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Comparing Methods for Assessing Forest Soil Net Nitrogen Mineralization and Net Nitrification

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ABSTRACT

A variety of analytical techniques are used to evaluate rates of nitrogen (N) mineralization and nitrification in soils. The diversity of methods takes on added significance in forest ecosystem research where high soil heterogeneity and multiple soil horizons can make comparisons over time and space even more complex than in agricultural Ap horizons. This study compares (a) the three most common incubation periods (7, 14, and 28 day) used in potential net

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N mineralization and nitrification measurements, and (b) three methods of net nitrification assessment (potential net nitrification, in situ net nitrification, and nitrification potential) in the context of a long-term, paired-watershed N manipulation experiment. Results suggest that the 28-day incubation may be most appropriate in these northeastern U.S. forest soils to allow time for patterns of N dynamics to emerge following the disturbance associated with sampling and incubation. All nitrification measurement methods studied showed similar effects of forest type and watershed treatments. Each method could be an appropriate choice depending on objectives. The nitrification potential method should be studied further to determine its sensitivity to detect alterations in N dynamics in forest soils.

INTRODUCTION

Researchers and managers frequently need to understand the amount and rate of cycling of soil nitrogen (N) in forest ecosystems to guide management and environmental decision making. Measuring total N in forest soils has become routine in many modern laboratories, but this measurement often tells us little about the rates of N cycling as reflected in leaching losses of N or biological uptake. Measuring labile N is more complex, and methods range from estimates of actual rates of N turnover in the field, to indices of N cycling rates from laboratory methods.[1–3] Interest in forest-soil N dynamics in the late 1900s came from an interest in optimizing tree nutrition and forest growth.[4,5] More recently, interest in N dynamics in forest soils has also resulted from concerns for atmospheric N deposition and the risks of ''N saturation,'' an ecosystem condition where inorganic N exceeds plant and microbial demand.[1,6] Because inorganic forms of N are the most labile in soils, techniques to evaluate the rate of N cycling commonly focus on N mineralization and nitrification processes. Measurements of net N mineralization or gross N mineralization are both techniques used to achieve these objectives,[7] with net N mineralization techniques more commonly used due to their ease and relative low cost. Common approaches to measuring net N mineralization include incubating soils in a laboratory or in situ incubations in the field for 1 or more weeks.[2,3] Researchers in tropical ecosystems often use 7-day incubations because N mineralization proceeds more rapidly under higher temperatures and moisture regimes.[8,9] Researchers in temperate forest ecosystems have more often used 14-, 28-day, or much longer (i.e., >30 weeks) incubations, both in situ and in the laboratory.[7,10–15] Therefore, methods are often

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inconsistent in the literature for measuring forest-soil potential net N mineralization. In addition, there is some question about the adequacy of laboratory rates as estimates of those obtained in the field.[16]

The objectives of this research were to (a) determine if there were operational advantages or disadvantages that could be identified among a set of commonly used methods for evaluating forest-soil N dynamics, and (b) determine the sensitivity of these methods to differences in forest-soil N dynamics at a long-term, whole-watershed N manipulation experiment.

MATERIALS AND METHODS

Soils Used in This Study

Soils from the Bear Brook Watershed in Maine (BBWM) were used in this study because they offered a contrast in forest types and long-term, whole-ecosystem N treatments. The BBWM is located in eastern Maine at 44°52′ north latitude and 68°06′ west longitude, approximately 60 km from the coast of Maine in the upper 210 m of the southeast slope of Lead Mountain. BBWM is a paired-watershed experiment begun in 1987 and established to evaluate whole-ecosystem response to elevated N and sulfur (S) deposition in a low-alkalinity forested stream watershed in northern New England.[17]

The vegetation at BBWM includes both hardwoods and softwoods, with hardwoods and mixedwoods dominating the lower ~60% of the watersheds. Hardwoods included American beech (*Fagus grandifolia* Ehrh.), sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), with minor occurrences of yellow birch (*Betula alleghaniensis* Britt.) and white birch (*Betula papirifera*). The higher elevations are nearly pure softwoods dominated by red spruce (*Picea rubens* Sarg.), but also include balsam fir (*Abies balsamea* L.) and hemlock (*Tsuga canadensis* L. Carr). Softwoods, hardwoods, and mixedwoods cover approximately 25, 35, and 40% of the total watershed areas, respectively.[17] Soil sampling for this study focused on the softwood and hardwood stands.

The soils were acidic, with low base saturation, cation-exchange capacity, and sulfate-adsorption capacity.[17] Bedrock geology consists of metamorphosed quartzite and calc-silicate gneiss. Further details of site characteristics can be found in Norton et al.[17] and Fernandez and Adams.[18]

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The Nitrogen Experiment

The BBWM site consists of two adjacent forested stream watersheds. The East Bear (EB) watershed (11.0 ha) serves as the reference watershed. The West Bear (WB) watershed (10.3 ha) has been treated bimonthly with ammonium sulfate [$(NH_4)_2SO_4$] starting in November 1989 as part of a whole-watershed manipulation experiment designed to investigate the effects of atmospheric deposition of N and S. Granular $(NH_4)_2SO_4$ has been aerially applied bimonthly at approximately 25.2 kg N ha^{-1} $year^{-1}$ resulting in estimated total N inputs (wet + estimated dry + treatment) of 33.6 kg N ha^{-1} $year^{-1}$. The reference East Bear watershed receives 8.4 kg N ha^{-1} $year^{-1}$ of ambient wet plus estimated dry deposition.[17] Within each watershed, four replicate 10×10 m plots were established with two of the four plots in each watershed in hardwoods and two in softwoods. Plots were chosen to have comparable slopes, dominant tree species, and proximity to streams between watersheds. Four soil samples were collected from each 10×10 m plot in September 2000 and June 2001. Two soil depth increment samples were collected at each sampling location that included (1) the whole O horizon and (2) the uppermost 15 cm of the B horizon. Samples were collected by laying down a 15×15 cm frame on the surface of the increment to be sampled, and then removing either all of the O horizon down to the top of the mineral soil, or the entire B horizon from the top of the B to a 15-cm depth. The E horizon was excluded when present. Samples were placed in labeled polyethylene bags in the field, transported on ice, and stored at 4°C prior to extraction. Field-moist O horizon soils were sieved through a 6-mm mesh sieve and mineral soils were sieved through a 2-mm mesh sieve. Soil extractions were completed within 24 h of collection. Extracts were frozen until they could be analyzed for NH_4^+-N and NO_3^--N. Additional details of the experimental design are described in Shah.[19]

In Situ Net Nitrogen Mineralization and Net Nitrification

In situ net N mineralization and net nitrification were assessed using the buried bag method of Eno.[10] Soils were incubated in the field for 35 and 28 days beginning in September 2000 and June 2001, respectively. Net N mineralization was defined as the difference between the sum of NO_3^--N and NH_4^+-N before and after incubation, while net nitrification was the difference for NO_3^--N alone. Soil samples were kept in plastic bags in a cooler for transport from the field to the

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laboratory. Replicate subsamples (~5 g) of field-moist soils were used to measure oven-dry moisture content (O horizon soils were dried at 65°C and mineral soils were dried at 105°C). At the initiation and during the experiment, subsamples were immediately extracted with 100 mL of 2 M KCl (solution-to-soil ratio m/v) to determine initial NO_3^--N and NH_4^+-N concentrations. Concentrations of NO_3^--N and NH_4^+-N were determined on an OI Analytic Dual-Channel Automated Ion Analyzer at the University of Maine's Analytical Laboratory and on a Perstop Flow Solutions 3000 Injection Analyzer at the Institute of Ecosystem Studies in Millbrook, New York.

Laboratory Net Nitrogen Mineralization and Net Nitrification Incubations

Net N mineralization and net nitrification were assessed using a 7-, 14-, or 28-day laboratory incubation period.[11] A field-moist soil sample was placed in a plastic cup and covered with Parafilm, perforated for ventilation, and incubated in the dark at ~22°C for 7, 14, or 28 days, after which a 15 ± 0.05 g subsample was removed. A separate ~5 g subsample of field-moist soil was used to determine oven-dry moisture content and to correct for the dry-mass equivalent of the incubating soils. Soil samples were extracted field moist with 100 mL of 2 M KCl and analyzed on an Automated Ion Analyzer for NO_3^--N and NH_4^+-N.

Nitrification Potential Assay

The nitrification potential assay of Hart et al.[2] was chosen because it measures the rate of nitrification in a soil by creating conditions where NH_4^+-N is no longer the limiting factor, with the assumption that rates of nitrification are then limited by the capacity of the nitrifying community itself. Acidic forest soils are thought to be dominated by autotrophic nitrifying populations as opposed to heterotrophic nitrifying populations, the latter for which assimilable organic-C could also limit growth.[20] Samples were handled according to the shaken-slurry method described by Hart et al.[2] A 15 ± 0.05 subsample of soil was taken from each sample and mixed with 100 mL of a solution containing 1.5 mM NH_4^+ and 1 mM PO_4^{3-} in a 250-mL flask. Slurries were shaken on a wrist-action shaker for 24 h. Approximately one-quarter of the slurry was removed at 2, 4, 22, and 24 h. These subsamples were then filtered through Whatman #40 filters that had been previously leached with deionized water to

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remove inorganic N contamination. Samples were analyzed on an Automated Ion Analyzer for NO_3^--N concentrations.

Statistical Analyses

The nitrification-potential data were analyzed using repeated measures ANOVA on the Statistical Analysis System[21] with an alpha level of 0.05. The statistical design for the laboratory and in situ incubations was a split-split plot among treatments, forest types, and time. In this design, factor A was the reference East and treated West Bear watershed soils, factor B was the hardwood- and softwood-forest types, and factor C was time. Spatial replication for plots per watershed and forest type was $n = 4$. Subsampling within plots ($n = 5$) provided precision to plot means. Analyses were performed separately on O horizons and mineral soils given the sharply contrasting characteristics of each horizon. All data except the nitrification potential required rank transformations.

RESULTS AND DISCUSSION

Importance of Incubation Time in Measuring Potential Net Nitrogen Mineralization

Figure 1 provides insight on the importance of incubation time by showing the influence of 7-, 14-, and 28-day incubations on potential net N mineralization for O horizon and mineral-soil grand means across watersheds and forest types. O horizons have significantly greater potential net N mineralization than mineral subsoils, and the numerical pattern of development in potential net N mineralization over time shows a large initial increase to day 7, followed by an asymptotic increase to days 14 and 28. Both the O and mineral-soil data can be fitted with highly significant regressions as represented in Figure 1. In contrast to the O horizon, the mineral-soil potential net N mineralization was much lower and shows marginal activity until after day 7, with continued numerical increases for days 14 and 28. These patterns of response were consistent for each forest type and watershed, and those results do not provide further insight on the behavior of these methods. Details on the response results for the interaction of these factors are reported in Jefts et al.[22] These results suggest that measuring potential net N mineralization with a 7-day incubation period in these northern U.S.

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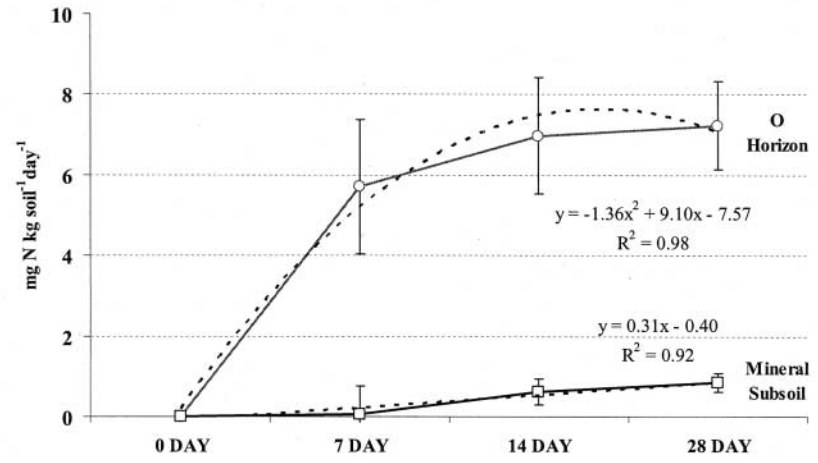

Figure 1. O horizon and mineral-soil net N mineralization means over 7-, 14-, and 28-day laboratory incubation periods. Solid lines are means and error bars represent standard error of the mean. Dashed lines are polynomial (O horizon) and linear (mineral subsoil) regressions for the data.

forest soils could underestimate the potential N cycling when compared with longer incubation times. Since the rate of change over time at 7 days is relatively high, it is possible that small differences in substrate quality or environmental conditions such as temperature or moisture could have disproportionate influences on the results. Although the 28-day results are higher than the 14-day results in the O horizon soils, the difference between these two longer incubation periods is relatively small, and the results from both are highly correlated ($r^2 = 0.94$) compared with a much weaker correlation ($r^2 = 0.29$) for the 14-day vs. 7-day results. For the O horizon soils, it seems that both the 14- or 28-day incubation periods might offer comparable assessments of potential net N mineralization, and other scientific or practical considerations could govern the final choice of technique. For the mineral soils, these data suggest that a 28-day or longer incubation period would be desirable to adequately assess mineral-soil potential net N mineralization. Given the precedent in the literature for 28-day net N mineralization incubations in forest-soils studies, and the advantages of a single uniform methodology for soil materials that grade from organic to mineral on the forested landscape, it is suggested that the 28-day incubation offers the best recommendation

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for incubation periods in these techniques. However, these results are for a single intensive research site, and similar comparisons should be conducted across a range of forest and soil types before drawing conclusions that might be applied to broad regions of climate and forest types.

Potential Net Nitrification, In Situ Net Nitrification, and Nitrification Potential

Potential net nitrification, in situ net nitrification, and nitrification potential all measure the capacity of soil to support nitrifier activity under varying field and laboratory conditions. While in situ net nitrification of forest soils is often taken to be an estimate of ''actual'' rates of nitrification in the forest, the other two methods are intended to be relative indices of N dynamics rather than absolute estimates of in situ rates. The three methods were applied to O and mineral horizon soils from the BBWM. Table 1 shows the means and associated standard error of the mean for each method, presented by both watershed and forest type from the BBWM experiment. All three methods showed significantly higher rates of nitrification for WB (treated) compared to EB (reference) for both O horizon and mineral soils. Differences between forest types were less consistent among the methods we used. The in situ method resulted in significantly higher nitrification rates in O horizons for hardwoods compared to softwoods, but no significant differences were evident for mineral soils. Both of the other methods showed similar numerical trends in the O horizon. The nitrification-potential assay resulted in no significant differences in the O horizon between forest types but resulted in significantly lower nitrification rates in the hardwoods' mineral soils compared to softwoods (Table 1). However, the nitrification-potential assay was the only method that detected numerically higher means in the mineral soils of the softwoods compared to the hardwoods (Table 1). The differences between horizons by forest types shown here for both in situ and 14-day laboratory incubations were consistent with results from more comprehensive studies of N dynamics at BBWM[18,19] and other northeastern U.S. forest-soil studies.[1,18,23,24] The nitrification potential may be more sensitive to incipient differences in soil N dynamics because it is carried out under ideal laboratory conditions, but further study is warranted to determine its scientific value in this regard.

Verchot et al.[7] compared gross and net N mineralization and nitrification and compared the sensitivity of laboratory incubations, in

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Table 1. Potential net nitrification and potential means (standard errors) by watershed and forest type.

	Watershed		Forest type	
Horizon	EB	WB	Hardwood	Softwood
Nitrification potential (mg N kg soil^{-1})				
O Horizon	5.68 (2.12)[a]	86.26 (18.05)	77.74 (19.33)	14.2 (4.59)
Mineral	9.31 (1.94)[a]	28.92 (3.31)	12.44 (2.02)[a]	26.66 (4.07)
14-Day potential net nitrification (mg N kg soil^{-1} d^{-1})				
O Horizon	0.18 (0.09)[a]	3.21 (0.56)	2.80 (0.61)	0.60 (0.16)
Mineral	0.21 (0.05)[a]	0.64 (0.31)	0.26 (0.30)	0.60 (0.09)
In situ potential net nitrification (mg N kg soil^{-1} d^{-1})				
O Horizon	0.06 (0.02)[a]	0.87 (0.20)	0.75 (0.20)[a]	0.19 (0.05)
Mineral	0.11 (0.02)[a]	0.16 (0.12)	0.08 (0.12)	0.19 (0.03)

[a]Indicates significant differences ($P = 0.05$) between watersheds or forest types within horizons.

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Table 2. Pearson correlation coefficients for 14-day PNN, in situ PNN, and nitrification potential for the combined data from both collections (9/2000 and 6/2001).

	In situ PNN	Nitrification potential
O Horizons		
14-day PNN	+0.81[a]	+0.62[a]
In situ PNN		+0.64[a]
Minerals soils		
14-day PNN	+0.44[a]	+0.76[a]
In situ PNN		+0.42[a]

[a]Indicates significant coefficients.

situ incubations, and the nitrification-potential assay for determining functional differences in net nitrification among hardwood-forest stands in Millbrook, New York and the Catskill Mountains of New York. They found that gross rates of N mineralization and nitrification were not good indicators of differences in forest types. Furthermore, they reported that net N mineralization and nitrification, particularly the laboratory incubation, proved to be better indicators of differences between forest types using a 14-day incubation technique both in the laboratory and in situ. Knoepp and Swank[16] compared 28-day laboratory incubations to both in situ buried bags and in situ incubated cores and found the in situ incubated cores to be preferable because they found them to best incorporate site-specific changes in moisture and soil temperature. Results reported by Verchot et al.[7] concur with this study in that nitrification potential appeared to be more sensitive to differences in forest types than net nitrification in laboratory incubations or in situ incubations.

All three methods for estimating nitrification rates were significantly correlated (Table 2), but the significance of the correlation varied considerably. Out of the three methods used (Table 2), the O horizons in the 14-day laboratory incubation and the in situ incubation were best correlated, however, in the mineral soils, the 14-day laboratory incubation and the nitrification-potential assay were best correlated. These data suggest possible relationships to explore in future studies but are limited in their applicability because of the narrow scope of the samples used in this study.

The three methods differed markedly in the absolute values of the means as shown in Table 1. The nitrification-potential assay had the highest rates, nearly an order of magnitude greater than either the 14-day

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laboratory incubation or the in situ incubation. For the latter two methods, the 14-day laboratory incubation rates were one to four times greater than the in situ incubation rates. The differences in magnitude of the results among the three methods were not surprising since the nitrification-potential assay supplies essentially unlimited NH_4^+-N to autotrophic nitrifying microbial communities. The difference in magnitude between the laboratory and in situ incubations was also expected since laboratory incubations use homogenized soils and higher laboratory temperature conditions (in this case $\sim$22°C) without the diurnal fluctuations in temperature that would be experienced in situ. Therefore, laboratory incubations are expected to provide more consistent rates of microbial activity during the incubation period that are higher than in situ rates, which do not optimize microbial activity because of cool night temperatures and perhaps the extreme heat of the warmest of days during the field season. Because of these differences, the laboratory incubation may be more effective at detecting differences in incipient N dynamics of sites, whereas in situ incubations are the logical choice for estimating actual rates of nitrification in the field. Even in situ methods are only estimates, as artifacts from the disturbance involved in the buried-bag method undoubtedly modify the accuracy of the results.

The Nitrification-Potential Assay

The nitrification-potential assay is designed to measure the potential maximum activity of soil nitrifiers by providing ideal conditions for nitrification where the limiting factor is no longer NH_4^+-N availability but the nitrifying population itself. Subsamples from each soil slurry taken periodically during the 24-h incubation period provide insight on the temporal pattern of NO_3^--N production over time (Figs. 2 and 3). Results from the repeated measures ANOVA for the O horizon indicated that there were significant differences over time, between watersheds, between forest types, and at all levels of interaction among the main effect variables in this analysis. Mineral-soil results indicated there were significant differences over time, between watersheds, between forest types, but interactions with time as a factor were not significant in this analysis. The nitrification-potential assay results for soils under hardwoods (Fig. 2) showed that O horizons from the treated WB had the highest rates of nitrification potential among the watershed-and soil-type combinations from the BBWM study, and O horizon rates were higher than mineral-soil rates. High rates of N cycling in the WB hardwood O horizons were first detected by Wang and Fernandez[15] and recently

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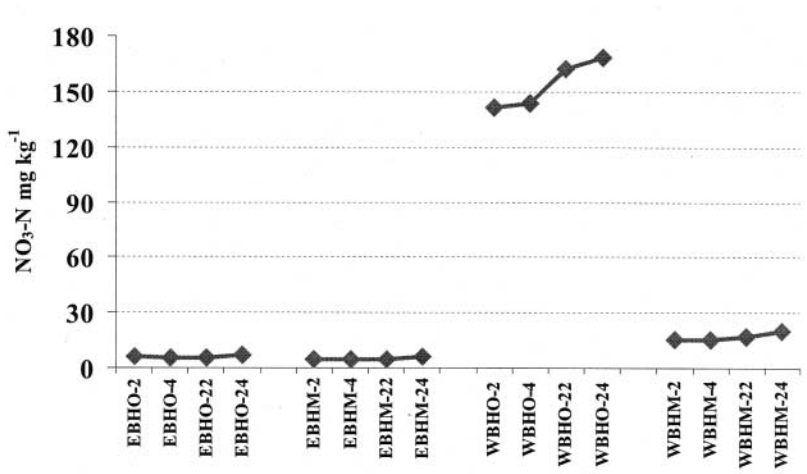

Figure 2. Hardwood-soils nitrification potential over the 24-h sampling period. EB = the East Bear watershed; WB = the West Bear watershed; H = hardwood-forest type; O = O horizons; M = mineral soils. Subsamples removed for analysis at 2,4,22, and 24 h after initiation of incubation.

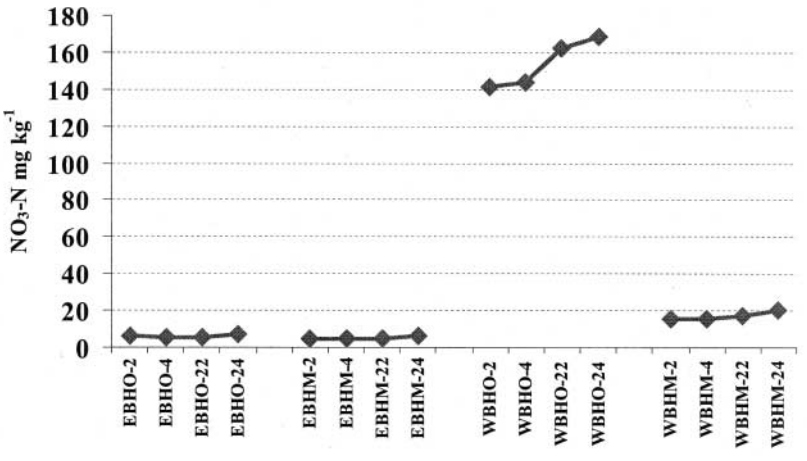

Figure 3. Softwood-soils nitrification potential over the 24-h sampling period. EB = the East Bear watershed; WB = the West Bear watershed; H = hardwood-forest type; O = O horizons; M = mineral soils. Subsamples removed for analysis at 2,4,22, and 24 h after initiation of incubation.

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reaffirmed by Shah.[19] Shah[19] attributed the higher rates in hardwoods from WB to a lower C/N ratio compared with softwoods, and a lower C/N ratio compared with EB hardwoods due to the treatments. The steep increase in nitrification rate to 24 h for WB O horizons suggests that nitrification would continue had the incubation period continued (Fig. 2). For the mineral soils (Fig. 3), WB had higher nitrification rates than EB, similar to the O horizon, but under softwoods it appears that the mineral soil has a greater capacity to nitrify under ideal conditions and the N treatments enhance this contrast among soil materials. Mineral soils under softwoods may possibly have slower rates of C turnover due to litter quality factors that slow rates of decomposition, leaving more mineral-soil C pools available for accelerated mineralization when exposed to N treatments.

CONCLUSIONS

Comparisons of 7-, 14-, and 28-day incubation periods for potential net N mineralization showed similar results in the O horizon for 14-day and 28-day incubation periods, although differences were greater in the mineral soils. Both 14-day and 28-day incubation periods resulted in increasingly higher mean nitrification rates beyond the 7-day incubation period. The relative differences in length of incubation appeared even more important for mineral vs. O horizon materials. The data suggest that the 7-day incubation period in these cool temperate forest soils could significantly underestimate potential net nitrification, particularly where mineral soils were included. While measurement programs limited to the O horizons might find either the 14- or 28-day incubation period satisfactory, it seems the 28-day incubation period would be most universally applicable across various soil materials for estimates of N dynamics if most temperate forest soils behaved similar to these findings.

All three methods used to assess net nitrification showed similar trends attributable to either forest cover or to the experimental N additions. Nitrification potential appeared to be the most discriminating in differentiating treatment, forest type, and soil effects and was particularly effective at eliciting differences in the mineral soils. Each of the three methods could be a suitable choice for determining qualitative differences in N dynamics, depending on conditions and objectives. The laboratory incubation for potential net N mineralization or nitrification is the easiest to perform since it requires a single site visit, is unconstrained by weather conditions once sampled, and is relatively

rapid and cost-effective. This method seems particularly well suited for studies of remote sites. The in situ method for net nitrification is the clear choice when the goal is to estimate actual rates of nitrification in the field since it incorporates the actual temperature variation of these sites. Finally, the less widely used nitrification potential may be more sensitive to incipient differences in soil N dynamics not revealed by the other methods studied, although further evaluation of its utility is warranted.

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