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EVIDENCE FOR ATMOSPHERIC DEPOSITION IMPACTS ON THE ENCHYTRAEID WORM POPULATION OF UK UPLAND OMBROTROPHIC PEATS

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The results of four experiments on acidification effects upon the Enchytraeid worm populations of ombrotrophic peats are reported. In the first, populations were measured in peats from *Calluna vulgaris*-dominated microcosms collected from along a gradient in N deposition in the UK and subjected for 18 months to simulated precipitation with a solute composition appropriate for their site of collection. There was a significant decline in Enchytraeid population along the N deposition gradient for *Calluna*-dominant microcosms, but when grasses took over from the *Calluna*, Enchytraeid numbers increased significantly. In the second experiment, two sets of peat moorland microcosms from a single site, supporting *Calluna* and *Calluna*-grass mixed vegetation, were subjected for 12 months to ambient and 2- and 6-times the ambient N deposition. Additional N was added in two forms, as ammonium sulphate and as nitric acid. The high N treatments significantly reduced the Enchytraeid populations for both vegetation types. In a third experiment, the pH preference for the Enchytraeids was assessed using interconnected tubes of peat covering the pH range 2.2–8.7. The preferred pH range after 8 months was 2.7–3.7. In the final experiment, it was found that recolonization with Enchytraeids after initial removal was more rapid under grass dominant vegetation than under *Calluna* dominant vegetation.

KEY WORDS: Enchytraeid, peat, acid deposition, ammonium deposition, *Calluna vulgaris*, grass.

INTRODUCTION

Enchytraeid worms have long been known to exist in large numbers in organic soils (Jegen, 1920; Moszynski, 1930), especially in moist acidic organic soils (Dawod and FitzPatrick, 1993). In acid organic peats, the Enchytraeids' contribution to faunal biomass is variable but, because of their food habits, their role in nutrient turnover is thought to greatly exceed their relative contribution to total biomass (Coulson and Whittaker, 1978). Toutain (1987) suggested that Enchytraeids strongly influence the physico-chemical characteristics of the humus layer and the cycling of plant nutrients in acid soils. Anderson *et al.* (1983), for example, reported that the presence of Enchytraeids decreased the leachate calcium concentration derived from decomposing oak leaves significantly, but had no effect on potassium, ammonium or nitrate concentrations. Addition of other soil animals to soil microcosms in the same study resulted in enhanced concentrations of ammonium in leachates compared to

those in leachates from the unamended controls. However, in another study, the presence of Enchytraeids was shown to enhance significantly ($p < 0.001$) mineral nitrogen leaching from Sitka spruce litter (Williams and Griffiths, 1989).

Thin sections of polished blocks of soils, in which the air and water in pores have been displaced by a hard setting resin prior to sectioning, are valuable for examining effects of soil animals on soil structure (FitzPatrick, 1993). At low pH, Enchytraeids were found to influence soil structure substantially (Dawod and FitzPatrick, 1993). Didden (1990) reported, from the microscopic study of such polished stabilized blocks of soil, that Enchytraeids increased pore continuity and pore volumes. They were also associated with a substantial occurrence of aggregates corresponding to their faecal pellet size.

It has been reported in early studies that there is no simple clear relationship between an Enchytraeid population and standard soil chemical properties (Abrahamsen, 1972). Lundkvist (1977) and Bååth *et al.* (1980a, b) reported a decrease in abundance of *Cognettia sphagnetorum* following application of either dilute sulphuric acid or lime to a podzol in a pine forest. Hågvar and Abrahamsen (1980) reported that liming of humus samples, unlike acidification of the same samples, resulted in a significant reduction in the numbers of *C. sphagnetorum*, but increases in the numbers of two other Enchytraeids studied. However, artificial acid rain treatments of pH 6, 4 and 3 did not affect the abundance of *C. sphagnetorum* or *Mesenchytraeus pelicensis* adversely, but pH 2.5 and 2 treatments and liming all had adverse effects (Abrahamsen, 1983).

Most work on Enchytraeids seems to have been carried out on forest soils, relatively little being reported for the peat soils below the predominantly *Calluna vulgaris* moorland vegetation that is so widespread in the UK uplands (Smith *et al.*, 1993). Standen and Latter (1977) found that worm numbers were not correlated significantly with soil pH for the blanket bog at Moor House National Nature Reserve in Cumbria, although their data suggested an optimum pH of around 3.6–3.8. They pointed out that the pH of peat could influence the worm population indirectly by modifying litter quality or decreasing the rate of litter decomposition.

Recently, Yesmin *et al.* (1995 a and b) reported loss of *Calluna vulgaris* from moorland peat microcosms with *Calluna*-grass mixed cover in an 18-month simulation experiment emulating a realistic nitrogen and sulphur deposition gradient in the UK. Lee and colleagues (1988) in Manchester demonstrated detrimental effects of supra-optimal nitrogen and sulphur deposition on the growth and existence of *Sphagnum* sp.. There are also indications of direct detrimental effects of a lower pH of peat as a consequence of acid deposition; for example, it has been shown that growth of *Calluna vulgaris* is depressed significantly (Sanyi, 1989). Both field studies and simulation experiments have shown decreases in litter decomposition rates at high levels of exposure to acid deposition (Killham and Wainwright, 1984; Brown, 1985; Sanger *et al.*, 1994). It has also been found recently from a simulation experiment that high levels of nitrogen deposition impacting upon peat microcosms result in a marked decline in the extent of ericoid mycorrhizal infection of *Calluna* roots (Yesmin *et al.*, 1995b). Dighton *et al.* (1986) reported small changes in mycorrhizal species composition in a study of effects of acid treatment at pH 3 on pine mycorrhizae, with a reduction for three types and no significant change for three other

types, and fewer root tips in the surface (<5 cm) soil. Little effect was found overall on fine root material or on growth of *Pinus sylvestris* saplings.

These field studies of deposited acidity effects on terrestrial ecosystems and controlled exposure simulation experiments suggest that acid deposition may be affecting the food substrate of Enchytraeids adversely. From the point of view of ecosystem functioning, the faunal community in soil plays a very significant role in facilitating conversion of nutrients from complex organic forms to much simpler plant-available forms. Atmospheric deposition effects on the soil, such as pH fall, increases in base cation leaching, induction of nutrient imbalance or reduction in litter decomposition rate, might, possibly, cause direct and indirect effects on fauna by modifying normal biological processes. The build up of a deepening, porous surface horizon for peats under *Calluna* vegetation (Yesmin *et al.*, 1995a) could well be a consequence of acid deposition effects, either direct, indirect, or in combination, upon the Enchytraeid population.

To test whether there was any evidence for effects of acid deposition to soil upon Enchytraeids, it was decided to measure the Enchytraeid populations at the end of two long-term simulated acid rain and enhanced nitrogen deposition experiments. Both experiments had involved the use of substantial *Calluna vulgaris*-rich peat moorland microcosms subjected to acid deposition of controlled composition (Sanger *et al.*, 1994; Yesmin *et al.*, 1995 a and b). In the first experiment, turfs, complete with predominantly *Calluna* vegetation, were collected from along a nitrogen deposition gradient in the UK. The amounts of wet deposited ammonium- and nitrate-N at the sites are shown in Figure 1, the data being subdivided according to

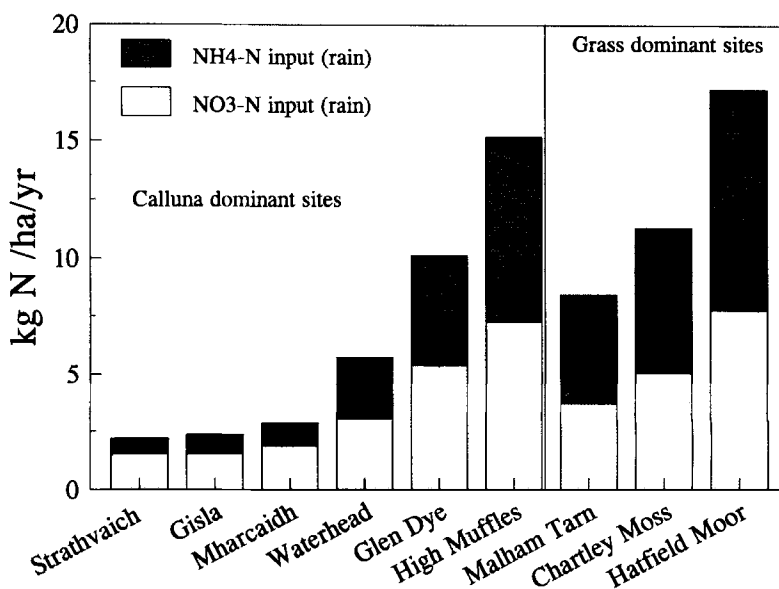


Figure 1 Annual wet deposited ammonium-N and nitrate-N fluxes along the N deposition gradient, plotted separately for the *Calluna* and grass dominant sites.

whether the vegetation was *Calluna*-dominant or grass dominant by the end of the experiment. The turfs were subjected, in Aberdeen, to simulated rainfall with compositions appropriate to their sites of collection.

In the second experiment, peat turfs, with *Calluna* and *Calluna*/grass mixed vegetation cover from a single site in north east Scotland were subjected to ambient and 2 and 6 times the ambient N deposition for the site, giving N application rates of 12, 24 and 72 kg ha⁻¹ yr⁻¹. The extra N was added over 12 months, as HNO₃ in one set of treatments and as (NH₄)₂SO₄ in another set, in simulated rain corresponding to the annual rainfall for the site, added *via* thrice weekly two- to three-hour showers of fine spray. A third experiment to quantify the pH preference of the Enchytraeids in a typical Scottish peat soil was set up and a fourth to estimate population recovery rates of Enchytraeids in peats from under the same vegetation types, following removal of the initial population.

METHODOLOGY

Sample Collection and Preparation

The site selection, turf collection and experimental design of the first experiment have been described fully elsewhere (Sanger *et al.*, 1994; Yesmin *et al.*, 1995a). Nine sampling sites with ombrotrophic peat were chosen close to UK Department of the Environment (DOE) precipitation monitoring sites (Sanger *et al.*, 1994), to provide a nitrogen (N) pollution gradient between Strathvaich in north west Scotland and Hatfield Moor in the English Midlands. Of the 9 sampled sites, 6 were *Calluna* dominant and the rest were grass dominant. For the first experiment, six replicate peat turfs, each 320 × 460 × 150 mm (depth) were cut from each site and artificial rain appropriate to each site, containing solutes corresponding to the volume-weighted mean solute composition (1986–88) for the nearest monitoring station, was applied for 18 months (Sanger *et al.*, 1994). To eliminate the effects of natural variability of rainfall amount and temperature, all the turfs were kept in a very well ventilated glasshouse and a uniform amount of rain was applied to each turf. After 18 months, the population of Enchytraeid worms was counted in peat from each site.

For the second experiment, deeper microcosms were thought to be desirable, so 20 approximately 200-mm deep turfs were collected from the top of peat profiles more than 0.5 m deep under both *Calluna* and grass dominant vegetation from Glen Dye (NO 642864) in north eastern Scotland. Artificial rain treatments were applied which contained solute corresponding to the volume-weighted mean solute composition for Glen Dye, viz. 63 µM NaCl, 7 µM MgCl₂, 1 µM MgSO₄, 4.5 µM CaSO₄, 2.3 µM K₂SO₄ and 27.5 µM H₂SO₄ respectively. The N treatments applied were ambient, 2 times and 6 times the ambient values for the site. The simulated rainfall events were applied *via* laboratory-built, gravity fed plastic and silicone rubber nebulizers, 3 times per week, each event lasting 2–3 hours and simulating 6.8 mm of precipitation. The worm populations were counted after 12 months.

The Extraction and Counting of Enchytraeid Worms

The technique used for the extraction of Enchytraeid worms was the modified Baermann funnel method developed by O'Connor (1955). Large (110 mm diameter) polyethylene funnels were attached to lengths of tubing, each closed by a small clamp. A nylon sieve with a 1-mm mesh was attached at 30 mm below the upper rim of each funnel. Peat sample blocks from the top 100 mm of the peat microcosms were each cut into three horizontal layers, corresponding to 0–20, 20–50 and 50–100 mm depths. Each layer was broken up separately by hand and the peat from each placed gently on to a sieve. Each funnel was then filled carefully with water to a depth of 10 mm above the peat sample. Heat was supplied above each funnel from a 275 W bulb for approximately 3 hours, until the peat surface temperature reached *ca.* 45°C. The worms migrate down into the funnel stem, from where they can be collected by opening the clamp.

Some small peat particles (<1 mm) passed through the sieve with the worms. To facilitate counting under the microscope, the worms were stained by adding aniline blue solution containing 1 part of 1% aniline blue solution to 15 parts of 5% phenol solution and 4 parts of glacial acetic acid. Dye solution (10 ml per 30 ml of sample) was added to each flask containing worms and drainage water, and the flask and contents were left for 10–15 minutes to stain the worms. The solutions were then filtered, leaving the worms and soil particles on the filter paper. The worm/soil particulate mix was removed from the filter paper to a Petri dish with a few ml of water, and the stained worms were counted under a low power (X10) microscope.

Method used to Assess the pH Preference of Enchytraeid Worms

All the worms were removed from a bulk Glen Dye peat sample by extraction as described above. Then worm-free peat was sieved through a 3-mm sieve. The pH of the peat at the Glen Dye site was measured and found to be 3.7 as a 1:2.5 soil:water paste. To assess the amounts of $\text{Ca}(\text{OH})_2$ needed to raise the peat pH to selected values, the pH of 30 g sieved moist peat sub-samples was raised by mixing in different amounts of $\text{Ca}(\text{OH})_2$. Similarly pH was lowered by adding different amounts of dilute 1% HCl to peat sub-samples. Graphs of peat pH against amounts of $\text{Ca}(\text{OH})_2$ or HCl added were plotted, and these were used to quantify the treatments needed to achieve the desired range of pH value, 2.2, 2.7, 3.7, 4.7, 5.7, 6.7, 7.7 and 8.7.

After adjustment of the peat pH values of separate peat sub-samples to the required pH values, the pH-adjusted sub-samples were packed lightly into a series of black plastic tubes (syringe barrels) open at both ends. A set of eight tubes, covering the above pH range, was then fitted through holes in a metal foil box. The foil box was filled with peat at field pH containing a normal worm population (approx. 5000 dm^{-3}). Eight such boxes were assembled. Duplicate boxes were harvested and worms were extracted from each tube and counted after 1, 2, 4 and 8 months.

Method used to Assess the Rate of Recolonization by Enchytraeid Worms

Peat turfs from under two different vegetation types, *Calluna* and *Calluna* plus *ca.* 80% grasses, were collected from Glen Dye in September 1993. Sixteen peat blocks,

eight from each vegetation type, were cut square with a cross sectional area of 4900 mm² and a depth of 100 mm. Each block was further subdivided into 5 horizontal slices, each 20 mm thick. Worms were extracted from all slices without breaking them, and the layers were then put back together to reconstitute the original blocks. Blocks were then wrapped on four vertical sides with black polyethylene and kept in an unheated glasshouse. The blocks were subjected to simulated rainfall appropriate to the site to keep them moist. The composition of the simulated rainfall for Glen Dye has been given in an earlier section. Two replicates for each vegetation type were sampled, and worms were extracted and counted after 1 month (October), 2 months (November), 4 months (January) and 8 months (May). Extraction of worms and counting were carried out according to the procedure described earlier.

RESULTS

Transformation of the Data and Statistical Analysis

In the first and second experiments, peats were sampled as blocks with a surface 50 × 50 mm and 100 mm deep. These were subdivided into three unequal portions, 0–20, 20–50, and 50–100 mm. It is not meaningful to compare directly the numbers of worms in unequal thicknesses, so the results are expressed on a unit volume basis. The distribution of worms was clustered in these experiments, especially in the second experiment, and the differences between replicates were large. After examining several methods of transforming the data, it was decided that a logarithmic transformation gave the closest proximity to a normal distribution. For experiments 3 and 4, square root transformations were used for statistical analyses.

Analyses of variance were carried out on the transformed data to determine any significant differences in the size of the Enchytraeid populations for various nitrogen deposition rates, sites and vegetation types. LSD (least significant difference) values were calculated on transformed data and used to test significance of differences between pairs of means (Tables I–IV).

Experiment 1

The mean population results for the first experiment, showing the difference in Enchytraeid population in peats from along the nitrogen deposition gradient in the UK, are summarised in Figure 2 and Table I. A significant decrease was observed along the nitrogen deposition gradient under *Calluna*, and numbers declined significantly with depth. However, grass dominant sites, although subjected to more nitrogen deposition than the most exposed *Calluna* dominant site (High Muffles), showed higher populations generally than *Calluna* dominant sites.

Experiment 2

The results of the second experiment showed significantly smaller worm populations under both high nitric acid and high ammonium sulphate treatments (Fig. 3 and

Table I Summary of results of statistical analysis for experiment 1, for Enchytraeid population differences between sites and with depths for the same site.

| <i>Depth*</i> <i>Site x depth*</i> <i>Site (Calluna dominant)*</i> | <i>Depth*</i> <i>Site x depth*</i> <i>Site (Grass-dominant)*</i> |
|--|--|
| Strathvaich ^c | Malham Tarn ^B |
| Gisla ^c | Chartley Moss ^C |
| Mharcaidh ^c | Hatfield Moor ^A |
| Waterhead ^{bc} | |
| Glen Dye ^b | |
| High Muffles ^a | |

* denotes significant difference(s) observed at the 5% level. Populations were significantly different between *Calluna*-dominant sites with no common superscript lower case letter, or between grass dominant sites with no common superscript capital letter. Choice of superscript letter denotes relative size of significant differences, so that C > B > A and c > b > a. Sites are listed in order of increasing nitrogen deposition.

Table II Summary of results of statistical analysis for experiment 2, for Enchytraeid population differences between treatments and with depths for the same treatment.

| <i>Calluna</i> | <i>Grass</i> |
|---|---|
| <i>Depth*</i> <i>Treatment x depth*</i> <i>Treatment*</i> | <i>Depth*</i> <i>Treatment x depth*</i> <i>Treatment*</i> |
| Control ^c | Control ^B |
| Medium nitrate ^c | Medium nitrate ^B |
| Medium ammonium ^{bc} | Medium ammonium ^B |
| High nitrate ^{ab} | High nitrate ^A |
| High ammonium ^a | High ammonium ^A |

* denotes a statistically significant difference at the 5% level. Populations were significantly different between treatments with no common lower case superscript letter for *Calluna* dominant sites and between treatments with no common capital letter for grass dominant sites. Control, medium and high refer to ambient N deposition and 2 and 6 times ambient N deposition, respectively.

Table II). A reduced abundance of Enchytraeids with higher ammonium and nitrate deposition was found for both vegetation types. Population density also declined significantly with depth under both vegetation types. For the high ammonium sulphate treatment, however, more Enchytraeids were found per unit volume of peat at 20–50 mm than at 0–20 mm depth.

Experiment 3

The results of the Enchytraeid acid tolerance/pH preference test are presented in Figure 4 and Table III, which show that the worms prefer the pH range

Table III Summary of results of statistical analysis for experiment 3, for Enchytraeid population differences between peats at specified pH values and with time at the same pH.

| pH 2.2 | pH 2.7 | pH 3.7 | pH 4.7 | pH 5.7 |
|----------------------|----------------------|----------------------|----------------------|----------------------|
| Month 1 ^b | Month 1 ^a | Month 1 ^a | Month 1 ^a | Month 1 ^a |
| Month 2 ^b | Month 2 ^a | Month 2 ^a | Month 2 ^a | Month 2 ^a |
| Month 3 ^c | Month 3 ^b | Month 3 ^b | Month 3 ^a | Month 3 ^a |
| Month 4 ^a | Month 4 ^b | Month 4 ^b | Month 4 ^a | Month 4 ^a |

Absence of common superscript letters at a single peat pH denotes a significant difference between populations in different months. No worms were found at higher pH values.

Table IV Summary of results of statistical analysis for experiment 4, for Enchytraeid population differences over time and between vegetation types.

| Time* | |
|----------------------|----------------------|
| Vegetation* | |
| <i>Calluna</i> | <i>Grass</i> |
| Month 1 ^a | Month 1 ^A |
| Month 2 ^a | Month 2 ^A |
| Month 4 ^a | Month 4 ^A |
| Month 8 ^b | Month 8 ^B |

* denotes significant differences observed at the 5% level. Common lower case and capital superscript letters under *Calluna* and grass dominant vegetation types respectively denote no significant difference between populations in different months.

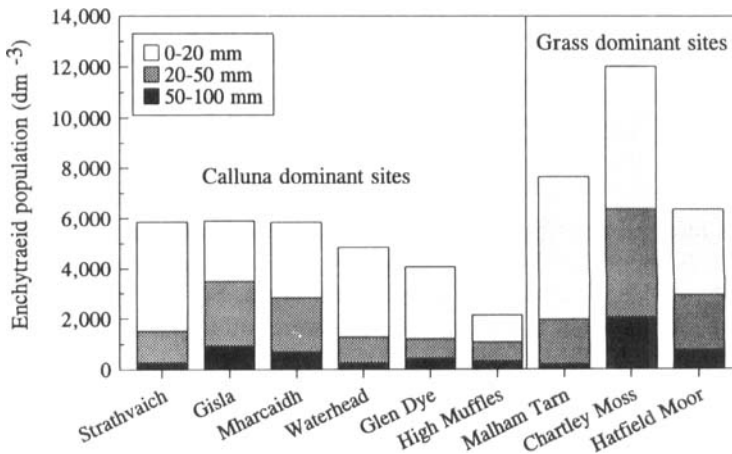


Figure 2 Populations of Enchytraeids at 0–20, 20–50 and 50–100 mm depths in the nine peats at the end of experiment 1. Results are plotted in order of increasing N deposition.

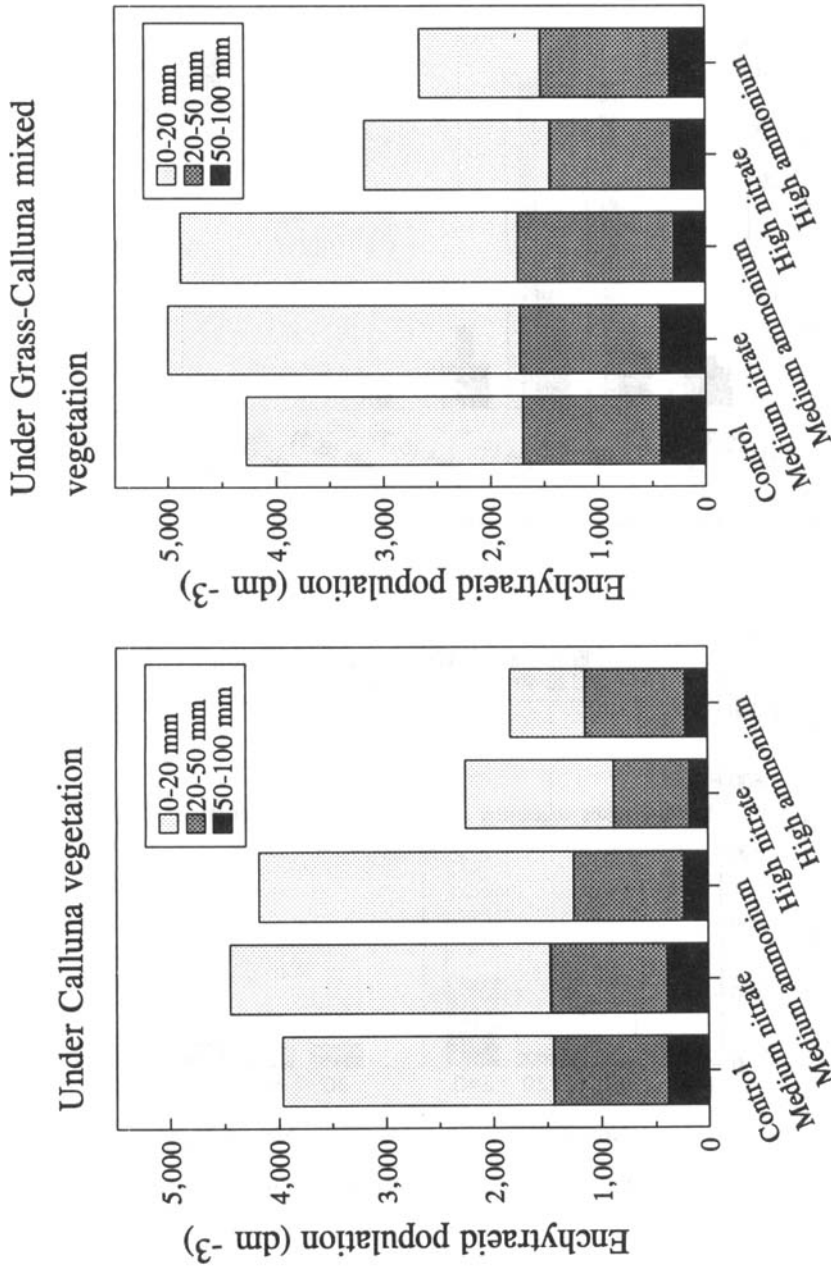


Figure 3 Effects of depth and N treatment upon Enchytraeid populations in peats under *Calluna vulgaris* and grass dominant vegetation, at the end of experiment 2.

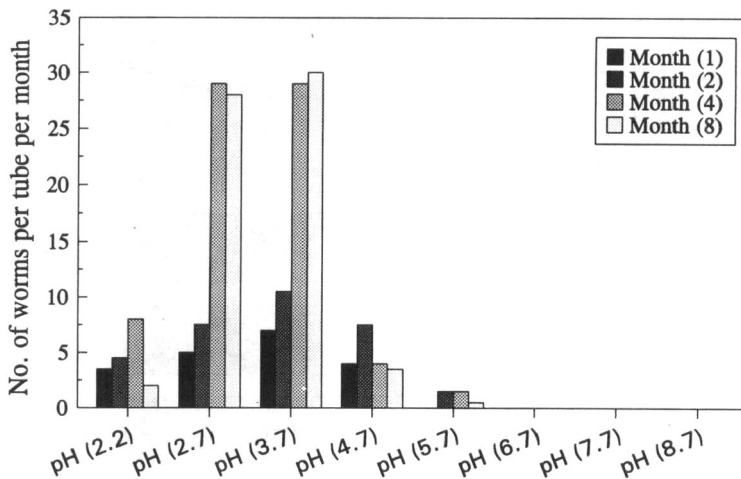


Figure 4 Effects of time and peat pH upon numbers of worms per tube in experiment 3.

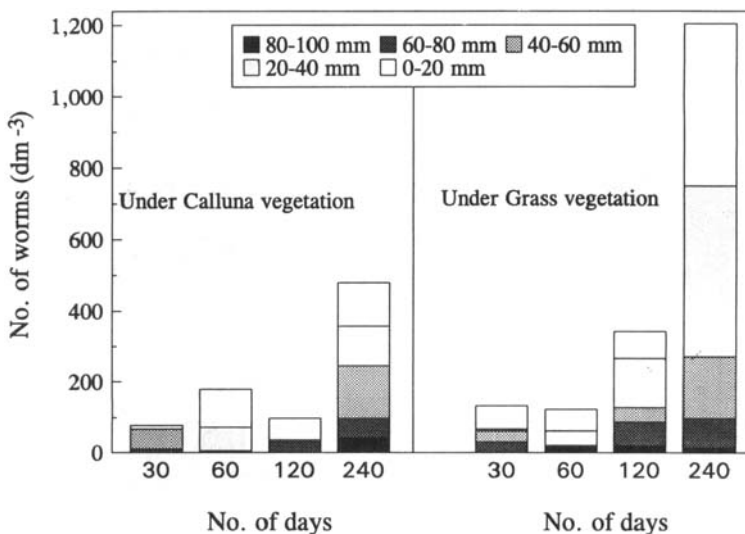


Figure 5 Effects of time, depth in peat and vegetation type on peat used on Enchytraeid population recovery in experiment 4.

2.7–3.7. Even though they are generally perceived as acid preferring species, their numbers were significantly lower under both the lowest and higher pH conditions by the end of the 8 months. No worms were found at pH 6.7 to pH 8.7.

Experiment 4

The results of the recolonization experiment are shown in Figure 5, and their statistical analysis is summarised in Table IV. Overall, the population growth was faster for the peat from under 80% grass vegetation than for the peat from under *Calluna*. For the grass dominant peat, the top 20 mm appeared to make a major contribution to the recolonization, although this effect was not apparent for the *Calluna* peat. At day 30, the lowest 20 mm studied (80–100 mm) appeared not to be contributing to the recolonization.

DISCUSSION

In the first experiment, a significant decreasing trend in Enchytraeid population along a nitrogen deposition gradient under *Calluna*-dominant vegetation was observed. The presence of accompanying gradients in vegetation type and in climate also might influence worm population. For example, in peat from the Malham Tarn site, the lower population of worms could be explained in terms of the heavy moss cover on the surface at this site. Standen and Latter (1977) showed that *Cognettia sphagnetorum* was more numerous in *Calluna* and *Eriophorum* litters than in *Sphagnum* litter. It might be that mosses decompose very slowly. Above ground vegetation cover determined the quality of litter and underlying peat, and worms were more abundant under grass than under *Calluna* in experiment 1. Even recolonization by worms in experiment 4 was higher under grass than under *Calluna*, again evidence that the worms prefer grass to *Calluna*. From the work of Sanger *et al.* (1994) and others, it is clear that grass litter decomposes faster than *Calluna* litter, which might reflect the preference of Enchytraeids for grass. Standen (1978) showed that one of the most important factors in Enchytraeid distribution in peat was the quality of litter. Enchytraeid population was always found to be greater in easily decomposable litter. The population was higher under sedge than under *Calluna* litter, for example, because the sedge leaves are less lignified than those of *Calluna*.

It is difficult to establish unequivocally any direct relationship between pollution gradient and population from experiment 1, as climatic and vegetational gradients occurred concurrently with the pollution gradient. In the second experiment, the population of worms decreased significantly under both high nitric acid and high ammonium sulphate treatments, and the effect was similar under both grass and *Calluna*. These results are a less equivocal indication of a direct effect of N deposition upon Enchytraeid population, since there were no climatic or vegetation type gradient effects. It is possible that the sudden increase of nutrient N concentration could affect worm population directly. Abrahamsen and Thompson (1979) showed that heavy fertilization affected the Enchytraeid population, although numbers recovered slowly after 2–3 years. Atmospheric deposition might act differently due to changes in soil chemical properties, for example *via* peat acidification (Skiba *et al.*, 1989; Smith *et al.*, 1993). In this experiment the peat has a very low pH and

all species are categorised as acidophilic. The pH preference experiment was conducted to give some insight into the extent to which a population might be affected by chemical change of the environment. The results in Figure 4 suggest that a value $\text{pH} < 2.7$ of peat soil solution would be required for acidity to affect the worm population size. It cannot be ruled out that peat soil solution pH values below 2.7 might occur, especially for sites strongly affected by acid deposition and during dryer periods, when the mobile anion effect, especially that associated with chloride deposition, may increase in importance. It should also be remembered that the pH preference experiment did not take account of any accompanying effects which acid deposition might have upon litter quality or upon the associated peat microflora.

Studies by Sanger *et al.* (1994) showed that reduced rate of litter decomposition may be related to atmospheric acidifying pollutant deposition. It could be that a reduced Enchytraeid population contributes to this slowing down in decomposition, especially if high ammonium deposition results in downward migration of Enchytraeids, as the second experiment suggests.

CONCLUSIONS

Although a significant decrease was observed in peat Enchytraeid population under *Calluna* along an increasing nitrogen deposition gradient, increasing grass dominance in vegetation was associated with higher populations, even at grossly polluted sites. Significantly higher recolonization was also found under grass than under *Calluna*. Short to mid-term changes in soil *via* enhanced nitrogen inputs, either as ammonium sulphate or nitric acid, in simulated rainfall significantly depressed Enchytraeid worm numbers under both grass and *Calluna*, which indicates that they can be affected by a change in acidifying pollutant deposition. The pH preference of the native Enchytraeid population in a typical acidic Scottish ombrotrophic peat was 2.7–3.7.

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