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Forest Ecology and Management xxx (2006) xxx–xxx
Forest Ecology and Management
www.elsevier.com/locate/foreco

One-day rate measurements for estimating net nitrification potential in humid forest soils

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Received 8 March 2006; received in revised form 12 April 2006; accepted 13 April 2006

Abstract

Measurements of net nitrification rates in forest soils have usually been performed by extended sample incubation (2–8 weeks), either in the field or in the lab. Because of disturbance effects, these measurements are only estimates of nitrification potential and shorter incubations may suffice. In three separate studies of northeastern USA forest soil surface horizons, we found that laboratory nitrification rates measured over 1 day related well to those measured over 4 weeks. Soil samples of Oa or A horizons were mixed by hand and the initial extraction of subsamples, using 2 mol L^{-1} KCl, occurred in the field as soon as feasible after sampling. Soils were kept near field temperature and subsampled again the following day in the laboratory. Rates measured by this method were about three times higher than the 4-week rates. Variability in measured rates was similar over either incubation period. Because NO_3^- concentrations were usually quite low in the field, average rates from 10 research watersheds could be estimated with only a single, 1-day extraction. Methodological studies showed that the concentration of NH_4^+ increased slowly during contact time with the KCl extractant and, thus, this contact time should be kept similar during the procedure. This method allows a large number of samples to be rapidly assessed.

Keywords: Forest soil; Nitrification; N mineralization; Ammonification; N transformation rate measurements

Net nitrification rate measurements in forest soils are extremely sensitive to sampling disturbance and, therefore, can only be considered potential rates (Hart et al., 1994; Ross and Hales, 2003). It is desirable, because of this, to choose the easiest procedure for measuring these potentials. Long-term field incubation of intact cores does not necessarily provide accurate estimates of rates because the soil is inevitably altered by sampling the core (e.g. cutting of roots and hyphae, and unavoidable physical disruption). Ollinger et al. (2002) demonstrated that there was little difference in 4-week rates between cores incubated in the field and the laboratory. Ross et al. (2004) found that 4-week rates measured in Oa and A horizons from laboratory-incubated intact cores were lower than, but closely related to ($R^2 = 0.88$), those measured in homogenized, composite (i.e. more disturbed) samples. Both studies were conducted in the relatively humid northeastern USA and the moisture content was not adjusted. It appears that, under these conditions, different sampling procedures and different incubation times provide rate measurements that are strongly correlated.

Ross et al. (2004) showed that repeated measurements in composite samples over the 4-week period often showed an initial rapid increase in NO_3^- and/or NH_4^+ concentrations. In samples with high 4-week net nitrification rates, there was always an initial peak in NH_4^+ followed by a rapid decrease, indicating that the rapid increase in nitrification was in response to the increase in NH_4^+ release (Ross and Hales, 2003). Thus in humid regions, the short-term (0–2 days) rate measurements may show similar results as the soil-slurry potential method described by Hart et al. (1994), except that no external source of NH_4^+ was added in our procedure. The purpose of this investigation was to compare rates measured over a short time

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doi:10.1016/j.foreco.2006.04.022

period (∼1 day) with the more traditional 4-week incubation. Additionally, we studied methodological issues arising from the need to perform initial extractions in the field.

Samples of Oa or A horizons were taken in three separate studies and slightly different methods were used. In Study 1, a number of different nitrification rate measurement techniques were performed on a small number of samples (2–4) from eight research sites, as detailed in Ross et al. (2004). Rate measurements were performed in triplicate and samples were incubated at 12 °C after return to the lab. In Study 2, a larger number of samples (12–38) were taken from each of four watersheds along pre-established transects or grids and rate measurements were performed only on mixed (or composite) samples taken by trowel from the sides of small pits and stored in polyethylene bags. Subsamples were extracted in duplicate in the field and then after 1 and 28 days of incubation at 10 °C. The watersheds sampled in Study 2 were Buck Creek in the southwestern Adirondacks of New York, Sleepers River W-9 in northeastern Vermont, a watershed just outside the Lye Brook Wilderness Area in southwestern Vermont and watershed W-7 at the Hubbard Brook Experimental Forest (HBEF) in central New Hampshire. Study 3 utilized replicated plots to examine temporal changes in rate measurements at two watersheds in northern Vermont (Fredriksen, 2005). Eight different 1 m^2 plots were sampled monthly from May to October in 2003 from an area just outside of Brush Brook watershed G in central Vermont and nine plots were sampled at Sleepers River W9-A over 5 months. The replicates were arranged in blocks and rates were determined in triplicate subsamples as described for Study 2 above. Statistical analyses were performed using SAS version 8.0 (SAS Institute, 2001).

The soils were typical of surface horizons from northern hardwood and mixed conifer forests (Table 1), although a number of relatively high pH samples were taken from Sleepers River. For the three studies, 1-day rates were between 2.7 and 3.0 × higher than 4-week rates (Fig. 1). The relationship found in the first study (Fig. 1a) was stronger than that found in the second study (Fig. 1b), probably a byproduct of differences in study design. In Study 1, sampling sites were selected with a bias towards well-drained soils representative of the watershed, and having a relatively thick Oa or A horizon. Rate measurements were done in triplicate with all sampling performed by one investigator. In Study 2, sites were selected by their position on transects, which included sites that were wet, extremely stony, or had thin surface horizons. A large number of samples were taken in 1 day (30–44) and extractions were performed in duplicate by multiple researchers. Thus, the soils of Study 2 were more diverse, especially in drainage class, and there was probably more experimental error. In the temporal study (performed by a single investigator), the relationship was also weaker than in Study 1, possibly a

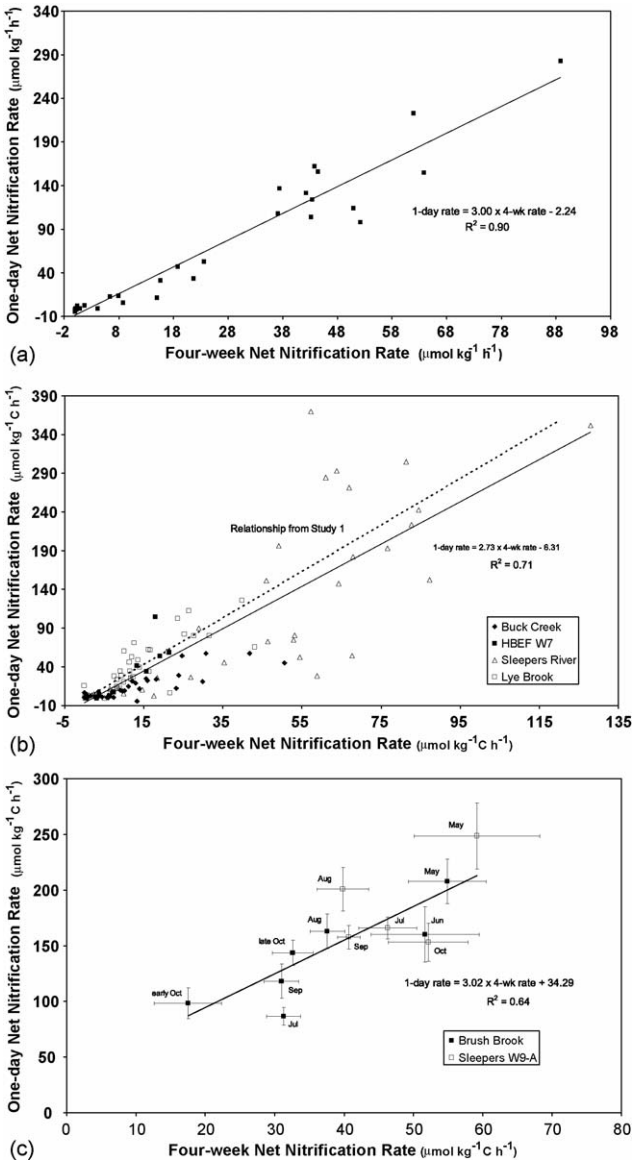

Fig. 1. One-day vs. 4-week net nitrification potential rate measurements from three separate studies. Study 1 (a) was performed at selected points in eight research sites in New York, Vermont and New Hampshire. Study 2 (b) represents a subset of samples taken along transects at four research sites. Study 3 (c) was a replicated temporal study comparing rates in two research sites in Vermont. Error bars represent the standard error.

Table 1
Characteristics of the Oa and A horizon samples taken from the three studies

	n	C (g kg^{-1})			C/N			pH		
		Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Study 1	30	322.3	81.0	512.8	19.4	12.2	34.1	3.77	3.37	4.54
Study 2	114	296.0	51.5	520.8	18.3	9.1	27.6	4.16	2.56	6.60
Study 3 Brush	54	175.7	83.8	289.5	13.6	11.1	18.9	4.35	3.65	4.93
Study 3 Sleepers	45	120.2	47.4	314.7	16.1	12.1	27.5	4.02	3.66	5.34

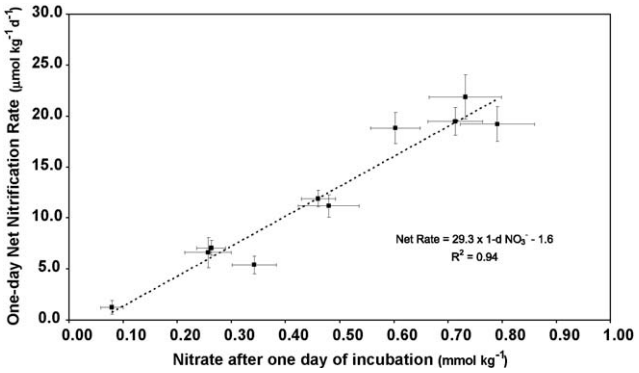

Fig. 2. One-day net nitrification potential rate measurements (calculated from the difference between NO_3^- concentrations in field and 1-day extractions) vs. the NO_3^- concentration after 1 day of incubation at 10 °C. Points represent means of 57–130 samples from 10 different research watersheds with error bars representing the standard error.

function of less of a range in values. However, seasonal variation in rate measurements was reflected in both incubation times (Fig. 1c). This study also showed little difference in variability between the incubation lengths. The standard error averaged 10.4% of the mean for the 1-day rate and 11.1% for the 4-week rate measurements. These three studies demonstrate that the shorter incubation time provides an index of potential net nitrification that is just as robust as that of more traditional, longer incubations.

Actual nitrification rates are presumably more similar to those measured in less disturbed intact cores but it is not possible to measure how similar. Many soils appear to be quite sensitive to sampling disturbance (Ross and Hales, 2003). The 4-week rates in the samples shown in Fig. 1a were about twice those of the 4-week rates in intact cores from the same sites but again strongly related ($R^2 = 0.88$) (Ross et al., 2004).

One might question if 1-day incubations are adequate to measure rates in soils with low net nitrification. In this and similar studies (Ross and Hales, 2003; Ross et al., 2004), we have not observed a time lag in the onset of net nitrification; soils with low initial net nitrification rates always had low 4-week net nitrification rates. Such a time lag has been observed in other forest ecosystems (e.g. Vitousek et al., 1982) and such a phenomenon would affect the relationships found in Fig. 1. However, the soils we studied had low rates both after 1 day and 4 weeks. In Study 2, the samples with 4-week rates <1 μmol kg^{-1} C h^{-1} ($n = 14$) averaged 10 times lower in net nitrification rates compared to their 1-day counterpart (0.3 versus 3.0 μmol kg^{-1} C h^{-1}). The higher rates in the shorter incubations help limit possible experimental error more common at low concentrations of NO_3^-.

There was not a significant relationship ($P > 0.05$) between 1-day and 4-week net ammonification rates but there was for the sum of ammonification and net nitrification (R^2 for the Studies 1–3 were 0.80, 0.60 and 0.85, respectively). In samples with high net nitrification, NH_4^+ accumulated over the first 1–2 days but dropped rapidly thereafter to below initial concentrations. In these cases, initial net ammonification rates were similar to initial net nitrification rates but the longer term nitrification was higher, consuming the transient NH_4^+.

It is important to start the initial extraction shortly after sampling if accurate individual rates are desired because sampling may cause rapid increases in net nitrification. However, extracting samples in the field is not always considered convenient. If watershed-wide rates are of interest, rather than individual samples, it is possible to omit the field extraction and still base the rates on a 1-day measurement (Fig. 2). Initial concentrations of NO_3^- in the field were generally quite low and the sampling-induced stimulation of net nitrification caused relatively high concentrations after 1 day, making the average rate calculations similar whether or not the initial field-value was subtracted. Caution should be applied when using this approach because low field concentrations of NO_3^- may not be universal. Again, it is only applicable when the goal is to obtain an average rate measurement for a large sample size.

Filtering in the field has not been found to be practical and thus the time of contact between the KCl extractant and the soil may be several hours. We have found that extended contact time does not change the concentration of NO_3^- but can cause an increase in NH_4^+ (Table 2). This study was performed on an A horizon (C 175 g kg^{-1}, C/N 11.8) taken just outside of watershed G at Brush Brook in an area mapped as Typic Haplorthods. The extraction procedure given below was

Table 2
Change in extractable NO_3^- and NH_4^+ in an Oa horizon approximately 2 h and 1 day after KCl addition

Time of KCl addition (hours after sampling)	Time of filtering[a] (hours after KCl addition)	Solution:soil ratio (L:kg)	Temperature after KCl addition	NO_3^- (μmol kg^{-1})	NH_4^+ (μmol kg^{-1})
0	0	10:1		70 a[b]	435 d
0	2.5	10:1	On ice	67 a	550 d
0	2.2	10:1	Warm	60 a	807 c
0	3.1	37:1	On ice	127 b	595 d
0	27	10:1	On ice	80 a	923 bc
0	27	10:1	Warm	57 a	2229 a
28	0	10:1		1296	514

Samples were incubated at two different temperatures with the warm treatment reflecting ambient conditions (high of 27 °C). All samples had KCl added in the field except for the last treatment in which the bulk soil was incubated 28 h at 12 °C before KCl addition.

[a] Initial sample was filtered in the field. All others were centrifuged in the lab followed by filtering.

[b] Values followed by the same letter within a soil and column were not significantly different using the Student–Newman–Kuels test. The sample that had KCl added after 28 h was not included in the statistical analysis.

followed except that one treatment was filtered (same paper as below) in the field after 15 m of intermittent shaking with KCl. About 5 mL of filtrate was obtained and immediately put on ice. Sample slurries (KCl + soil) were kept on ice throughout their incubation period except for a ''warm'' treatment in which the samples were incubated at ambient temperature (high of 27 °C). The accumulation of NH_4^+ was significant after 1 day on ice and sooner under warm incubation conditions (Table 2). We also tested the time until filtration on five Oa samples from HBEF W-7 (average C 340 g kg^{-1}, C/N 18.3, pH 3.7), centrifuging subsamples after ~5.5 or 22.5 h of KCl contact time on ice. Significant differences in NH_4^+ and NO_3^- were not found with the mean solution concentration of NH_4^+ varying from 34.8 to 39.4 μmol L^{-1} and of NO_3^- from 11.5 to 12.1 μmol L^{-1}. Overall, the increase in NH_4^+ with KCl contact time was relatively slow and probably not a concern if contact times during the 0- and 1-day extractions are kept similar. However, anecdotal evidence suggests that NH_4^+ concentrations in filtered KCl extracts can increase after either prolonged refrigerated or frozen storage. More work is needed in this area.

A solution:soil ratio of 10:1 is recommended for extraction of NH_4^+ from soils (Mulvaney, 1996). If organic rich horizons are sampled, 25 mL of KCl and 10 mL of soil should result in a ratio of about this magnitude. In the watershed studies (Fig. 2), the average solution:soil ratio across sites was 11.4:1. However, there was a wide range from 3.0:1 to 30.4:1. The low ratios in low organic soils may not be adequate to extract all the exchangeable NH_4^+. Conversely, because the CEC of these soils is a function of organic carbon content, the low solution:soil ratios may be sufficient. The high ratios foster greater error if the N concentrations are low. In the KCl contact-time study (Table 2), increasing the ratio from 10:1 to 37:1 did not result in significantly more NH_4^+ being extracted (the increase in NO_3^- may be due to greater measurement error because the concentration was approaching the detection limit of our analyzer). It would be advisable for users of this method to determine the optimum solution:soil ratio for their particular soils, again a balance between efficient extraction of NH_4^+ and the detection limits for NO_3^-.

Moisture content was not adjusted during the 4-week incubations because samples were stored in sealed, polyethylene bags at 10 °C. Rewetting air-dried soil has been shown to cause rapid increases in net nitrification (e.g. Haney et al., 2004). Our study sites may experience seasonal dry periods but soils do not generally become desiccated. Because we have not tested our method on soils that have experienced such conditions, we can only recommend it for forest soils in humid northeastern regions.

In summary, potential net nitrification rates can be measured on humid forest soil samples with only a 24-h incubation, if sampled and mixed (i.e. not intact cores). While 1-day rates are higher than 4-week incubations, they are linearly correlated. The advantages of this method include the ease and rapidity of the procedure coupled with increased sensitivity at sites with low nitrification potential. It is important to keep in mind that all rate measurements should be considered potential rather than actual rates. The 1-day procedure is presented below in a format that should be easily adaptable for usage:

1. Obtain about 500 mL of the desired horizon and mix gently by hand on a clean plastic sheet, discarding coarse roots and stones.
2. Using a calibrated spoon and spatula, put 10 mL of firmly packed soil into each of two or three 50-mL centrifuge tubes, add 25 mL of 2 mol L^{-1} KCl, record the time, and shake intermittently for 15 min. Place the tubes in an ice-filled cooler for transport back to the laboratory. To obtain a higher solution:soil ratio (recommended for B and C horizons low in organic matter), either decrease the sample size to 5 mL or increase the volume of the added KCl.
3. Put the remaining soil in a polyethylene bag and store in a cooler with ice packs until returning to the lab for incubation at a standard temperature (e.g. 10 °C).
4. Upon return to the lab, centrifuge the samples and pass through N-free filter paper to remove floating debris. We used a fast, qualitative paper (Schleicher & Schuell SA710, Keene NH).
5. Extract fresh subsamples of the incubated soil the following day, recording the time. To avoid possible artifact in the amount of NH_4^+ extracted before centrifuging, store the samples on ice for approximately the same number of hours that the initial extracts were stored.
6. Determine the NO_3^- and NH_4^+ concentrations in the extracts as soon as feasible after extraction (or freeze until just prior to analysis). We used the Cd-reduction method for NO_3^- and the salicylate–nitroprusside method for NH_4^+ on a Lachat QuikChem AE autoanalyzer (Hach Instruments, Loveland, CO).
7. Determine the dry weight and moisture content of the soil on additional 10-mL subsamples. We air dried the samples for at least 1 week and then further dried at 80 °C for 2 h in a forced-air oven before recording final weights.
8. Calculate the soil concentration of NO_3^- and NH_4^+ by using the solution:soil ratio of:

$$25\,\text{mL} + (\text{mL of } H_2O \text{ in } 10\,\text{mL of soil})/\text{dry weight of soil}$$

Calculate the rate by the difference between the concentrations at each time divided by the difference in time.

Acknowledgments

We thank Sandy Wilmot and the Vermont Dept. of Forest, Parks, and Recreation for permission to perform the research within Camels Hump State Park; and Nancy Burt and the Green Mountain and Finger Lakes National Forests for supporting the research at Lye Brook. The Hubbard Brook Experimental Forest is operated and maintained by the Northeastern Research Station, U.S. Department of Agriculture, Newtown Square, PA. This study was partially funded by award 99-35101-8235 from the USDA NRICGP and partially funded by the Northeastern States Research Cooperative (USDA award 02CA11242343110).

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