

Sampling-Induced Increases in Net Nitrification in the Brush Brook (Vermont) Watershed

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ABSTRACT

Accurate measurement of nitrification rates in disturbance-sensitive soils can be problematic. A series of experiments illustrated the extent of net nitrification increases and explored possible causes. In highly disturbed soils, weekly leachates of columns containing homogenized Oi/Oe material had NO_3^- concentrations over 5 mmol L^{-1} after 20 wk and over 1 mmol L^{-1} after 2 yr. Small-scale impact disturbance in the field (footprints) resulted in a tripling of NO_3^- concentrations after a 2-wk incubation. After disturbance by sampling, bulked Oa horizons showed rapid increases in NO_3^- concentration, with change detectable within 1 h. Initial net nitrification rates were as high as $74 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ ($300 \text{ mg N m}^{-2} \text{ d}^{-1}$). Ammonium concentrations increased over the first 10 h but decreased afterwards. Incubation with acetylene stopped nitrate accumulation but NH_4^+ continued to increase. Intact cores also showed increases in NO_3^- after 1 to 3 d but at a much lower rate than bulked samples. Gross rates of nitrification in intact cores were much lower than net rates in bulk samples. The addition of NH_4^+ to intact cores caused an increase in net nitrate after 20 h. These results suggest that sampling stimulates nitrification rapidly through an increase in NH_4^+ availability. The NH_4^+ increase may be because of a stimulation of ammonification caused by mixing, a decrease in spatial heterogeneity, or a disturbance in other types of NH_4^+ consumption. In soils that exhibit these disturbance effects, nitrification rate measurements will not be accurate but still may represent the potential for nitrification with increased NH_4^+ supply.

FOREST SOIL DISTURBANCE has been found to increase net nitrification creating artifact in field plot studies (Miller et al., 1992), soil column studies (Johnson et al., 1995; Nelson and Robarge, 1996), root dynamics studies (Joslin and Wolfe, 1999), and soil sampling (Ross and Bartlett, 1990; van Miegroet, 1995). Disturbance-induced increases in stream NO_3^- export have been demonstrated in watershed studies (e.g., Likens et al., 1969; Vitousek et al., 1979) and after episodes of severe freezing (Mitchell et al., 1996). Observation of this phenomenon is not new. In a treatise on the humus layer of northeastern U.S. forest soils, Romell (1935, p. 6) observed: "Even very heavy and pronounced northeast-American forms of mor (belonging to or approaching the greasy type) react with an ease surprising to a European observer. They seem to be activated by the slightest disturbances. The reason for their greater reactivity has not been explained." This "activation" refers to the onset of net nitrification and eventual conversion of a "bothersome" mor to a more mull-like condition. A complete explanation for the cause of the disturbance effect is still lacking.

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Nitrification in forest soils is of special interest in ecosystems that may be impacted by increased N deposition. Changes in nitrification rates could have a profound effect on NO_3^- leaching. Thus, the potential magnitude and rapidity of NO_3^- release after experimental or sampling disturbance present problems for monitoring and research efforts aimed at measuring ecosystem N fluxes. For example, Johnson et al. (1995) found extremely high soil solution NO_3^- concentrations (peaking at $\approx 5 \text{ mmol L}^{-1}$) in a controlled-environment pot study using the Ap horizon of an Aiken clay loam (Xeric Haplohumult). Miller et al. (1992) found O horizon solution concentrations as high as 1.8 mmol L^{-1} in a trenched plot experiment on a Typic Cryohumud. These elevated concentrations would almost certainly mask the effect of any reasonable treatment.

Dramatic changes in soil NO_3^- concentrations take place shortly after sampling, even when soils are stored near freezing. For example, we found that the NO_3^- concentrations in soil solution from two Oa-horizon samples nearly doubled after 24 h of storage at 3°C (Ross and Bartlett, 1990). Van Miegroet (1995) studied changes after sampling the top 10 cm of mineral soil taken in the Great Smoky Mountains National Park. She found as much as a seven-fold increase in NO_3^- concentrations between extracting in the field immediately after sampling and extracting in the laboratory after <24 h of storage on ice. This rapid increase obviously dictates field extraction if in situ soil NO_3^- concentrations are required.

The mechanism for the rapid increase in NO_3^- after sampling is not clear. An increase in net nitrification could result from either a decrease in NO_3^- consumption (e.g., microbial assimilation, plant uptake, abiotic fixation, denitrification), an increase in the gross rate of nitrification, or a combination of both. The gross rate of nitrification may be controlled by the availability of NH_4^+ or by the presence of inhibitory factors. Davidson and Hackler (1994) and Korsaeht et al. (2001) hypothesized that in situ spatial heterogeneity of available NH_4^+ limits the ammonia oxidizers even when bulk NH_4^+ concentrations are relatively high. The chemolithoautotrophic ammonia oxidizers use inorganic C, while the heterotrophic decomposers use organic C. These communities may be spatially separated. Tight cycling among the heterotrophs and higher plants would limit the NH_4^+ available to the autotrophs. Disturbance may stimulate nitrification by decreasing this spatial heterogeneity. It may also stimulate NH_4^+ production (and resulting nitrification) simply through mixing and providing the heterotrophs with more accessible substrate. Numerous responses to disturbance are possible (Vi-

tousek et al., 1982) and it is likely that no single mechanism explains the rapid increase in net nitrification.

It is important to understand both the extent and the magnitude of disturbance-induced net nitrification. Are field studies inherently limited on sites with soils displaying this phenomenon? This study examined a range in disturbance, from the homogenization that occurs when samples are bulked to simple heavy footprints. Different common approaches for measuring nitrification rates were compared—for example, incubation of intact cores vs. bulk samples. Intact core sampling should create less disturbance, although both methods obviously cause cessation of plant uptake. Experiments were also designed to investigate the effect of spatial heterogeneity by adding NH_4^+ to cores already containing substantial concentrations of extractable NH_4^+ . Gross rates of both NO_3^- and NH_4^+ production were measured to compare with net rates. Additionally, autotrophic ammonia oxidation was inhibited to reveal the pattern of net NH_4^+ production in the absence of nitrification. The experiments document the rapidity of disturbance-induced net nitrification and attempt to elucidate a mechanism.

MATERIALS AND METHODS

All experiments were performed in Camels Hump State Forest in Huntington and Duxbury, VT (44°19'N lat., 72° 53'W long.). Previous work in this area showed rapid increase in soil NO_3^- after sampling (Ross and Bartlett, 1990). Except for the column study, all soils were mapped as Houghtonville sandy loams (Coarse-loamy, isotic, frigid Typic Haplorthods) or variants with deeper lithic contact. Vegetation was mixed northern hardwoods (primarily *Betula alleghaniensis* and *Acer saccharum*) with a contribution of *Picea rubens* and *Abies balsamea*, and pockets of *Tsuga canadensis*. More details on the research watershed can be found in Ross et al. (1994). For convenience, experiments are numbered and site conditions summarized in Table 1.

Reconstructed Column Study (Exp. 1)

This study illustrated the effect of a high amount of sample disturbance. A large bulk sample of mixed Oi

and Oe horizon was collected from a Saddleback sandy loam (Loamy, isotic Lithic Humicryods). After about 1 mo of storage at 4°C, the sample was macerated briefly in a food processor, thoroughly mixed, and packed into columns 30 cm high with a 6.4-cm diam. Columns were stored vertically at 10°C and treated weekly with 80 mL of either 1×10^{-4} mol L^{-1} H^+ as HNO_3 and H_2SO_4 (equimolar NO_3^- and SO_4^{2-}) or $\text{Ca}(\text{NO}_3)_2$ and CaSO_4 at the same anion concentration. Leachate was collected the same day and analyzed for NO_3^- by ion chromatography using a Dionex 2000i series instrument (Dionex Corp., Sunnyvale, CA) and NH_4^+ by the indophenol-blue method (Dorich and Nelson, 1983). Four cores of each treatment were leached for 102 wk. Soil pH was determined using 0.01 M CaCl_2 (2:1 v/v). Carbon was estimated from weight loss-on-ignition at 450°C using the conversion equation of David (1988). Nitrogen was measured by standard Kjeldahl digestion and distillation. The experiment was designed to observe differences between treatments, but leachate concentrations of all measured constituents were not significantly different and the results presented are averages of eight different cores.

Small-Scale Impact (Footstep) Study (Exp. 2)

This study was designed to determine if a relatively minor disturbance in the field would have an effect on soil inorganic N status. The experiment was initiated by establishing a series of ten alternating footprints approximately 70 cm apart ("giant steps"). Each print was made by two impacts of sufficient force to leave a 0.5- to 1.0-cm heel impression. A flag was inserted just off the front inside corner of the heel. After 2 wk, an identical set of footprints 25 cm to the left or right of the original prints were made so that 10 pairs of prints were established over a distance of about 6.5 m. A 4 by 4 cm square, 2 to 4 cm thick, sample of the A horizon from under the heel area was taken from each new print and the associated old print within 2 min. of the second treatment. After mixing, duplicate 5-mL subsamples were added to 50-mL centrifuges tube containing 20 mL of 1 M KCl. An additional 5-mL subsample was saved to obtain composite samples of each treatment. Samples

Table 1. Date and site characteristics for all experiments.

Experiment	Sampling date	Elevation	Soil Temperature	Thickness of Oa or A horizon	Dominant canopy
		m	°C	cm	
1	Column soil Oi/Oe	820	nd†	nd	<i>Betula alleghaniensis</i> , <i>Abies balsamea</i>
2	Footstep study	790	nd	3	<i>Acer saccharum</i> , <i>Betula alleghaniensis</i>
3a	Timed extractions	~650	12	11	<i>Tsuga canadensis</i> , <i>Picea rubens</i>
3b	Timed extractions	700	14	5	<i>Betula alleghaniensis</i> , <i>Acer saccharum</i>
3c	Timed extractions	700	5	5	<i>Betula alleghaniensis</i> , <i>Acer saccharum</i>
4	Core vs. bulk study	~680	14	6	<i>Acer saccharum</i> , <i>Betula alleghaniensis</i>
5a	NH_4^+ addition	660	10	6	<i>Betula alleghaniensis</i> , <i>Picea rubens</i>
5b	NH_4^+ addition	670	nd	4	<i>Acer saccharum</i> , <i>Betula alleghaniensis</i>
5c	NH_4^+ addition	670	15	4	<i>Betula alleghaniensis</i> , <i>Picea rubens</i>
6a	Gross rates	same as Exp. 3b			
6b	Gross rates	700	15	5	<i>Betula alleghaniensis</i> , <i>Acer saccharum</i>
6c	Gross rates	same as Exp. 3c			
6d	Gross rates	700	7	5	<i>Betula alleghaniensis</i> , <i>Acer saccharum</i>
6e	Gross rates	670	11	5	<i>Acer saccharum</i> , <i>Betula alleghaniensis</i>

† Not determined.

were returned to the lab and centrifuged. Concentrations of NO_3^- and NH_4^+ in this and all subsequent experiments were determined using the Cd-reduction method and the salicylate-nitroprusside method, respectively, on an automated flow-injection analyzer (Lachat Quik-Chem AE, Milwaukee, WI). The dry weights of the soil samples were determined by rinsing the extracted samples with distilled water to remove excess KCl and drying at 55°C. In this and all subsequent experiments we measured pH in H_2O (2:1 v/v) and %C and %N by total elemental analyzer.

Short-Term Net Nitrification (Exp. 3)

These experiments were designed to determine the rapidity of the disturbance response and observe changes in the hours following sampling. Oa horizon bulk samples were taken from the side of small pits and homogenized by hand. Volumetric subsamples were put into a 50-mL centrifuge tube with 25 mL of KCl solution. Experiment 3a used duplicate 5-mL subsamples and 1 M KCl, and Exp. 3b and 3c used triplicate 10-mL subsamples and 2 M KCl. In Exp. 3a, soil solution was also extracted with the syringe-pressure method (Ross and Bartlett, 1990) and NO_3^- determined by ion chromatography. After the initial sampling, soils and centrifuge tubes were placed in a cooler until the return to the lab (2–3 h later). Samples from Exp. 3a were stored at 4°C and those from Exp. 3b and 3c were stored at 12°C. Additional subsamples were taken and extracted over a 10-h period, and after 1 and 2 d. For the acetylene treatment in Exp. 3c, 10-mL subsamples were incubated in 100-mL gas-tight glass bottles fitted with septa. The treatment was prepared in the field within about 30 min of sampling by adding 1 mL of acetylene with a syringe. At each extraction time, 25 mL of KCl was added directly to each of three bottles and the samples shaken. Dry weights and moisture content were measured on separate volumetric subsamples.

Intact Core vs. Bulk Sample Study (Exp. 4)

This experiment examined the difference in the disturbance effect between two sizes of intact cores and bulk samples. Cores were inserted flush with the forest floor by carefully cutting around the outside edge with a sharp knife as downward pressure was applied. Cores were either open-ended metal cans (11.5 cm high with an 8.5-cm diam.) or 125-mL polyethylene bottles with the bottoms removed (6 cm high with a 4.8-cm diam.). Six blocks containing two each of the different cores were established. Within each block, the distance between core centers averaged 11 cm. Treatments, intact or disturbed, were assigned randomly for each pair of similar cores. The intact cores were lifted from the soil, put in a polyethylene bag, and returned to their original position. The disturbed cores were taken apart and the Oa horizon was separated and thoroughly mixed by hand. Duplicate subsamples (10 mL for the larger and 5 mL for the smaller cores) were extracted with 25 mL of 1 M KCl for NO_3^- and NH_4^+ analysis as described above. The remaining Oa sample was put in a polyethyl-

ene bag, replaced in the original sampling hole and covered with the Oi/Oe layer from that core. After 3 d, all remaining intact cores were taken apart and all treatments were extracted in the field as described above. Dry weights of 5 or 10 mL of soil and soil moisture content were determined on separate subsamples.

Ammonium Addition Studies (Exp. 5)

In these studies, NH_4^+ was added to intact cores to determine if net nitrification could be increased. The hypothesis was that the native NH_4^+ was not as available to the ammonia oxidizers, probably because of heterogeneity. Intact cores were sampled in open-ended metal cans (11 cm high with an 8.5-cm diam.) as described above. Each of five blocks contained four cans with treatments randomly assigned. Two cans were treated with 10 mL of $(\text{NH}_4)_2\text{SO}_4$ solution (2.0 mmol L^{-1} in Exp. 5a and 2.5 mmol L^{-1} in the final two) and two with 10 mL of distilled H_2O . The amount of added NH_4^+ was intended to increase the soil NH_4^+ concentration by about 0.3 mmol kg^{-1} . The solutions were added using 10-cm needles on a 5-mL syringe. A series of 8 to 10 repeated injections were made between 4- and 8-cm depth in a pattern around the volume of the core. Within 20 min of treatment, one core of each type of solution was taken apart and the Oa horizon separated and mixed by hand. Triplicate 10-mL samples were extracted in the field with 1 M KCl for analysis of NH_4^+ and NO_3^- as described above. All remaining Oa samples and remaining intact cores were put into polyethylene bags, placed in coolers with ice-packs and transported to a cold laboratory room (12–13°C). The intact cores were taken apart after about 20 h and all treatments were extracted as described above.

Gross Rate Measurements (Exp. 6)

Gross rates of ammonification and nitrification were measured using the isotope dilution method (Hart et al., 1994) to determine if there was high internal cycling. Blocks of four intact cores (11 by 8.5 cm) were carefully cut into the forest floor and two each were treated with 5 μmol of ^{15}N as either $(\text{NH}_4)_2\text{SO}_4$ or KNO_3 . Four blocks were used in Exp. 6a and 6b and five blocks used in the others. The solution was added evenly between a depth of 4 and 8 cm. One set of cores was dismantled and extracted in the field and the second set extracted after about 24 h of incubation at 12°C in the laboratory. The Oa horizon from the depth-treated was homogenized and 120 mL extracted with 240 mL of 2 M KCl. The extracted inorganic N was prepared for isotopic analysis using the Mason-jar diffusion method of Khan et al. (1998). Each sample was spiked with 100 μg of N as $(\text{NH}_4)_2\text{SO}_4$ to provide sufficient N for analysis. Concentrations of inorganic N were measured as described above and gross rates were derived using the calculations and assumptions described in Hart et al. (1994). Net rates in disturbed samples were measured in composite bulk samples of the Oa or A horizon taken from the immediate area of the intact cores. Repeated extrac-

tions for NO_3^- and NH_4^+ were performed as described above for Exp. 3b.

Statistical Analysis

Statistical analysis was performed using the general linear models procedure of the SAS Institute (1994). A complete random block design was used to test for treatment differences using the average of the replicated measurements. All data was log transformed to create normal distributions. Because the footstep treatment in Exp. 2 was not randomized, a separate ANOVA was performed to test the effect of order (left-right vs. right-left), and no effect was found. Experiments 4 and 5 had four cores per block but only two 0-time measurements (the disturbed treatment). The ANOVA was performed only on the second set of complete measurements. Experiments 5a through 5c were combined into one ANOVA by treating each as a replicate. Pairwise contrasts were used to examine the effect of added N in each soil treatment (intact cores or disturbed).

RESULTS AND DISCUSSION

Reconstructed Column Study (Exp. 1)

The disturbed-column leaching study showed an enormous conversion of organic N to NH_4^+ and NO_3^- (Fig. 1). Weekly leaching of Oi/Oe horizon samples held at 10°C resulted in peak NO_3^- concentrations of $\approx 6 \text{ mmol L}^{-1}$ and NH_4^+ of $\approx 4.5 \text{ mmol L}^{-1}$ within the first 20 wk. Nitrate concentrations were still $> 2 \text{ mmol L}^{-1}$ after 75 wk of leaching. Nitrogen loss was calculated both by summing the export of inorganic N (16.4% loss) and by measuring differences in total N before and after treatment (22.7% loss). Overall, the Oi/Oe columns lost 11.8% of their original weight. Treatment effects (simulated acid vs. nonacid rain) were masked by both the high ionic strength and the acidification from nitrification (pH declined by 0.7 units). Other researchers have found similar problems with reconstructed soils (Johnson et al., 1995; Neslon and Robarge, 1996). In these cases, disturbance is high and the effects are dramatic.

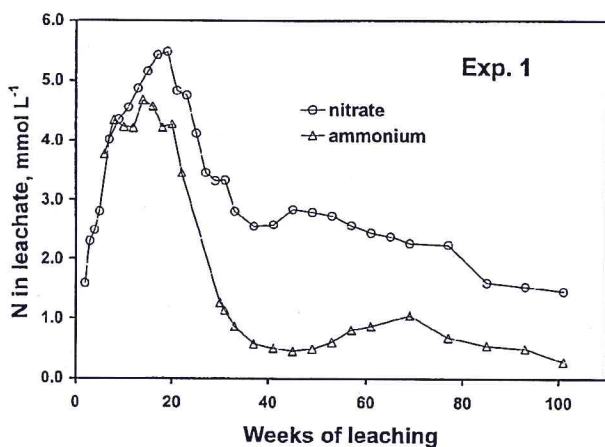


Fig. 1. Inorganic N in weekly leachates from a laboratory study of highly disturbed soils (Exp. 1). Soil columns contained a homogenized mixture of Oi and Oe horizon material.

No conclusions can be drawn from such experiments except that the columns do not mimic field conditions. This study demonstrated an extreme in disturbance probably now avoided by most researchers.

Small-Scale Impact (Footstep) Study (Exp. 2)

In the "footstep" experiment, the 2-wk old "stepped-on" A horizon contained $0.84 \text{ mmol kg}^{-1} \text{ NO}_3^-$, almost three times the concentration in the freshly stepped-on samples ($0.27 \text{ mmol kg}^{-1}$). In all associated pairs of footsteps, the NO_3^- concentration was greater in the 2-wk treatment and the difference was significant at $P = 0.003$. No block or left vs. right effect was observed ($P > 0.15$). There was no significant difference in NH_4^+ concentration (0.37 and $0.59 \text{ mmol kg}^{-1}$ in the 0-wk and 2-wk treatments respectively, $P = 0.20$). Subsamples for extraction were taken by volume and no treatment differences were found in the mass of soil extracted. Differences between the composite treatment samples in C, N, and pH were 1.5, 4.8 and 0.0% respectively (overall averages are given in Table 2). Because each treatment could only be sampled once, it was not possible to determine the rapidity or persistence of the NO_3^- increase. This simple experiment did demonstrate that a relatively minor disturbance, such as heavy footprints, could cause measurable change in soil NO_3^- status.

Short-Term Net Nitrification (Exp. 3)

In measuring short-term rates of net ammonification and nitrification after disturbance, the initial increase in both NO_3^- and NH_4^+ was rapid and nearly linear over the first 10 h (Fig. 2-4). Because of the time needed for sampling and mixing, the first extraction occurred approximately 20 min after initial disturbance. Extrapolation back to Time 0 gives a NO_3^- concentration of approximately 0.18, 0.005, and $0.12 \text{ mmol kg}^{-1}$ for the three soils. The extremely low in situ concentration of NO_3^- in Exp. 3b was in sharp contrast to that found after 10 h of disturbance (higher in all three experiments by factors ranging from 3.4 to 159). Two methods, soil solution extraction or KCl addition to subsamples, were used to extract soil NO_3^- in the first experiment (Exp.

Table 2. Carbon, N, and pH of the soils used in the different experiments.

Exp. No.	Experiment	C		C/N ratio	pH
		%			
1	Column study Oi/Oe	51.3†	2.42	21.2	3.5‡
2	Footstep study	13.4	1.04	13.0	4.6
3a	Short-term nitrification	35.8	1.80	19.9	3.45
3b	Short-term nitrification	29.8	1.48	20.2	3.75
3c	Short-term nitrification	18.4	1.13	16.3	3.66
5a	NH_4^+ addition	33.7	2.09	16.1	4.04
5b	NH_4^+ addition	28.2	1.72	16.4	4.11
5c	NH_4^+ addition	23.3	1.58	14.7	3.88
6a	Gross rates	34.3	1.65	20.7	3.74
6b	Gross rates	19.7	1.29	16.3	3.62
6c	Gross rates	24.5	1.45	16.9	3.66
6d	Gross rates	12.3	0.96	12.7	4.11
6e	Gross rates	17.7	1.22	14.5	3.72

† Calculated from organic matter (Exp. 1 only).

‡ pH in 0.01 M CaCl_2 —All other pH readings are in water.

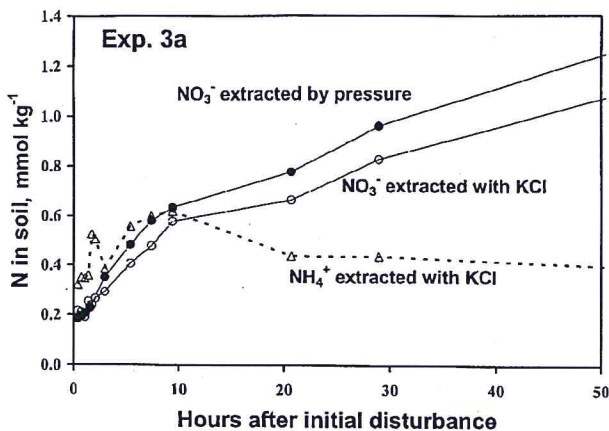


Fig. 2. Repeated measurements of inorganic N in a bulked Oa sample (Exp. 3a), beginning shortly after sampling. Separate subsamples were extracted over time by two different methods.

3a; Fig. 2), and the agreement between the two suggests that the increases found were not an artifact of the methodology. With the addition of acetylene, NO_3^- concentrations remained low and NH_4^+ continued to increase after 10 h (Fig. 4). While it needs to be kept in mind that these are only net changes, the temporal changes seen in these experiments suggest that the disturbance effect begins with an initial rapid increase in NH_4^+ , followed shortly by an increase in nitrification. Within the first 24 h, the concentration of soil NO_3^- became higher than that of NH_4^+ . In Exp. 3c, the sum of NO_3^- and NH_4^+ with or without acetylene was similar, both treatments showing decreased rates of net accumulation after 24 h. This suggests that the continued rapid rate of disturbance-induced nitrification is limited by the rate of NH_4^+ supply.

Intact Core vs. Bulk Sample Study (Exp. 4)

After relatively short field incubation (3 d), significantly higher NO_3^- ($P < 0.05$) was found in disturbed bulk samples compared with intact cores (Fig. 5). No differences in NH_4^+ were found. In contrast to a dis-

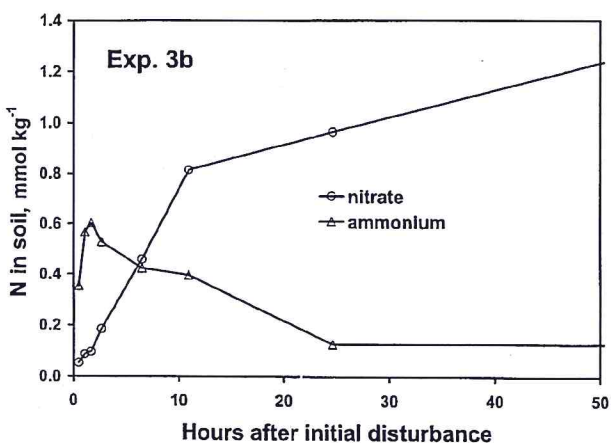


Fig. 3. Repeated measurements of inorganic N in a bulked Oa sample (Exp. 3b). Separate subsamples were extracted over time with 2 M KCl.

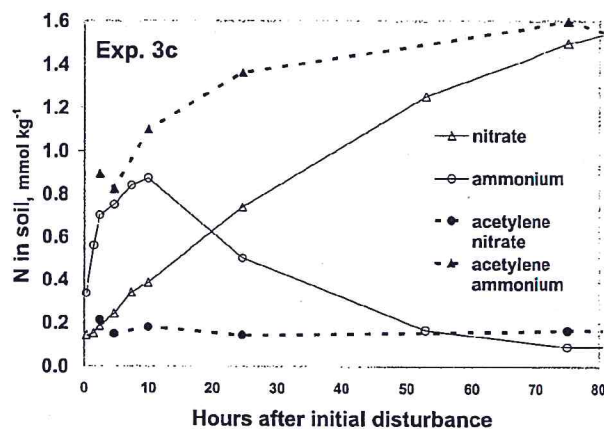


Fig. 4. Repeated measurements of inorganic N in a bulked Oa sample (Exp. 3c). Separate subsamples were extracted over time with 2 M KCl. An additional set of subsamples was incubated in a 1% acetylene atmosphere before extraction.

turbed bulk sample, a carefully sampled intact core should preserve the soil's structure, except near the edges. Plant uptake of N is stopped but mixing is minimized. Roots are killed during the coring process, most likely altering the activity of the soil microbiota. Two sizes of cores were used to examine possible edge effects (the ratio of interior surface area/edge length was 2.4 and 4.25 for the small and large cores respectively) but no significant differences were detected between the 4.8- and 8.5-cm diam. cores (Fig. 5). Although it is clear that net nitrification was higher in the bulk samples because of disturbance, it is not possible to ascertain if the increase in NO_3^- in the intact cores was sampling related.

Ammonium Addition Studies (Exp. 5)

In three separate experiments (5a, 5b, 5c), added NH_4^+ caused a significant NO_3^- increase in the intact cores ($P < 0.05$) but not in the disturbed samples (although a trend is evident, Fig. 6). This increase occurred

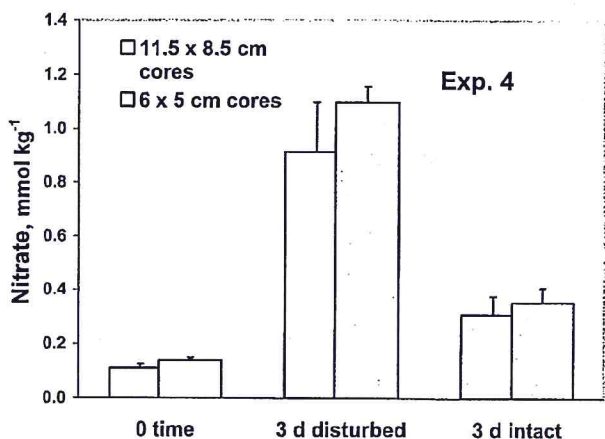


Fig. 5. Nitrate in the Oa horizon from two sizes of cores (Exp. 4). The disturbed treatment consisted of bulked Oa samples from cores dismantled just after sampling. Error bars represent the standard error of each treatment across blocks. The difference between 3 d disturbed and intact cores was significant ($P < 0.05$).

even though there was extractable NH_4^+ at Time 0 without added N (Table 3). The increase in 0-time extractable NH_4^+ because of N treatment was 0.3, 0.7, and 0.4 mmol kg^{-1} (0.3 was the target). The trends in net NH_4^+ change were variable, but in all cases, there was less extractable NH_4^+ in the N-treated samples after 20 h. This could be the result of nitrification, immobilization and other consumption of the added NH_4^+ . The increase in net nitrification because of sample disturbance was again shown in these experiments ($P < 0.05$). Because the added NH_4^+ stimulated net nitrification, it appears that it was more available to the ammonia oxidizers than the native NH_4^+ . This is based on the supposition that the 0-time concentration of NH_4^+ (Table 3) was adequate if accessible. There could be spatial separation of the native NH_4^+ from the ammonia oxidizers, causing the KCl extraction to provide a poor estimate of availability. This has important implications for uses of the isotope dilution method, which assumes that added NH_4^+ is similarly available to ammonia oxidizers as native NH_4^+ .

Gross Rates (Exp. 6)

Because of spatial variability, gross rate measurements in intact cores inherently have more uncertainty than repeated measurements on a bulk, homogenized sample. Notwithstanding, our results suggest that the high rates of net nitrification in disturbed samples are not a result of high undisturbed gross rates masked by high NO_3^- consumption (Table 4). If this were the case, gross nitrification rates in intact cores should be as high as or higher than the net nitrification rates in the disturbed samples. However, intact-core gross nitrification was much lower than net in disturbed samples taken from around the cores (even though it was greater than intact-core net). Thus, overall rates of nitrification must have increased following disturbance. Although Table 4 only presents gross nitrification results from two sites, similar results were found in samples from four other research sites in Vermont and New York that showed the disturbance effect (D.S. Ross, Univ. of Vermont, unpublished data, 2000). On the other hand, gross rates of NH_4^+ production and consumption were relatively high while net intact rates were quite low, often negative. Thus, it is possible that the disturbance effect is because of an increased NH_4^+ pool through an interruption of consumption, not a stimulation of ammonification. It is interesting that we found relatively low gross rates of nitrification in soil samples showing high net nitrification upon disturbance, while Stark and Hart (1997) found high gross rates in soils showing low net rates. The initial short-term net nitrification rates shown in Fig. 2 through 4 are about $300 \text{ mg N m}^{-2} \text{ d}^{-1}$ (calculated assuming the depth of the Oa in Table 1 and the bulk density of the disturbed sample), similar to the high gross rates found by Stark and Hart (1997).

Causes of Disturbance-Induced Nitrification

These studies showed a rapid, large response in net nitrification to disturbance. Very large releases of ni-

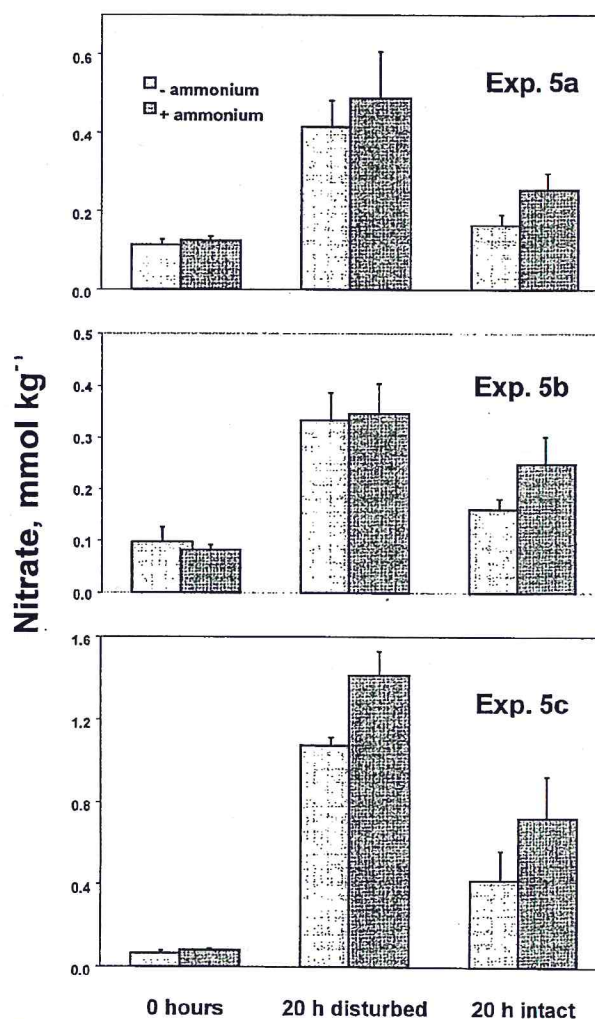


Fig. 6. Nitrate in the Oa horizon of intact cores incubated with and without added NH_4^+ (Exp. 5a-c). Each graph represents a different replication of the experiment (see Table 1). Error bars represent the standard error of each treatment across blocks. With the three experiments combined, there was a significant ($P < 0.05$) increase in nitrate in the intact cores in response to added NH_4^+ .

trate occurred in the case of extreme disturbance (column study in Exp. 1, Fig. 1), and a significant increase in NO_3^- was found 3 wk after relatively light disturbance in the field (footsteps in Exp. 2). We found higher net nitrification after 3 d in bulk samples compared with intact cores but both showed relatively high increases (Exp. 4, Fig. 5). In disturbed bulk samples, there did not appear to be a lag period in the increase in net NO_3^- and in situ concentrations were quite low compared with concentrations found after only 10 h (Exp. 3, Fig. 2-4). In these same experiments, NH_4^+ increased initially but then decreased rapidly over the first 24 h. Inhibition of nitrification resulted in continued increase in NH_4^+ concentration (Exp. 3c, Fig. 4). Ammonium added to intact cores increased net nitrification (Exp. 5, Fig. 6) and gross rate measurements showed relatively high rates of ammonium production and consumption but low nitrate production. These results suggest that

Table 3. Ammonium concentration in ammonium-addition experiments.

	Exp. 5a		Exp. 5b		Exp. 5c	
	Control	+(NH ₄) ₂ SO ₄	Control	+(NH ₄) ₂ SO ₄	Control	+(NH ₄) ₂ SO ₄
	mmol kg ⁻¹					
0 time	1.23	1.48	0.29	0.99	0.52	0.92
20 h disturbed	0.29	0.44	0.40	0.82	0.36	0.39
20 h intact	0.46	0.50	0.49	0.71	0.77	0.67

the phenomenon may be a simple response to increased NH₄⁺ availability to the ammonia oxidizers.

Thus, it appears that disturbance increases NH₄⁺ supply and decreases spatial limitation. The high rates of intact-core gross ammonification indicate that a sufficient rate of NH₄⁺ supply may exist before disturbance. Alternatively, the initial increase in NH₄⁺ could be a simple response to the creation of a readily available C source caused by mixing. This concept is not new. Schloesing (1897) demonstrated that mixing increased decomposition independently of aeration. A portion of the readily available C may be from bacterial and fungal cells lysed by the disturbance (Piatek, 1999). Nitrification has often been found to respond to the NH₄⁺ supply (Vitousek et al., 1982; Robertson, 1982). If this hypothesis is valid, higher in situ NO₃⁻ concentrations should be found after disturbances (e.g., freeze/thaw) and when competition for NH₄⁺ is reduced (off-season). Watershed-scale disturbances have been found to increase NO₃⁻ concentrations in streams, as have episodes of severe freezing (Mitchell et al., 1996). Seasonal fluctuations in stream NO₃⁻ export are well documented with concentrations increasing through the fall and winter. This could be explained by having significant nitrification only during periods of low plant uptake and greater NH₄⁺ supply (rather than having greater NO₃⁻ consumption during the growing season). Significant net nitrification can occur at low temperatures (Ross and Bartlett, 1990; van Miegroet, 1995). It is likely that the ammonia oxidizing community is adapted to the cycle of available NH₄⁺.

Alternate hypotheses exist to explain the disturbance phenomenon and need to be explored. Different scenarios for the inhibition of nitrification in the field have been offered over the years (e.g., Rice and Pancholy, 1972; Bremner and McCarty, 1988; Paavolainen et al., 1998). Gaseous or volatile inhibitors would be dissipated by disturbance, allowing nitrification to proceed. Another intriguing possibility is the recently confirmed oc-

currence of anaerobic ammonia oxidation (to N₂ gas), found in sewage treatment plants (Jetten et al., 1999). Introduction of oxygen into this system immediately stops the process and NH₄⁺ accumulates. An interaction with denitrification is also possible, that is, the introduction of oxygen stops the process and NO₃⁻ accumulates. These alternative hypotheses cannot easily explain all our findings but they do need further investigation.

Relationship to Other Studies

Differences in the extent of disturbance-induced nitrification among soils are still not well understood. Vitousek et al. (1982) found a wide range in net nitrification in disturbed samples from 14 sites across the USA, with many sites showing no net NO₃⁻ accumulation. Recent research has linked nitrification rates to various parameters, including landscape position (Ohri et al., 1999), land-use history (Goodale and Aber, 2001), elevation (Bohlen et al., 2001), moisture and temperature variation (Gilliam et al., 2001), and tree species (Lovett et al., 2000). The factors that contribute to differences in nitrification rates are complex and are interrelated. For example, landscape position can affect species composition (Peterjohn et al., 1999), elevation may have a moisture interaction (Bohlen et al., 2001) and land-use history may determine soil C/N ratios (Goodale and Aber, 2001). Differences in rates may also relate to methodology, as measurements in "reactive" soils may be sensitive to disturbance. Most of the studies cited above measured initial nitrate concentrations 1 to 2 d after sampling. If the soils behaved in a similar manner to those in the Brush Brook watershed, this initial reading could be much higher than that found in the field and interpretation of differences between studies may be difficult. Differences in sampling and incubation procedures could also result in variations in the results

Table 4. Gross and net nitrification and ammonification rates (Exp. 6). The disturbed rates were calculated over the first 10 h when the nitrification rate was linear. The intact core rates were measured over a 20- to 25-h period. Values in parentheses are the standard errors.

	Nitrification				Ammonification			
	Disturbed		Intact Cores		Disturbed		Intact Cores	
	Net	Net	Gross	Consumption	Net	Net	Gross	Consumption
	μmol kg ⁻¹ h ⁻¹							
a	73.8†	1.9 (3.2)	10.9 (4.2)	9.0 (5.4)	3.9‡	-9.7 (2.7)	16.4 (14.6)	26.1 (14.9)
b	71.2	6.1 (2.1)	4.9 (5.9)	-0.5 (8.2)	-12.5‡	-16.9 (9.7)	31.9 (7.6)	48.8 (11.8)
c	27.3	5.4 (1.4)	nd§	nd	48.1†	-1.2 (1.3)	62.2 (13.4)	63.4 (12.6)
d	32.9	7.0 (2.0)	nd	nd	48.4†	5.1 (1.2)	29.0 (4.8)	23.8 (3.6)
e	54.7	10.4 (4.5)	nd	nd	5.9‡	6.1 (5.4)	50.5 (13.6)	44.5 (9.1)

† Slope of regression from repeated measurements over 10 h, for example, Fig. 3.

‡ Calculated from two points because regression was not significant.

§ Not determined.

from sensitive soils, again making comparisons between studies difficult.

It should be reiterated that not all sites show a similar response. Christ et al. (1995) found little nitrification of added NH_4^+ in a beech (*Fagus grandifolia*)-dominated stand in the Hubbard Brook Experimental Forest, about 150 km from the study site. Some European sites have not exhibited significant nitrification even with added NH_4^+ (e.g., Emmett et al., 1995). In a study of forested sites in Sweden and Denmark, Persson et al. (2000) found little difference in net N mineralization between intact cores and sieved soils. Similar results were found by Laverman et al. (2001) in samples from a forested site in the Netherlands. Reasons for the differences between sites may be related to the C/N ratio. Using sites in a European north-south transect, Persson et al. (2000) found a good relationship between Oe/Oa C/N ratio and relative net nitrification below a C/N ratio of about 25. Above this, no net nitrification occurred. Working in New Hampshire's White Mountains, Goodale and Aber (2001) showed a similar relationship between total soil profile C/N ratio and net nitrification rates with little or no net nitrification above a ratio of 20. Gundersen et al. (1998) and Dise et al. (1998) working in Europe, and Goodale and Aber (2001) in New Hampshire, all found a relationship between nitrification and stream or soil water NO_3^- export, again with a critical ratio C/N between 20 and 25. A common theme with most of these results is high variability, especially below the critical C/N value. The average C/N ratio of the soils that we studied was 16.8, well below the critical value. Thus, these soils would be expected to exhibit net nitrification.

More work is needed to clarify the mechanism of the response and the cause of differences between sites. The task is difficult because experimentation invariably causes disturbance. Regardless of the cause of the phenomenon, the implications are serious for research on these soils. How does one study them without producing artifacts? Is a soil that produces large concentrations of NO_3^- upon disturbance a soil that is a good nitrifier or one in which nitrification in the field is limited by factors that we do not understand? The correlation found between stream export and C/N ratio (Gundersen et al., 1998; Dise et al., 1998; Goodale and Aber, 2001) suggests that soils that are sensitive to sampling disturbance will also produce NO_3^- without disturbance. Although absolute rate measurements may not be possible, perhaps the potential rate measurements are useful for predicting ecosystem response to perturbations.

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