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Effects of increased nitrogen deposition on soil nematodes in alpine tundra soils

E. Lokupitiya[1], N. L. Stanton[1], R. S. Seville[1] and J. R. Snider[2]

[1] Department of Zoology and Physiology and
[2] Department of Atmospheric Science, University of Wyoming, Laramie, WY 82071 USA

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Summary

Because of anthropogenic activities, atmospheric deposition of nitrogen has increased on some high elevation ecosystems in North America. On Elk Mountain in SE Wyoming, USA, we found that ice, formed by the impaction of supercooled cloud droplets, contains nitrogen levels (NH_4^+ = 58 ± 47 µmole/l, NO_3^- = 52 ± 40 µmole/l) significantly higher than snow (NH_4^+ = 7 ± 5 µmole/l, NO_3^- = 14 ± 7 µmole/l). At this site the impaction process (riming) occurs on krummholz and is an important mechanism of water and nutrient deposition. We sampled nematodes in alpine soil for two seasons under this rime ice deposition and in adjacent meadow and krummholz soil with only snow deposition. No significant difference was found in nematode density and trophic composition between snow and rime ice deposition zones in krummholz; and nematode densities were significantly higher in meadow soil than in the krummholz rhizosphere. Densities of active nematodes were highest immediately after snow melt and a positive correlation was found between nematode density and percent soil moisture. With subsequent soil drying, nematodes gradually entered anhydrobiosis and this process began earlier in the exposed meadow than under the krummholz canopy.

In a subsequent microcosm experiment we exposed nematodes in two alpine soils to four different nitrogen treatments [ammonium nitrate (NH_4NO_3) at 0, 20, 40 and 80 kg N/ha/yr] and two temperatures (5 and 25°C). None of the three variables had a significant effect on nematode density. But at the first sampling (1 month) the interaction between soil type and nitrogen level was significant, and at the second and final sampling (6 months) the interaction between soil type and temperature was significant. Nematode response to increasing nitrogen deposition rates varied in a com-

Corresponding author: N. L. Stanton, *e-mail:* stanton@uwyo.edu

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plex way with soil type and temperature. Under the microcosm conditions, nematode community composition shifted to opportunistic rhabditid species. Our work indicates that higher levels of nitrogen deposition (>80 kg N/ha/yr) were necessary to produce detectable effects on nematode density in this alpine region.

Key words: nematodes, alpine soil, nitrogen deposition, microcosms

Introduction

Because of human activities such as fossil fuel burning, atmospheric deposition of nitrogen on some high elevation forest soils has increased, and ecosystem dynamics has shifted from a nitrogen-limited to a nitrogen-saturated system (Aber et al. 1989; Tamm et al. 1995; Williams et al. 1996). Since the late 1980's, nitrogen levels in NADP (National Acid Deposition Program) sites at Niwot Ridge in Colorado and on the Snowy Range in Wyoming have increased dramatically. For example in the Snowy Range, annual nitrate (NO_3^-) loading increased by 300 % over 4 years – from 3.3 kg/ha in 1986 to 10 kg/ha in 1990 (Williams et al. 1996).

These alpine areas at treeline consist of a patchwork of islands of stunted coniferous trees (krummholz) interspersed in meadow. Here one striking form of nitrogen deposition is in cloud droplets that freeze on krummholz as rime ice. With warm temperatures, the rime ice melt is localized on the leeward side of the kummholz. Since rime ice contains much higher concentrations of ammonium, nitrate and sulfate than snow (Borys et al. 1988), local differences in the intensity of atmospheric deposition are expected to occur between alpine meadow and krummholz islands: i.e., the effect of riming should be localized where melt water enters the krummholz soil.

Since high elevation ecosystems can be more sensitive to chemical changes than those at lower elevations, small changes in atmospheric deposition may cause large changes in ecosystem dynamics (Williams et al. 1996). Any effect from increased nitrogen levels on soil processes should be most readily detectable in locations directly receiving rime ice melt water.

Nematodes are the most abundant metazoan in alpine soils and analysis of nematode communities has been used by a number of investigators to monitor soil conditions in a variety of other ecosystems (See Bongers & Ferris 1999 and references therein).

Effects of nitrogen addition (Bååth et al. 1978, 1981; Rodriguez-Kabana 1986; Sohlenius & Bostrum 1986) and soil acidification (Hyvönen & Persson 1990; Ruess & Funke 1992; Dmowska 1993; Ruess et al. 1996) on soil nematodes have been measured in several laboratory and field studies. Bååth et al. (1978, 1981) demonstrated in microcosms with nitrogen addition, bacterial biomass increased, resulting in more bacterial feeding nematodes and fewer fungal feeders such as *Aphelenchoides*. Sohlenius & Bostrum (1986), however, reported increases in both bacterial and fungal feeding nematodes with nitrogen fertilization of a barley field. Results from these studies vary but, in general, the predaceous and omnivorous taxa were reduced; bactivore response depended upon the level of acidity; and the fungivores appeared most tolerant of acidification.

We sampled soil nematodes in both rime ice and snow deposition zones on Elk Mountain in SE Wyoming, where the Atmospheric Science Department, University of Wyoming, maintains a facility and where snow and rime ice chemistry have been sampled occasionally beginning in the mid 1980s. Two inferences are evident from our data summary (Table 1). First, the concentrations of nitrate and ammonium contained in rime ice are substantially higher than that in snow. This finding is consistent

Table 1. Average winter nitrogen concentrations (±SD) in rime ice and snow at Elk Mountain and in snow at the Snowy Range, Wyoming

	$[NH_4^+]$ micromole/liter	$[NO_3^-]$ micromole/liter
Snow, Elk Mountain *(1985–1992)*	7 ± 5 (n=14)	14 ± 7 (n=14)
Rime-ice, Elk Mountain *(1989–1996)*[1]	58 ± 47 (n=142)	52 ± 40 (n=197)
Snow deposition, NADP Snowy Range, WY[2] *(1987–1992)*	5 ± 5 (n=62)	10 ± 8 (n=130)

[1] Sample collection and analysis methods are in Snider and Vali (1994)
[2] Data obtained from the National Atmospheric Deposition Program (http://nadp.sws.uiuc.edu)

with work conducted at an alpine site which is 200 km south west of Elk Mountain (Borys et al. 1988). Second, concentrations of both nitrate and ammonium in snow at Elk Mountain are consistent with concentrations at the nearest NADP monitoring site (Snowy Range, WY). The comparison of the Snowy Range and the Elk Mountain sites is noteworthy. It shows that precipitation chemistry data from the Snowy Range can be used with some confidence to make estimates for Elk Mountain. Also of importance is the comparison of Elk Mountain rime ice and snow chemistry, which suggests that rime ice formation can be an important mechanism for transferring fixed nitrogen to some alpine ecosystems, particularly those that are frequently exposed to high wind speed in combination with wintertime cloud immersion events. A few authors have investigated these issues (Lovett et al. 1982; Politovich and Vali 1983; Snider and Vali 1987) but a comprehensive study of the phenomenon and its biological consequence has not yet been attempted.

We hypothesized that nematode densities would be higher in soil receiving rime ice melt because of the high nutrient pulse during the first part of the growing season. Although several studies have measured the effect of increased nitrogen deposition on alpine plants (Bowman et al. 1993, 1995; Bowman 1994; Lipson et al. 1996; Theodose et al. 1996) and microbial populations (Fisk & Schmidt 1996), to our knowledge the effects of increased nitrogen on nematode communities in alpine soil have not been measured.

Subsequently in a microcosm study, we measured the effect of three nitrogen deposition levels on nematodes: 1) 20 kg N/ha/yr which is approximately four times the

current annual levels of nitrogen in wet deposition at Elk Mountain, 2) 40 kg N/ha/yr which approximates nitrogen deposition in certain forests in Europe and California (Bytnerowicz & Fenn 1996), and 3) 80 kg N/ha/yr which simulates the highest nitrogen deposition rates in parts of Europe (Heij & Schneider 1991).

Materials and Methods

Field study – Study site

Sampling was conducted in an alpine ecosystem on Elk Mountain (elevation 3,486 m; 41°38'N,106°31'W), SE Wyoming, USA. Common alpine plant species included *Carex rosii*, *Arenaria obtusiloba*, *Geum rosii*, *Potentilla diversifolia*, *Erigeron* sp., *Trifolium dasyphyllum*, *Cerastium arvense*, *Achillia millifolia*, *Sibbaldia procumbens* and *Festusa* sp. Krummholz islands of subalpine fir (*Abies lasiocarpa*) and Englemann spruce (*Picea englemannii*) are distributed throughout the transitional ecocline between the coniferous forest and alpine meadow. Winters are cold with heavy snow and strong winds. Complete snowmelt can occur as late as July and frost can occur any month of year.

Sampling scheme

For nematode extractions, soil was sampled (coring bit: 4.8 cm diameter by 10 cm) in June, July 1995 and March and June 1996. In June 1995, two 10×10 m plots were established in two rime deposition sites leeward to krummholz islands and the prevailing southwest wind and downslope of the krummholz. Within each of the two plots, three samples were cored at each of nine points evenly spaced at 5 m apart. In the adjacent meadow, which experienced snow but no rime ice deposition, nine points in one 10 by 10 m plot were sampled similarly. This yielded 27 meadow and 54 rime ice samples. These plots were resampled in July 1995; however, only one sample was taken at each point (i.e., 54 samples). In March 1996, because several meters of snow covered the ground, only four rime ice and three meadow cores could be taken (i.e., 7 cores).

The sampling scheme described above clearly confounds rime ice deposition with a possible rhizosphere effect of tree roots. Therefore, in June 1996, this sampling scheme was changed to control for rhizosphere effects: i.e., all samples were collected from krummholz tree rhizosphere. From each of three patches of krummholz, 20 m apart, we collected five random samples (each of a single core) from tree rhizosphere both above (upwind and upslope) and beside the rime ice deposition zone and five random samples from the rime ice deposition zone downslope of each of the three krummholz islands (i.e., a total of 45 cores).

Soil processing and analyses

Soil samples were transported on ice and stored in a refrigerator at 7°C until processed. Small rocks larger than 1 cm were removed from each sample by hand. Percent moisture was measured gravimetrically for each sample using a 10 g subsample. Another 10 g subsample was separated from each and bulked for chemical analyses. Nematodes were extracted from the remaining soil by sieving (#325 mesh, 355μm) and subsequent extraction on Baermann funnels for 48 hrs at 25°C. Nematodes were counted in three 1-ml subsamples from each Baermann extract of 25 ml.

Nematodes were classified as (Mai et al. 1996): saprobes or bactivores (Orders Rhabditida and Monhysterida), fungivores (Order Aphelenchida), root/ fungal feeders (Order Tylenchida), omnivores (Order Dorylaimida), and predators (Order Mononchida). Unidentified orders were categorized as 'unknown'. Nematode numbers are reported per 100 g dry soil after correcting by the percent moisture levels in soil samples.

Since July soil samples had a much lower moisture content (rime ice soil, 12 % and meadow soil, 8 %) than the June samples (rime ice soil, 36 % and meadow soil, 30 %), we hypothesized that some nematodes may have entered anhydrobiosis. Therefore, we saturated 15 additional July samples (5 rime ice and 10 meadow samples) with distilled water and refrigerated them (7°C) for 7 days prior to extraction.

Student's t-tests were used to detect differences between total densities and orders of nematodes (*ln* transform) in the rime ice zone and the reference (non-rime ice) soils for each sampling time. To detect correlations between percent moisture and total number of nematodes, multiple regression analyses was done on rime ice and reference soils from the rime ice zones with percent moisture as the predictor variable and *ln* transformed total number of nematodes/ 100 g dry soil as the response variable. For the first sampling scheme (June 1995–March 1996), data from July 1995 were not included because of very low percent moisture levels (8–12 %). Data from June 1996, the second sampling scheme, were analyzed separately as described above.

Soil subsamples (10 g) were analyzed for organic matter content (OM), nitrate, phosphate, and potassium (Soil Testing Laboratory, University of Wyoming).

Microcosm study – Microcosm construction

We established small microcosms with alpine soils containing nematodes and applied four levels of NH_4NO_3 equivalent to deposition levels of 0, 20, 40 and 80 kg N/ha/yr. Soil for microcosms was collected from a wet alpine meadow on Medicine Bow Peak (elevation 3,780 m; 41°21'N, 106°19'W) of the Snowy Range, Wyoming, USA, which has similar climate to Elk Mountain but with easier accessibility. This sampling site is approximately 1 km NW of the Snowy Range NADP site. Plant species common in both sites included *C. rosii*, *P. diversifolia*, *A. milifolia* and *T. dasyphyllum*. Soil was homogenized by removing plant parts and rocks followed by sieving (with meshs of 1 cm, 0.5 cm and 1.6 mm). Soil from one sod was strongly aggregated and more difficult to sieve. Because of this difference in aggregate size, the soils were treated as two separate types: fine textured with no aggregates (F) and coarse textured and aggregated (C).

For pre-treatment density estimates, nematodes were extracted and sampled (as described in the previous section) from 10 samples (8 g wet soil) from each soil type. The initial number of nematodes per 100 g dry soil was calculated using percent moisture of each sample.

One hundred and twenty microcosms were constructed from plastic cups (7.5 cm in diameter) with 5–6 holes drilled in lids and bases to allow evaporation and leaching. Wet soil was added to each microcosm: 150 g of fine soil and 100 g of coarse soil (less coarse soil was available). Prior to setting up microcosms, chemical analyses on both soils were conducted.

Eighty microcosms, 40/soil type, were maintained for 6 months at 25°C. Each of 10 replicates/treatment received one of the four NH_4NO_3 deposition rates applied once/week (solution volume of 20 ml on fine soil and 10 ml on coarse soil) based upon amount of water required to saturate each soil type, but both soils received the same level of nitrogen application. To measure possible effects on nematodes at a colder temperature, the remaining 40 microcosms were maintained at 7°C: 20 received distilled water and 20 received NH_4NO_3 equivalent to a deposition of 80 kg N/ ha/ yr.

Sampling and analyses—nematodes and soil

Ten replicates from each soil type were taken/treatment at 1 month (Jan. 1997) and 6 months (June, 1997) after initiation of the experiment. One soil sample (8 g) was taken from each microcosm and nematodes were extracted in Baermann funnels for 48 hrs at 25°C. Nematodes were counted, identified to order and expressed as number/100 g dry soil.

In eight multiple regression analyses (4 N concentrations, two sampling times, two tempe-

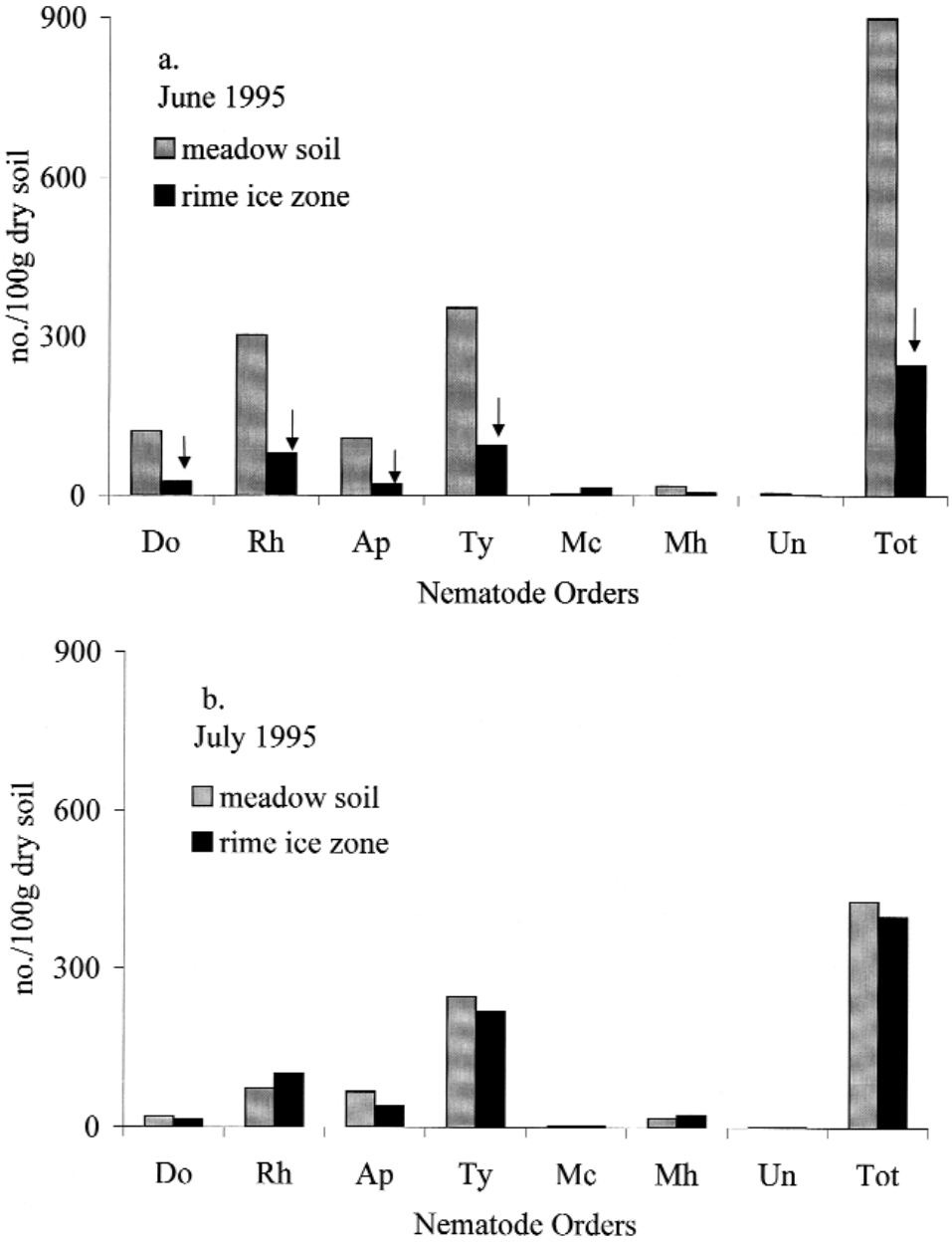
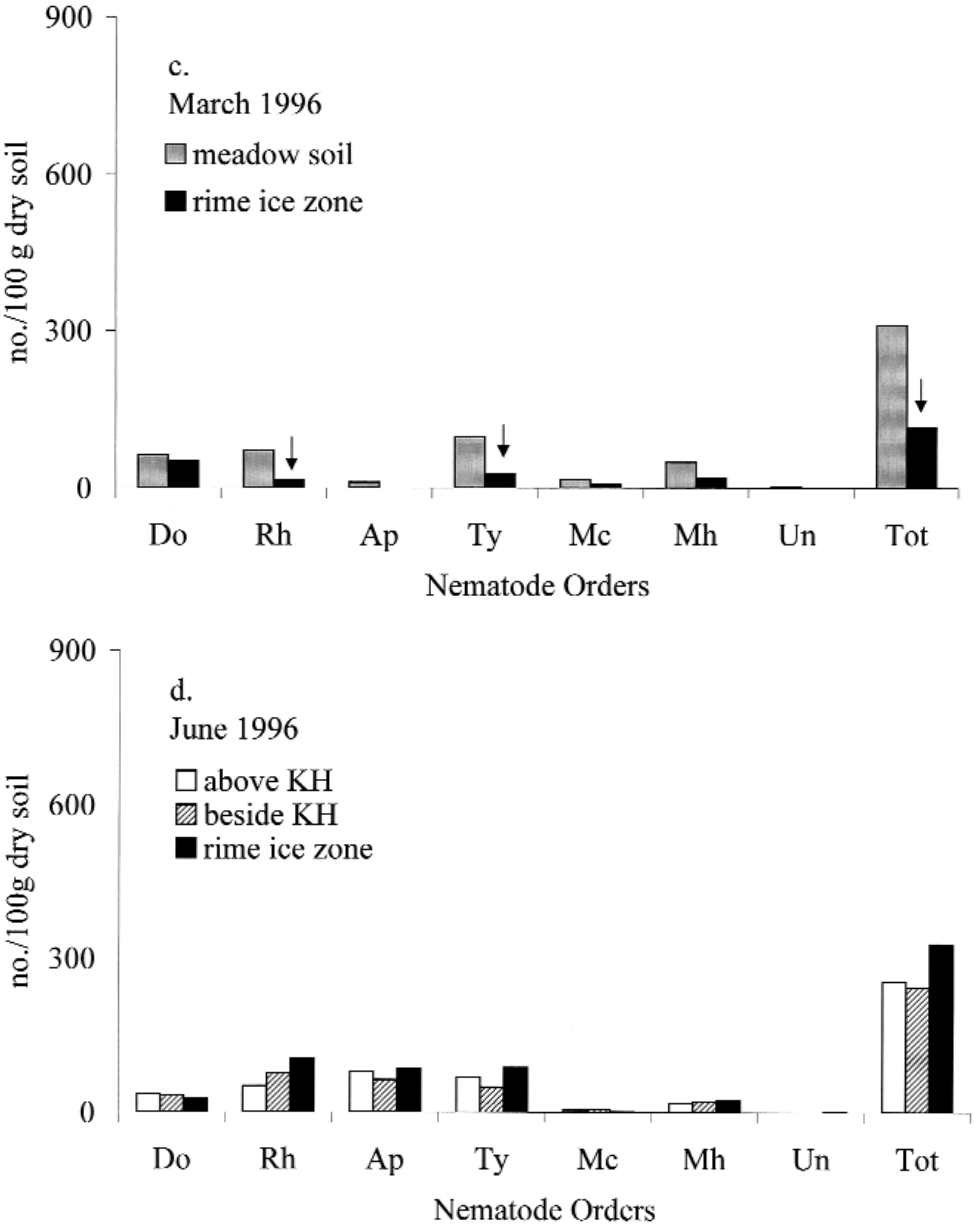


Fig. 1. Nematodes/100 g dry soil in meadow soil and the rime ice zones collected in (a) June 1995, (b) July 1995, (c) March 1996 and (d) June 1996 in tree rhizosphere zones above and below krummholz (KH) islands and in the rime ice zone: Do=Dorylaim-



ida, Rh=Rhabditida, Ap=Aphelenchida, Ty=Tylenchida, Mc=Mononchida, Mh=Monhysterida, Un=Unknown, and Tot=Total (arrows indicate a statistically significant difference at $p<0.05$, Student's t-tests for *ln*-transformed nematode numbers)

ratures and two soil types), nitrogen deposition rate, soil type and temperature were used as predictor variables and the total number of nematodes (*ln* transformed due to high variability) was the response variable.

Soil samples from the different treatments were analyzed at the end of the second sampling (after 6 months) for ammonium using 10 g samples (pooled 1g samples from each microcosms).

Microbial plate counts

Bacteria and fungi were plated from the fine soil treated with 0, 20, 40 and 80 kg N/ha/yr at 25°C using a 10 g sample (pooled 1 g subsamples) per treatment. Soil dilutions of 1×10^{-5}, 1×10^{-6} and 1×10^{-7} were prepared and 10 plates with Thorton's medium were inoculated with each dilution from each different nitrogen treatment (1 ml from each dilution per plate, 30 plates for each nitrogen treatment; 120 plates total). Inoculated plates were incubated at 25°C for one week.

Colonies of bacteria and fungi were counted and the average number of bacterial and fungal colonies per g soil were calculated using the soil dilution factors. To approximate the diversity of bacteria and fungi, the number of different colony types was recorded. Pure cultures were established using Mineral Salts Medium for bacteria and Malt Extract Agar for fungi. Isolated bacteria were gram-stained and counted and fungi were identified to species by M. Christensen, Botany Dept., University of Wyoming.

Any effect from increasing nitrogen deposition rates on bacteria and fungi was assessed by regression analyses on the *ln* transformed bacterial and fungal counts/g wet soil.

Results

Field study – Nematode abundance and diversity

The total number of nematodes was significantly lower in the rime ice zone than in the meadow in **June 1995**, immediately after the snow melt ($t = 7.81$; $p < 0.0001$; Fig. 1a). Rhabditida ($t = 8.78$, $p < 0.0001$), Tylenchida ($t= 4.29$, $p < 0.0001$), Aphelenchida ($t = 3.91$, $p < 0.001$) and Dorylaimida ($t = 7.08$, $p < 0.0001$) were also significantly lower in the rime ice zone. Bacterial feeding Rhabditida and the root/ fungal feeding Tylenchida were the most abundant, and Mononchida and Monhysterida the least abundant in both meadow soil and rime the ice zone.

In **July 1995**, the total number of nematodes and the number per order (Fig. 1b) were not significantly different between the rime ice zone and meadow soil ($p > 0.05$). In addition, the total number of nematodes extracted from meadow soil had declined from 900 in June to 400/100 g dry soil; but conversely the total number in the rime ice zone had increased from 240 to 400/100 g dry soil (Fig. 1a, b).

Samples that were wetted and incubated for 7 days (7°C) yielded far more nematodes than field samples directly extracted: the total was five times higher in wetted meadow soil (430 to 1910; Fig. 2a) and over two times higher in the wetted rime ice zone (400 to 900; Fig. 2b). Also the total number of nematodes extracted from the meadow soil was significantly higher than that from the rime ice zone ($t = 2.95$, $p < 0.01$); and the difference was also significant for the orders Rhabdita ($t= 2.46$, $p < 0.05$), Tylenchida ($t = 2.98$, $p < 0.01$) and Aphelenchida ($t= 3.52$, $p < 0.01$). Densities in the orders Dorylaimida, Mononchida and Monhysterida were low in both zones (Fig. 2, a, b).

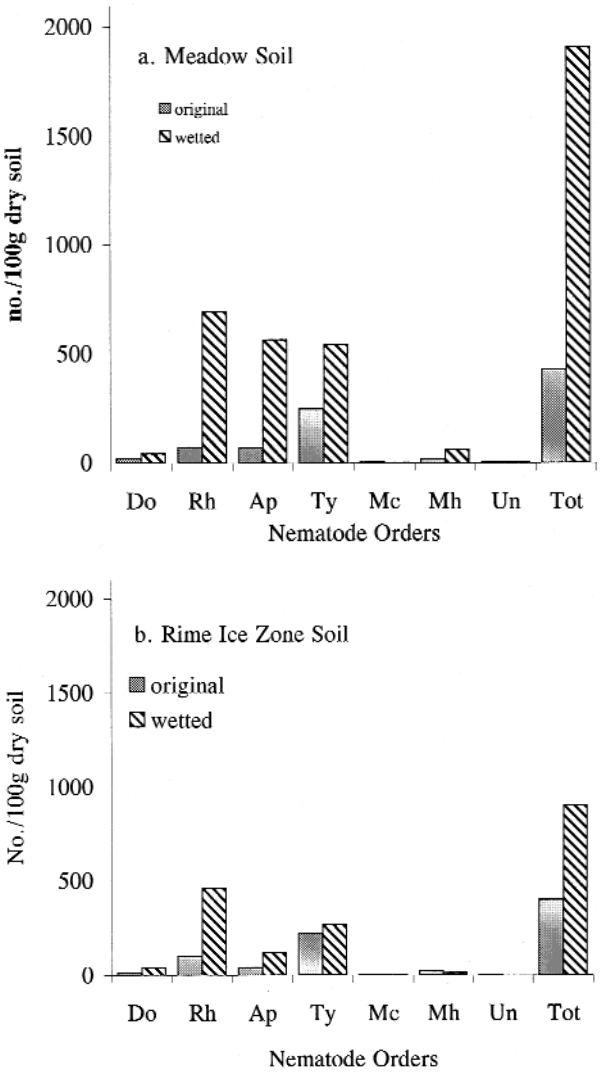


Fig. 2. Nematodes/100 g dry soil in (a) meadow soil and (b) in the rime ice zone wetted before extraction compared to the numbers extracted from the original (unwetted) soil collected July, 1995. See caption Fig. 1 for nematode taxa

In **March 1996**, the rime ice zone had significantly lower nematode densities than the meadow soil ($t = 3.20$, $p < 0.05$); and this difference was also significant for the orders Tylenchida ($t = 4.08$, $p < 0.01$) and Rhabditida ($t = 2.64$, $p < 0.05$) (Fig. 1c).

However, with the changed sampling regime (samples taken only in tree rhizosphere) in **June 1996**, the total number of nematodes in the samples collected from above, beside and below rime ice deposition zones did not differ significantly (above rime vs rime, $t = 0.55$, $p > 0.05$; beside rime vs rime, $t = 1.22$, $p > 0.05$). Nor did any of the orders differ significantly among the three sites ($p > 0.05$). Total nematode numbers were just slightly higher in the rime samples (Fig. 1d).

Nematodes in relation to percent moisture

In **June 1995**, the average percent moisture in the rime ice zone (36 %) was only slightly higher than the meadow zones (31 %). In **July 1995**, soils form the rime ice zone (12 %) and meadow (8 %) had a lower percent moisture compared to the other

Table 2. Percent soil moisture (± SD) in the meadow (control) and the rime ice zones.

Time of sampling		*Control Samples*	*Rime Ice Samples*
1995	June	31± 0.9	36 ± 1.2
	July	8 ± 0.7	12 ± 0.7
1996	March	28 ± 1.5	32 ± 2.0
	June	37 ± 1.3	43 ± 1.8

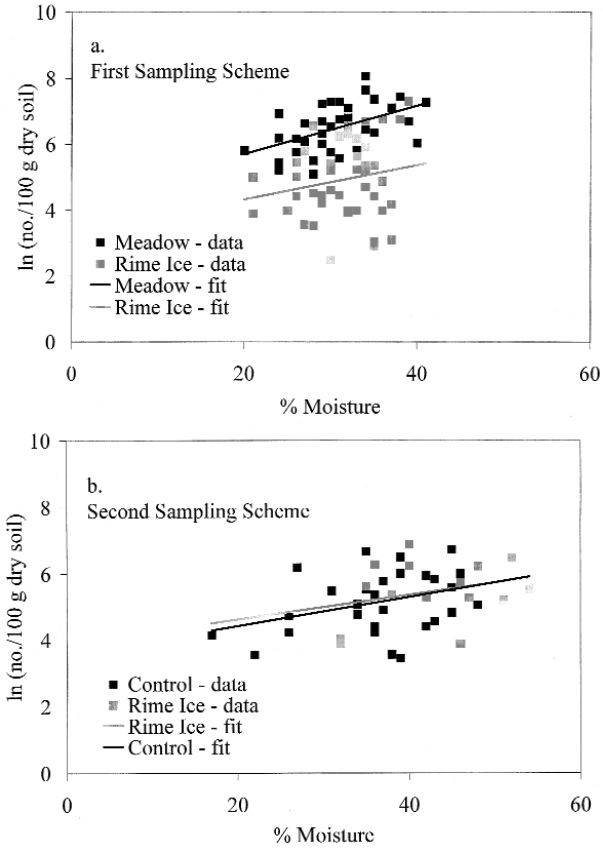


Fig. 3. Regression of *ln* transformed total number of nematodes/100 g dry soil versus percent moisture for (a) meadow soil and the rime ice zone sampled June, 1995 and March 1996 and for (b) controls (above rime and beside rime zones combined) and the rime ice zone sampled in June, 1996. Trend lines illustrate relationships between nematodes and % moisture for the rime ice zone and meadow soil

sampling times (Table 2). Multiple regression analysis of *ln* transformed total nematode numbers with soil zone (S) and percent moisture (M) under the **first sampling scheme** (June 1995, March 1996) yielded a significant correlation [(r^2 = 43.2 %, $p_{overall} < 0.001$, $p_{soil\ zone} < 0.001$, $p_{\%moisture} < 0.05$); Fig. 3a] with a regression equation of *ln* total nematodes = 4.54 - 1.62S + 0.0634M. The trend lines in Fig. 3a reflect the higher densities of nematodes in the meadow soil.

Under the *second sampling scheme* (June 1996), multiple regression analysis yielded a significant correlation of ln total number of nematodes with percent moisture, but not with soil zone [r^2 = 13.2 %, $p_{overall} = 0.05$, $p_{soil\ zone} > 0.05$, $p_{\%moisture} < 0.05$); Fig. 3b]. The regression equation was ln total nematodes = 3.62 + 0.062S + 0.0416M. The nearly isotonic trend lines in Fig. 3b reflect the nearly identical density of active nematodes in meadow soil and the rime ice zone.

Although nematode numbers differed between the rime ice zone and meadow soils in June and July 1995, chemical analyses reflected no consistent difference in any of the parameters measured (Table 3).

Table 3. Chemical analysis of soil samples collected in June and July 1995 from meadow soil and the rime ice zones and in June 1996 from tree rhizosphere beside, above and in the rime ice zone

	June 95		July 1995		June 1996		
	Meadow	Rime	Meadow	Rime	Above Rime	Beside Rime	Rime
% OM	7.4	8.2	7.0	9.3	9.8	10.9	8.4
mg NO3N/kg	31.0	25.0	52.0	51.0	118.0	180.0	118.0
mg PO4P/kg	8.0	4.0	8.0	7.0	10.0	10.0	10.0
mg K/kg	138.0	122.0	193.0	146.0	154.0	204.0	160.0
pH	5.4	5.3	4.8	4.6	4.4	4.3	4.6

Microcosm study – Nematodes

Initial nematode numbers were different in the two soil types: 5600/100 g dry soil in fine-textured and 2100/100 g dry soil in coarse-textured soil. In the four control microcosms (Fig. 4a–d), numbers of all taxa declined through the course of the experiment except rhabditids in fine-textured soil at 25°C (Fig. 4a), where rhabditid numbers recovered at 6 months exceeded the counts after one month and only rhabditids were present at the end. In the microcosms with course-textured soil (Fig. 4 b,d), nematode numbers declined over time and only rhabditids and tylenchids were present at 25°C at the last sampling. These orders plus aphelenchids and monhysterids also lingered on in both soils at 5°C d (Fig. 4c, d).

All nitrogen application treatments mirrored the controls (Fig. 4a–d and are not illustrated) with one exception – in the 80 kg N/ha/yr application at 25°C, the number of rhabditids at the last sampling was lower than the initial count.

At the first sampling, multiple regression analysis of ln transformed total number of nematodes with soil type [fine (S = 0) and course (S = 1) textured], nitrogen depo-

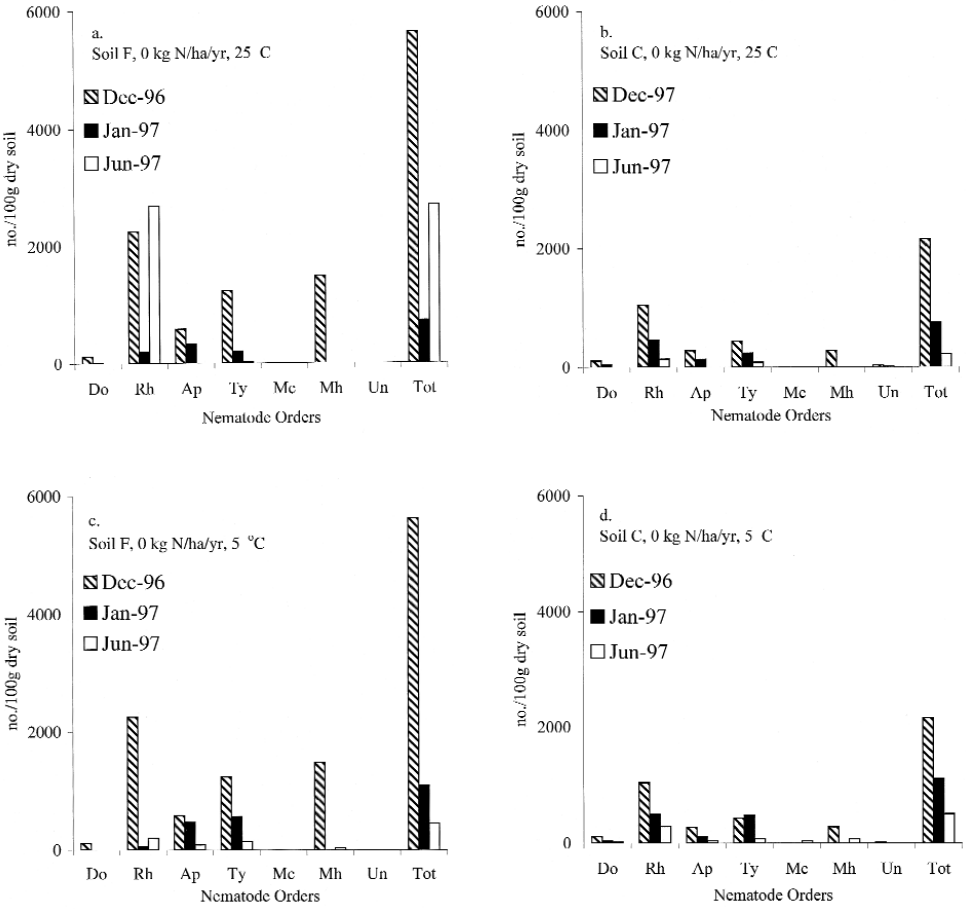


Fig. 4. Nematode numbers from control microcosm (no N addition) at the initiation of the experiment (December 1996), and at the two subsequent samplings (January 1997, and June 1997) in a) finel and b) coarse soil at 25°C and in c) fine and d) coarse soil at 5°C. See caption Fig. 1 for nematode taxa abbreviations

sition rate (N: 0, 20, 40 80 kg N/ha/yr), and temperature [T: 5°C (T = 0) and 25°C (T = 1)] yielded a significant result only when the interaction of S*N (soil type and nitrogen deposition rate) was added to the above three independent variables ($p_{overall} < 0.001$, $r^2 = 19\%$; ln total no = 6.85 – 0.056S – 0.316T – 0.00418N + 0.0116S*N). The correlation of S*N interaction with ln total number of nematodes was further verified by doing separate regression analyses of N deposition rate vs ln total number of nematodes for the two different soil types: this gave significant p values (fine-texture: a positive correlation with $p<0.05$, $r^2 = 13\%$; course-texture: a negative correlation with $p<0.05$, $r^2 = 11\%$, Fig 5a).

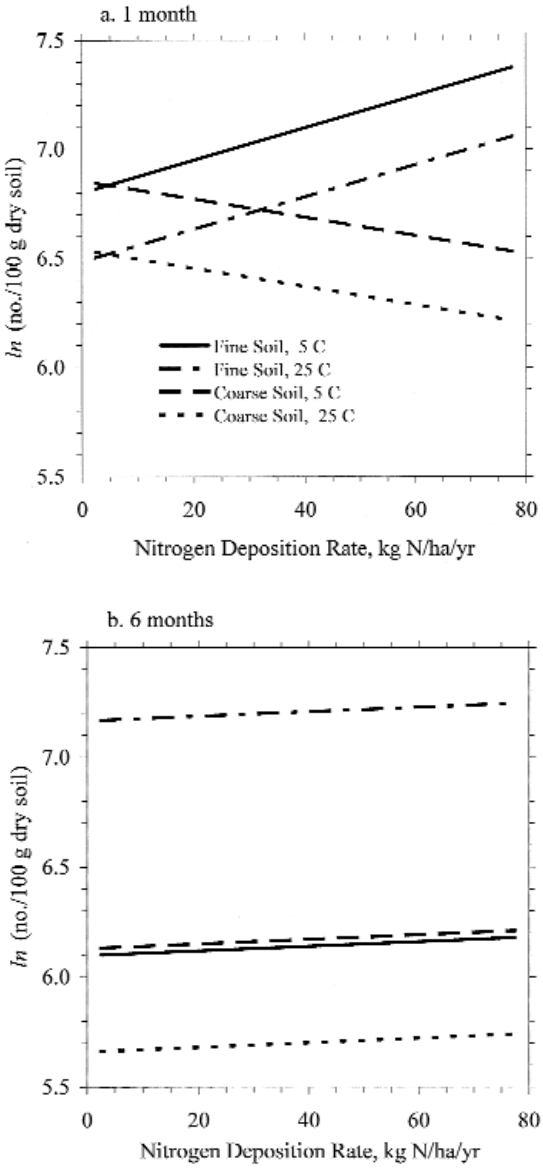


Fig. 5. For the microcosm experiment, fitted lines for the regression of ln total number of nematodes versus nitrogen deposition rate: a) first sampling, b) second sampling (solid line = fine soil at 5°C, dashed line = coarse soil at 5°C, dash-dot = fine soil at 25°C and dotted = coarse soil at 25°C

At the second sampling, S*T alone had a significant effect on nematode numbers ($p < 0.001$, $r^2 = 31.6\,\%$; *ln* total no = 6.13 – 0.032S – 0.468T + 0.00105N + 1.54S*T, Fig. 5b). However, at this sampling, nematode numbers did not vary with nitrogen deposition levels (Fig. 5b)

Chemical analysis indicated that the fine-textured soil had a lower pH level (4.7) and a higher organic matter content (11.6 %) compared to the course-textured (pH, 5.4; OM, 8.3 %) at the start of the experiment. By the end, ammonium concentration

in soil increased with increasing nitrogen application. This was very much evident for the coarse textured soil left at room temperature, where N increased with increasing nitrogen deposition rates (ammonium concentrations in the soil at the end were 9, 54, 66 and 136 mg N/kg for the deposition rates of 0, 20, 40 and 80 kg N/ha/yr respectively).

Bacterial and fungal plate counts

Bacteria and fungi were sampled only at the end of the second sampling in the microcosm with fine-textured soil at 25°C. Nitrogen deposition levels had no effect on bacteria and fungi under microcosm conditions ($p>0.05$ for the regression analyses done on *ln* transformed data). Gram-staining revealed bacteria had gram negative, gram positive and gram variable forms that included bacilli, cocci and spore forming bacillicocci. Fungi observed included *Chaetomium erraticum* (a rarely reported species), *Paecilomyces cladosporioides*, *Cladosporium cladosporioides*, *C. herbarum*, *Penicillium lilacinum*, *P. griseofulvu* and an undescribed or very rare species of *Penicillium* (Christensen, pers. comm.).

Discussion

Field study

In the first sampling (June 1995), the total number of nematodes was significantly higher in meadow soils compared to rime-ice zones; but at the second sampling (July 1995) no difference was detected. But when nematode counts from wetted July samples were pooled with counts from unwetted samples, meadow soil again had significantly higher counts. Our original hypothesis that rime ice soil has higher nematode numbers of all trophic categories was not supported: nematode numbers were lower in soil under krummholz rime ice zones than in alpine meadow soil.

During the second summer (1996), we changed our sampling scheme to control for the difference in rhizosphere affects of meadow versus the krummholz zone. Rime ice zone samples were collected, as previously done, on the leeside and down hill from the krummholz islands; but the "control" samples were collected from krummholz rhizosphere lateral to, and uphill from, rime ice deposition. No difference was found between nematode abundance in rime soil versus this control soil. Furthermore, nematode counts from krummholz rhizosphere soils were about the same in both June 1995 and June 1996 (200 and 250/100g dry soil). We conclude that the differences detected in abundance in 1995 were an artifact of our sampling scheme, and that nematode density was higher in the alpine meadow soil than in kummholz soil.

A comparison between the two initial sampling sites (rime vs meadow) across the first two sampling times (June vs July 1995) reveal temporal differences by microsite in total number of active nematodes. From June to July, the average number of active nematodes in meadow soil decreased (900 to 400/100g dry soil), while the number in the rime ice zone increased (200 to 400/100g dry soil). These trends may be best explained by results from the laboratory experiment in which some soil was wetted seven days before extraction to stimulate the nematodes out of anhydrobiosis. The wetted rime samples yielded an average of 900/100g dry soil versus 400/100 g dry soil

from unwetted soil, while the meadow samples yielded almost 2000/100g dry soil versus 400 from unwetted soil. Thus about 80 % of the nematodes in meadow soil and 55 % in the rime ice zone were in anhydrobiosis in July.

We speculate that nematode numbers may be affected by the temporal variation in the pulse of snowmelt. After snowmelt, the intense solar radiation quickly dries out the meadow soil. But this drying is delayed under the krummholz canopy. By the July sampling time, most of the nematodes in the meadow had become anhydrobiotic; but in krummholz only about half had entered this state. This argument is supported by our gravimetric soil moisture data: values declined from 31 % in June to 8 % in July in the meadow and from 36 % to 12 % in the krummholz. We also found statistically significant positive correlations between ln active nematode counts and percent soil moisture.

The taxa composition of all soils collected was essentially the same: tylenchids, rhabditids and aphelenchids had the highest relative abundance; mononchids, monhysterids and dorylaimids, the lowest.

Soil chemical analyses for both years revealed no consistent difference among meadow soil, rhizosphere reference soils and the rime ice zone, with the possible exception that organic matter was slightly lower in meadow soil. Thus, the higher chemical load in rime ice versus snow was not evidenced by the soil analyses, probably due to the high buffering capacity of the soil (Clayton et al. 1991).

Therefore from our field sampling, we conclude first that nematode numbers were higher in meadow soil compared to coniferous rhizosphere soil. The reasons for this difference are unclear but the pattern is consistent with Petersen & Luxton's data compilation (1982) in which they reported that of all "non-wooded ecosystems", temperate grasslands support the highest number of nematodes, and that coniferous forests have lower nematode numbers than grasslands and tundra. Second, it appears that nematode activity was high after snowmelt and continued until moisture became limiting and the nematodes began entering anhydrobiosis. Therefore, the pattern of snowmelt apparently creates a mosaic of soil microhabitats that differ in plant phenology (although not measured) and nematode activity.

Microcosm study

The primary variables of nitrogen deposition, soil type and temperature did not have significant effects on nematode numbers at either sampling time. At the first sampling only the Soil*Nitrogen interaction was significant and the regression model became significant when this interaction term was included. In the fine soil, the regression model predicted higher nematode densities with increased nitrogen additions and in the coarse soil, lower densities with increased nitrogen (although the r^2s were low). At the second sampling time, nematode numbers were significantly affected by Soil*Temperature interaction. Across all nitrogen concentrations at 5°C, the regression yielded almost the same nematode counts in both coarse and fine soils. In other words, at 5°C by the second sampling, nematode densities were reduced in both soils and converged to the same density across all nitrogen treatments. At 25°C, densities in the fine soil were higher and densities in the coarse soil were lower than the regression lines for the almost isotonic lines of fine and coarse soils at 5°C. At 25°, densities in the fine soil increased from time 1 to time 2 but proportionally more in the lower N treatments. In the coarse soil, just the opposite occurred: densities decreased

from time 1 to 2, and proportionately more in the lower N treatment. This difference may reflect both the change in taxa composition from time 1 to 2 (essentially only rhabditids were present at time 2) plus the differential response of rhabditids to soil type observed only at 25°C. We conclude that nematode response to nitrogen and temperature depends on interactions with soil type, temperature, and faunal composition and these interactions change over time. But since only two samplings were conducted, statistical trends in these interactions of soil type with nitrogen and temperature could not be addressed.

Nematode numbers were lower at the first sampling compared to the initial numbers in both soil types probably because of the soil homogenization in preparing microcosms, which had a slightly delayed effect on nematode numbers and/or resource availability. Schouten (1995) reported that homogenization of mineral soil samples can cause a significant delay in nematode recovery. Therefore mixing and homogenization may have had an initial negative effect. And only rhabditids in the fine soil in the three lowest nitrogen treatments exceeded initial counts after 6 months. In contrast, the bacterial-feeding monhysterids, initially abundant, were totally eliminated over time in most microcosms. Sohlenius (1985) noted that monhysterids are able to grow only under very specific climatic conditions. Dorylaimids, tylenchids and aphelenchids also declined over time. The absence of plants in the microcosms is an obvious reason.

Bååth et al. (1978) reported that microcosms with plants had higher nematode numbers (even compared to the field samples), and that bacterial feeding nematodes increased with nitrogen addition (1.5 mg/pot). According to Bottner et al. (1988), microbial biomass in soils with plants is about 2–4 times greater compared to soil without plants. Clarholm (1985) also found similar results.

The nitrogen deposition rates we used did not have any effect on bacteria and fungi. Fungi were found only on a few petri dishes, which may partially account for the low numbers of fungal feeding nematodes observed. Fisk &Schmidt (1996) found increased microbial activity (nitrification and net mineralization) in alpine soil from Niwot Ridge, Colorado, with a much higher deposition rate of nitrogen (250 g N/ha/yr) than used in this experiment.

In conclusion, nematode response to increasing nitrogen deposition rates varied in a complex way with soil type and temperature although nitrogen deposition level alone did not have a significant affect on nematode density. Under the microcosm conditions, nematode community composition shifted to opportunistic rhabditid species: i.e., to a lower Maturity Index (Bongers 1990; Bongers & Ferris 1999). Much higher levels of nitrogen deposition (>80 kg N/ha/yr) would have been necessary to produce a detrimental affect on rhabditids. Further, our results suggest that extremely high N deposition rates would be required to directly affect nematode communities in alpine systems.

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