to make the moisture content of the in situ covered core method more comparable with the in situ buried bag method as described by Matson and Vitousek (1981). In the buried bag technique, PVC cores were used to collect the soil. The soil was removed from the core with minimal disturbance, placed in a bag, and returned to the collection point. Buried bags have a distinct disadvantage in that the soil moisture content remains unchanged during the incubation period. The result is often incubating soil that is extremely wet or dry unless one chooses sampling days when soil water content is optimized. This makes the PVC cores more suitable for in situ measurements. We found that even without perforations, soil moisture content within the core changes during the incubation period. Capillarity moves water into the cores, and since the cores were not tightly sealed, evaporation results in some water loss. The resulting net soil moisture changes within the PVC cores, as measured gravimetrically at $t = 0$ and $t = 1$, were lower but positively correlated with net plot soil moisture changes measured weekly with TDR during the same 28-d period (Topp et al., 1980). These measurements would not be expected to correlate precisely since cores are 10 cm long, while the TDR readings are for the surface 30 cm of soil. The slope of this regression was 0.57 ($P = 0.0001$) with a 1.7 intercept ($r^2 = 0.38$, $n = 249$). Decreased net moisture changes within the core were expected since the core prevented plant water uptake and inputs were limited to capillary water movement. The correlation analysis used soil core gravimetric moisture content converted to volumetric moisture content using bulk density measurements.

¹ The use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture for any product or service.

In Situ Buried Bag Incubation

Buried bag in situ incubations were carried out as described by Matson and Vitousek (1981). Two intact 10-cm soil cores were collected using the sampling procedure and PVC tubes described above, one from each transect. Soil cores were plunged from the PVC tube into 15 by 15 cm, 0.072-mm-thick, interlocking polyethylene bags. The bags were sealed and returned to the hole, then collected after 28 d. Gordon et al. (1987) found that polyethylene bags with thickness ranging from 0.015 to 0.032 mm had no effect on N mineralization. While these bags are considerably thicker, rates of N mineralization in bags were comparable to covered core and laboratory incubations, suggesting that gas permeability was adequate.

Aerobic Laboratory Incubation

One 50-g sample of each composite $t = 0$ soil (sieved <6 mm) was placed in a 0.95-L canning jar, and the moisture content was adjusted. The desired moisture content was 45% for JW and 30% for JE, which is equivalent to -0.03 MPa pressure measured on intact A horizon soil cores. Soil plus jars were covered tightly with 0.01-mm-thick plastic wrap, to allow gas exchange with minimal water loss, and weighed. Soils were incubated at 20°C for 28 d; this was approximately equal to the maximum 10-cm soil temperature. Water was added weekly as necessary to maintain constant moisture content.

Tension Lysimeters

Two porous-cup tension lysimeters were installed on each of the four control and five treated plots, one at a depth of 30 cm and one at 60 cm. Lysimeter placement was determined using the pedon description for the Cowee-Evard soil types (USDA Natural Resource Conservation Service, 1993). Located above the Bt horizon and within the rooting zone, the 30-cm lysimeter was intended to estimate nutrients in soil solution available to plants. The 60-cm lysimeter was located below the main rooting zone and Bt horizon to collect soil solution potentially leaving the site. Solution samples were collected weekly, at which time lysimeters were evacuated to -0.03 MPa. Analyses of soil solutions for NO_3^- and NH_4^+ were performed on volume-weighted monthly composite samples using the autoanalyzer methods described above.

Environmental Parameters

Soil temperature was measured weekly at 10 cm in each plot with a digital meter and thermistor probe. Soil moisture, percentage by volume at 0 to 30 cm was measured weekly on each plot using the TDR method (Topp et al., 1980). Data used in correlation analyses were both $t = 0$ and mean monthly soil temperature and moisture values.

Statistical Analysis

Statistical analysis of the data was conducted with several objectives. The first objective was to determine the ability of each method to discriminate the effect of the burn treatment on net N mineralization and nitrification or on the other N availability indices. The t-test analysis was used on data for each method using the t-test procedure of SAS (SAS Institute, 1985). Variance homogeneity was tested with a folded F statistic. When variances were unequal, approximate t-test and Satterthwaite's approximation for computing degrees of freedom were used (SAS Institute, 1985). The second objective was to compare the results of the three soil incubation methods. The SAS general linear models procedure was used to perform

an analysis of variance that compared rates of net N mineralization and nitrification for the in situ buried bag, covered core, and laboratory incubations. The third objective was to examine the relationship between the soil incubation methods and lysimeter N, extractable soil N, and soil climate. Correlations were conducted with data from both treated and control plots using Pearson standard and Spearman rank correlation analyses (SAS Institute, 1985).

RESULTS AND DISCUSSION

Treatment Effect Discrimination

All methods, soil incubations, extractable soil N, and soil solution N, measured significantly greater ($P < 0.1$) net N mineralization rates, and $\text{NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N}$ concentrations in the prescribed burn plots compared with the control plots (Fig. 1). This was also the case for net nitrification rates and $\text{NO}_3^- \text{-N}$ concentrations in soils and soil solution ($P < 0.1$) with the exception of in situ buried bags ($P = 0.35$; Fig. 1). Elevated N transformation rates following site disturbance are well documented (Burger

and Pritchett, 1984; Knoepp and Swank, 1993). Significant site and treatment differences in N availability have been measured with laboratory incubations, in situ incubations (both buried bag and covered core), lysimeters, and various chemical methods (Adams and Attiwill, 1986; Carlyle and Malcolm, 1986; Montagnini et al., 1986; Hart and Firestone, 1989; Binkley and Matson, 1983).

The three soil incubation methods gave similar results for mean net N mineralization rates on both the prescribed burn and control plots (Fig. 1). However, rates of net nitrification measured in burn-plot soils using laboratory incubation were significantly greater than rates measured with in situ incubations.

Although mean rates of net N mineralization did not differ among soil incubation techniques, they were not well correlated. Covered core and buried bag in situ measurements of net N mineralization showed the only significant correlation ($r = 0.40$; $P = 0.002$). Net nitrification rates measured using the three soil incubation methods also showed no significant correlation ($P < 0.1$). These results differ from earlier studies, which showed a better correlation for indexes of NO_3^- availability than for indexes of NH_4^+ availability (Hart and Firestone, 1989).

Soil incubation measurements of N transformation rates had some significant relationships with the other measurements of N availability. Soil solution concentrations of $\text{NO}_3^- \text{-N}$ and $\text{NO}_3^- \text{-N}$ plus $\text{NH}_4^+ \text{-N}$ correlated well with all rates of N transformations, with the exception of buried bag nitrification (Table 1). Previous research has also shown that soil solution collections are good indicators of N availability and potential leaching losses (Hansen and Harris, 1975; Montagnini et al., 1986; Hill and Shackleton, 1989). Net mineralization measurements were also correlated with soil inorganic N content at the time of sampling, with r values ranging from 0.28 to 0.60 (Table 1). While this general relationship has been identified in other studies (Donaldson and Henderson, 1990; Knoepp, 1994, unpublished data), the difference in controlling factors, makes extractable soil N concentration a poor predictor of N transformation rates.

Method Response to Soil Climate

Sample date affected the three soil incubation measurements of net N mineralization at $P = 0.006$ for covered core, 0.08 for buried bag, and 0.11 for laboratory incubations. The date effect is probably related to observed variations in soil temperature and moisture. Net N mineralization rates were correlated with soil temperature at 10 cm for all soil incubation methods (Table 1). Adams and Attiwill (1986) measured net N mineralization in situ and in the laboratory for eight eucalypt forests in a wide range of climates and soil types. Both methods identified site differences but only the in situ incubations produced seasonal patterns of mineralization. This result was attributed to field temperature fluctuations. Carlyle and Malcolm (1986) found greater rates of net N mineralization for laboratory-incubated soil samples than for in

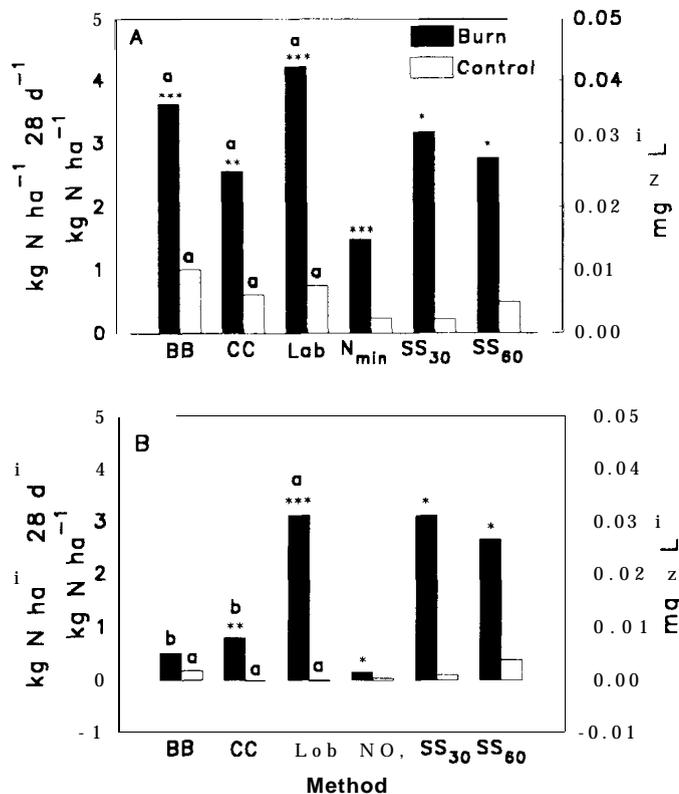


Fig. 1. Means for prescribed burn and control plots for (A) mineralization and (B) nitrification indices. Mineralization indices include in situ buried bag (BB), in situ covered core (CC), and laboratory incubation (Lab) rates of net $\text{NH}_4^+ \text{-N}$ plus $\text{NO}_3^- \text{-N}$ production, $t = 0$ $\text{NH}_4^+ \text{-N}$ plus $\text{NO}_3^- \text{-N}$ (N_{min}) concentration, and soil solution concentrations of $\text{NH}_4^+ \text{-N}$ plus $\text{NO}_3^- \text{-N}$ collected at 30 (SS₃₀) and 60 cm (SS₆₀). Nitrification indices include in situ BB, in situ CC, and Lab rates of net $\text{NO}_3^- \text{-N}$ production, $t = 0$ $\text{NO}_3^- \text{-N}$ (NO_3) concentration, and soil solution concentrations of $\text{NO}_3^- \text{-N}$ collected at SS₃₀ and SS₆₀. Values represent means of three dates for two sites on burn ($n = 30$) or control ($n = 24$) plots. *, **, *** Significant difference between burn and control plot means at $P < 0.1$, 0.05, and 0.01, respectively. Soil incubation burn or control plot means with different letters indicate significant differences between methods ($P < 0.05$).

Table 1. Correlation coefficients between buried bag (BB), covered core (CC), and laboratory (Lab) measurements of net N mineralization (Min) and nitrification (Nit) and soil solution N (mg N L⁻¹) from 30- and 60-cm lysimeters, initial soil NO₃⁻ and NH₄⁺ plus NO₃⁻ content, soil temperature, and soil moisture (values in parentheses represent probability of values greater than *r*).

Other parameters	Soil N transformation measurements					
	BB-Min	CC-Min	Lab-Min	BB-Nit	CC-Nit	LabNit
Lysimeter, 30 cm	0.28 (0.05)	0.57 (<0.01)	0.29 (0.03)	nst	0.53 (<0.01)	0.24‡ (0.08)
Lysimeter, 60 cm	0.32‡ (0.02)	0.53 (<0.01)	0.39 (<0.01)	ns	0.49 (<0.01)	0.50‡ (<0.01)
NO ₃ ⁻ , kg N ha ⁻¹	na§	na	na	-0.34‡ (0.01)	ns	0.42 (<0.01)
NH ₄ ⁺ + NO ₃ ⁻ , mg N kg ⁻¹	0.49‡ (<0.01)	0.28 (0.04)	0.60 (<0.01)	ns	ns	0.51‡ (<0.01)
Soil temperature at 10 cm, °C	0.52‡ (<0.01)	0.37‡ (<0.01)	0.36 (<0.01)	ns	0.32‡ (0.02)	0.55‡ (<0.01)
H ₂ O, g kg ⁻¹ , <i>t</i> = 0	3.2 (0.02)	ns	ns	3.6 (<0.01)	ns	ns

† ns = correlation coefficients where *P* > 0.1.

‡ Indicates Spearman rank correlation coefficient; all others are Pearson correlation coefficient.

§ na = correlations between those variables were not performed.

situ samples. They corrected the laboratory rates for temperature by using a *Q*₁₀ of 2, thereby removing the difference. All our measurements took place during the growing season when average monthly soil temperatures at 10 cm ranged from 16 to 22°C (Swift et al., 1993).

Soil water content, *t* = 0, was positively correlated with net N mineralization and nitrification only for the buried bag in situ incubation (Table 1). This agrees with the findings of Matson and Vitousek (1981); in their laboratory incubations, as soil moisture increased from 15 to 35 %, net mineralization also increased. Soil moisture content did not change in the buried bags, making the initial moisture levels important. Net mineralization and nitrification rates measured using laboratory and in situ covered core incubations were not significantly correlated (*P* < 0.1) to initial soil moisture, since soil moisture changed from *t* = 0 levels for both. Moisture content was adjusted and maintained at a constant, experimentally derived, optimal moisture content for laboratory-incubated soil samples. The moisture content of soil within the in situ covered cores, as measured gravimetrically at *t* = 0 and *t* = 1, varied with the moisture content of the surrounding soil, as measured weekly for each plot using TDR (*r*² = 0.38; *n* = 249), although net changes were less. Capillarity results in water movement into the core from below when bulk soil moisture increases. The cores are not sealed, so water can be lost by evaporation (see Materials and Methods for further discussion).

All methods tested detected differences in rates of net N mineralization and nitrification between prescribed burn and control plots. The mean rates for the three soil incubation methods were not statistically different from each other (data not shown). However, a pattern emerged when we compared the mean of individual methods to overall mean rates of net N mineralization and nitrification. For the control plots, all methods gave fairly uniform results (Fig. 2). However, for the burn plots, laboratory incubation measurements of N transformation rates were consistently the greatest, while the in situ covered core method gave values below overall mean rates. Laboratory incubations are performed under near-optimal conditions of soil temperature and moisture, which may result in elevated N transformation rates. In contrast, in situ incubations are conducted under ambient environmental conditions, which frequently are not ideal.

Our results show that all the methods examined are adequate for identifying treatment differences in indexes of N availability. However, their poor correlation indicates the need for caution when comparing N transformation rates measured by different methods. We believe that N transformation rates measured using the covered core in situ incubation method best reflects site N transformation rates. Of the methods tested, this method best incorporates site-specific temperature and moisture variations into net N mineralization and nitrification measurements.

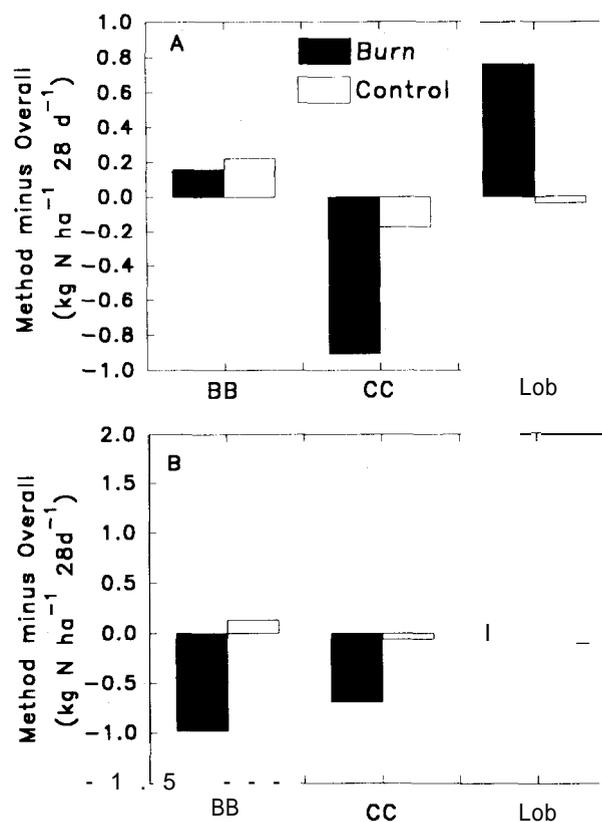


Fig. 2. Means for each soil-based method minus the overall mean for all soil incubation measurements of net (A) N mineralization and (B) nitrification. Methods include in situ buried bag (BB), in situ covered core (CC), and aerobic laboratory (Lab) incubations. Overall means of all soil incubations are 3.5 and 0.8 kg N ha⁻¹ 28 d⁻¹ net N mineralization and 1.5 and 0.04 kg N ha⁻¹ 28 d⁻¹ net nitrification for prescribed burn and control plots, respectively.

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