



## Impact of $\text{NH}_4\text{NO}_3$ on microbial biomass C and N and extractable DOM in raised bog peat beneath *Sphagnum capillifolium* and *S. recurvum*

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**Abstract.** Regular bi-weekly additions of  $\text{NH}_4\text{NO}_3$ , equivalent to a rate of  $3 \text{ g N m}^{-2} \text{ yr}^{-1}$ , were applied to cores of *Sphagnum capillifolium*, inhabiting hummocks and *S. recurvum* a pool and hollow colonizer, in a raised bog in north east Scotland. Microbial biomass C and N, both measured by chloroform extraction, showed similar seasonal patterns and, for most depths, the effects of added N on microbial biomass C and N changed with time. The addition of inorganic N had greatest effect during October when the water table had risen to the surface and microbial C and N in the untreated cores had decreased. Microbial C and N were maintained at  $75 \text{ g C m}^{-2}$  and  $8.3 \text{ g N m}^{-2}$  above the values in the untreated cores and far exceeded the amounts of N that had been added up to that date ( $1 \text{ g N m}^{-2}$ ) as  $\text{NH}_4\text{NO}_3$ . This increased microbial biomass was interpreted as leaching of carbonaceous material from the  $\text{NH}_4\text{NO}_3$  treated moss resulting in greater resistance of the microbial biomass to changes induced by the rising water table. Treatment with N also caused significant reductions in extractable dissolved organic N (DON) at 10–15 cm depth, beneath the surface of the moss, but at lower depths to 25 cm no changes were observed. Extracted dissolved organic carbon (DOC) was not affected by N treatment and showed less seasonal variation than DON, such that the C:N ratio of dissolved organic matter (DOM) in all depths increased from approximately 4 in July to around 30 in December.

### Introduction

Peatlands sequester and accumulate carbon (C) and are perceived as sinks for nitrogen (N), derived largely from the atmosphere, though the retention of N during the period of peat accumulation appears to vary widely between bogs (Damman 1988). As concentrations of N in wet and dry deposition increase (INDITE 1994) it has been suggested that peatland ecosystems, through their capacity to retain N, are susceptible to changes in vegetation and in the rates of accumulation of organic matter (Aerts et al. 1992).

*Sphagnum* mosses are among the principal peat forming plants in raised bogs and have been the focus of attention in relation to the impact of N and other pollutants (Woodin & Lee 1987). These mosses can assimilate more N than they currently receive in atmospheric deposition (Li & Vitt 1997; Williams & Silcock 1997). However, there is little information about the transfer of N that has been assimilated and its effects on the underlying peat. *Sphagnum* species receiving inorganic N accumulate free amino acids (Baxter et al. 1992) and increased concentrations of dissolved organic nitrogen (DON) in water extruded from the moss have been detected (Silcock & Williams 1995). A study of the  $\text{NH}_4^+$ , DON and microbial N pools beneath two moss species treated with  $^{15}\text{NH}_4$   $^{15}\text{NO}_3$  showed that, on average, 72 per cent of the added  $^{15}\text{N}$  was taken up by the moss in a 2 week period (Williams et al. 1999). The amount of DON in the water extracted from the moss increased linearly with the amount of inorganic N added, but the proportion of  $^{15}\text{N}$  found in the DON was very small,  $< 0.1$  per cent, and very little was found in extractable DON and microbial biomass N pools in the underlying peat. Williams and Silcock (1997) reported increased microbial biomass C in peat beneath *S. magellanicum* which had shown a growth response to  $\text{NH}_4\text{NO}_3$  additions at  $1 \text{ g N m}^{-2}$  over a period of 4 months, whereas  $10 \text{ g N m}^{-2} \text{ yr}^{-1}$  failed to show any effects.

It was concluded from these previous studies, that if there is an impact of N in atmospheric deposition on peat beneath the vegetation, then the effect is transmitted via the plant, possibly as carbon substrates. In this paper, we test this hypothesis in a field experiment in which inorganic N was applied regularly to two *Sphagnum* moss species occupying contrasting habitats on the bog surface. The impact of the applied N was examined by measuring the seasonal dynamics of microbial biomass C and N, and easily extractable organic C and N in the underlying peat.

## Materials and methods

### *Experimental site*

The experimental site is a raised bog, the Moidach More (National Grid Reference NJ 030420) in the north-east of Scotland, located at an altitude of 275 m above sea level. The mean annual rainfall is approximately 800 mm and the mean annual temperature  $8^\circ\text{C}$ . The average depth of the peat is 2.1 m and peat more than 0.5 m thick extends to 760 ha. The dominant vegetation comprised *Sphagnum* species such as *Sphagnum magellanicum* Brid., *S. papillosum* Lindb., *S. capillifolium* (Ehrh.) Hedw. and *S. recurvum* var. *mucronatum* (Russ.) Warnst., and *Erica tetralix* L. and *Trichophorum*

*cespitosum* (L.) Hartm.. *Calluna vulgaris* (L.) Hull occupied areas where there had been disturbance from peat cutting or burning. The experiment was carried out on carpets of *Sphagnum capillifolium* (Ehrh.) Hedw. and *S. recurvum* var. *mucronatum* (Russ.) Warnst. where relatively undecomposed moss extended to 20 cm depth (Williams et al. 1999).

### *Field experiment*

*Sphagnum capillifolium* and *S. recurvum* were chosen for the experiment because they colonised contrasting sites, *S. capillifolium* is a hummock forming species whereas *S. recurvum* occupies hollows and pools (Daniels & Eddy 1985). During June 1994, hollow pvc cylinders, (length 30 cm, internal diam. 7.5 cm) were inserted into the moss carpet at 216 points (108 for each moss species) on the bog surface and vascular plants removed. The cylinder top was level with the surface of the moss carpet. The cores were set out in three replicate areas (approx. 50 m<sup>-2</sup>) for each species, where the moss species were concentrated. Using a randomising procedure, cores within each block were assigned harvest dates and treatments such that adjacent cores were not removed at the same time. At two week intervals, the controls received deionised water (200 cm<sup>3</sup>) and an aqueous solution of NH<sub>4</sub>NO<sub>3</sub> containing 0.51 mg N core<sup>-1</sup> (equivalent to 0.115 g N m<sup>-2</sup> every 2 weeks or 3 g N m<sup>-2</sup> yr<sup>-1</sup>) was added to the N treated cores. The solutions were applied in 200 cm<sup>3</sup> aliquots using a syringe modified to include 6 needle outlet ports to simulate the dropwise addition of rain. To avoid edge effects, solutions were applied evenly over a quadrat, 20 cm × 20 cm, that contained the core at its centre. Two weeks before a set of cores was due for harvesting the regular addition of NH<sub>4</sub>NO<sub>3</sub> at natural abundance was replaced by the same quantity of N as <sup>15</sup>NH<sub>4</sub><sup>15</sup>NO<sub>3</sub> at 99.8 atom% (ISOTEC Inc., Ohio). Regular N additions were postponed if cores became waterlogged or iced-over during the winter and additional amounts added later when conditions allowed and this occurred in both moss species. Initially, 12 cores (3 replicates × 2 species × 2 treatments) were harvested monthly and then less frequently between October and March. On two occasions in December and March, cores were removed from the bog to an open area adjacent to the laboratory for treatment with <sup>15</sup>NH<sub>4</sub><sup>15</sup>NO<sub>3</sub> and subsequent incubation. The bottoms of these cores were covered with nylon mesh and the cylinders placed inside tall 1 dm<sup>3</sup> beakers with an artificial water table maintained at the surface of the core as it had been in the field, by the addition of deionised water. Solutions were added and the cores incubated in the open for two weeks prior to sampling. The cores in the field experiment were harvested on nine different occasions between July 1994 and August 1995, the date referring to the month of harvest.

The water-table depth was measured at two week intervals at a central point in each of the three blocks for each of the two *Sphagnum* species. Rainwater was collected every two weeks at three locations at the field site adjacent to the cores.

#### *Core analysis*

Each peat core was removed from the pvc cylinder in the laboratory and sliced transversely into 5 cm sections for analysis. Some cores were incomplete after excavation, but in all cases the cores extended to 25 cm depth. Each section was weighed and stored at 4 °C prior to sub-sampling. The surface 5 cm of each core containing the live moss was removed for <sup>15</sup>N analysis, the results of which have been described previously (Williams et al. 1999).

Moisture contents of the cores were determined by drying weighed sub-samples at 105 °C. Bulk density was expressed as the weight of dry matter per unit volume as ash contents were between 2 and 3.1% of dry matter (Williams et al. 1999). The degree of decomposition of samples was assessed using the method of von Post (1929). Acidity was measured as the pH of suspensions of peat in 0.01 M CaCl<sub>2</sub> at a sample: solution ratio of 1:5 (w/v).

For inorganic and dissolved organic nitrogen (DON) in the peat below 5 cm depth, fresh samples (10 g) were shaken for 2 hr with 50 cm<sup>3</sup> 0.5 M K<sub>2</sub>SO<sub>4</sub> and filtered through glass fibre filters (Whatman GFA) under suction. The peat was washed with a further 50 cm<sup>3</sup> of extractant and after filtering again through Millipore filters (0.45 µm) extracts were made up to 100 cm<sup>3</sup>.

#### *Microbial C and N*

Microbial C and N were determined by the fumigation extraction (FE) method after fumigation for 18 h with chloroform vapour (Williams & Sparling 1984) followed by extraction with 0.5 M K<sub>2</sub>SO<sub>4</sub>. Microbial C was calculated from the flush of dissolved organic carbon (DOC) extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> using the recovery factor of 0.45 derived for peat soils by Sparling et al. (1990). Microbial C was also measured using the substrate induced respiration (SIR) method (Anderson & Domsch 1978) and using conductimetric measurements to determine CO<sub>2</sub> sorbed by 0.01 M KOH over a 4 h period (Chapman 1971). Microbial C was calculated using the relationship derived by Sparling et al. (1990): microbial C (mg) = 50 × evolved CO<sub>2</sub> cm<sup>3</sup> g<sup>-1</sup> dry weight h<sup>-1</sup>. Microbial N was calculated from the total N flush using the value 0.54 for k<sub>N</sub> (Brookes et al. 1985).

### *Chemical analyses*

Total dissolved N (TDN) concentrations in the  $K_2SO_4$  extracts of fumigated and fresh samples were measured as  $NO_3^-$  after oxidation of extracts with alkaline potassium persulphate (Williams et al. 1995). Extracts were made alkaline by addition of MgO and the resulting  $NH_3$  diffused, into 0.01 M  $H_2SO_4$  (Bremner 1965) and analysed colorimetrically (Crooke & Simpson 1971). Nitrate was determined colorimetrically after reduction to  $NO_2^-$  using copperised cadmium (Henriksen & Selmer-Olsen 1970). Dissolved organic nitrogen (DON) was calculated as the difference between the total N and the  $NH_4^+$  and  $NO_3^-$  -N concentrations.

For measurements of  $^{15}N$  abundance of the TDN and  $NH_4^+$  pools, the N in each pool was converted to  $NH_4^+$  and diffused into 0.001 M  $H_2SO_4$  at 30 °C which was then dried (Williams et al. 1999). Small amounts of  $NH_4^+$  were supplemented with 25  $\mu g$  N as  $(NH_4)_2SO_4$  at natural abundance. The ratios of  $^{15}N:^{14}N$  were measured in a mass spectrometer (Finnegan MAT, Hemel Hempstead, U.K.).

Dissolved organic carbon (DOC) in extracts of fresh and fumigated samples was determined by oxidation using a soluble carbon analyser (OI Analytical Model 700, OI Corp., College Station, TX, U.S.A.).

### *Statistical analysis*

The results were expressed on an area ( $g$  or  $mg\ N\ m^{-2}$  for each 5 cm thick layer) for statistical analysis. A comparison of the two N treatments and two species over nine sampling times was carried out at each depth by analysis of variance (Table 1). For skewed distributions, values were transformed to the natural logarithm, but for clarity, untransformed means and standard errors of difference from the ANOVA of untransformed data are presented. A repeated measures technique, using values from all five depths, was also used at each sample date to test for treatment effects throughout the cores to 25 cm depth (Kenward 1987). All statistical analyses were performed using the Genstat package (Genstat 5 Committee 1993).

## **Results**

The visual and physical characteristics of the peat profiles in the surface 25 cm beneath the two *Sphagnum* species were similar (Williams et al. 1999). Water table level fell between June and August 1995 to 35 cm below the surface at both sites rising again in September. From September until June the water table was at a significantly ( $P < 0.001$ ) lower depth beneath

Table 1. Analysis of variance table for log transformed values of microbial C in the 5–10 cm layer measured using the substrate induced (SIR) method.

Source of variation	Degrees of freedom	Sum of squares	Variance ratio	F probability
block.species stratum				
species	1	3.8668	5.31	0.082
residual	4	2.9103	1.38	
block.species units stratum				
ntrt	1	0.0442	0.08	0.773
time	8	74.1087	17.56	<0.001
species*ntrt	1	1.3489	2.56	0.115
species*time	8	17.3823	4.12	<0.001
ntrt*time	8	13.3215	3.16	<0.004
species*ntrt*time	8	5.9393	1.41	0.210
residual	66 (2)*	34.1820		
total	105 (2)	134.5656		

\*number of missing values.

*S. capillifolium* than *S. recurvum*, mean 4.7 cm compared with 2.2 cm (Table 2).

#### Microbial biomass

##### Microbial C (SIR)

Microbial biomass C (SIR) fluctuated significantly ( $P < 0.001$ ) with time (Table 1) at all depths mainly because of high values obtained during March 1995 and low values during August 1994 (Figure 1) when the water table reached its greatest depth (Table 2). Consistent differences between the species were not evident at any of the depths. At each depth, microbial C was increased by the N treatment at some time during September, October or December and decreased relative to the control at other dates. This interaction between N and time was significant ( $P < 0.05$ ) at 5–10, 10–15 and 20–25 cm depths and the positive effect of N occurred as the water table rose after the summer. The lower the depth in the profile the later this effect of N appeared suggesting that it was influenced from the surface moss downwards, by passage of rain.

At 10–15 cm depth, the effect of N averaged over all dates was significantly ( $P < 0.001$ ) different between the two species. Mean values decreased from 18.4 to 14.6 g C m<sup>-2</sup> under *S. capillifolium* and increased from 10.9 to 15.1 g C m<sup>-2</sup> under *S. recurvum* (sed = 2.7,  $n = 24$ ). In the 15–20 cm and

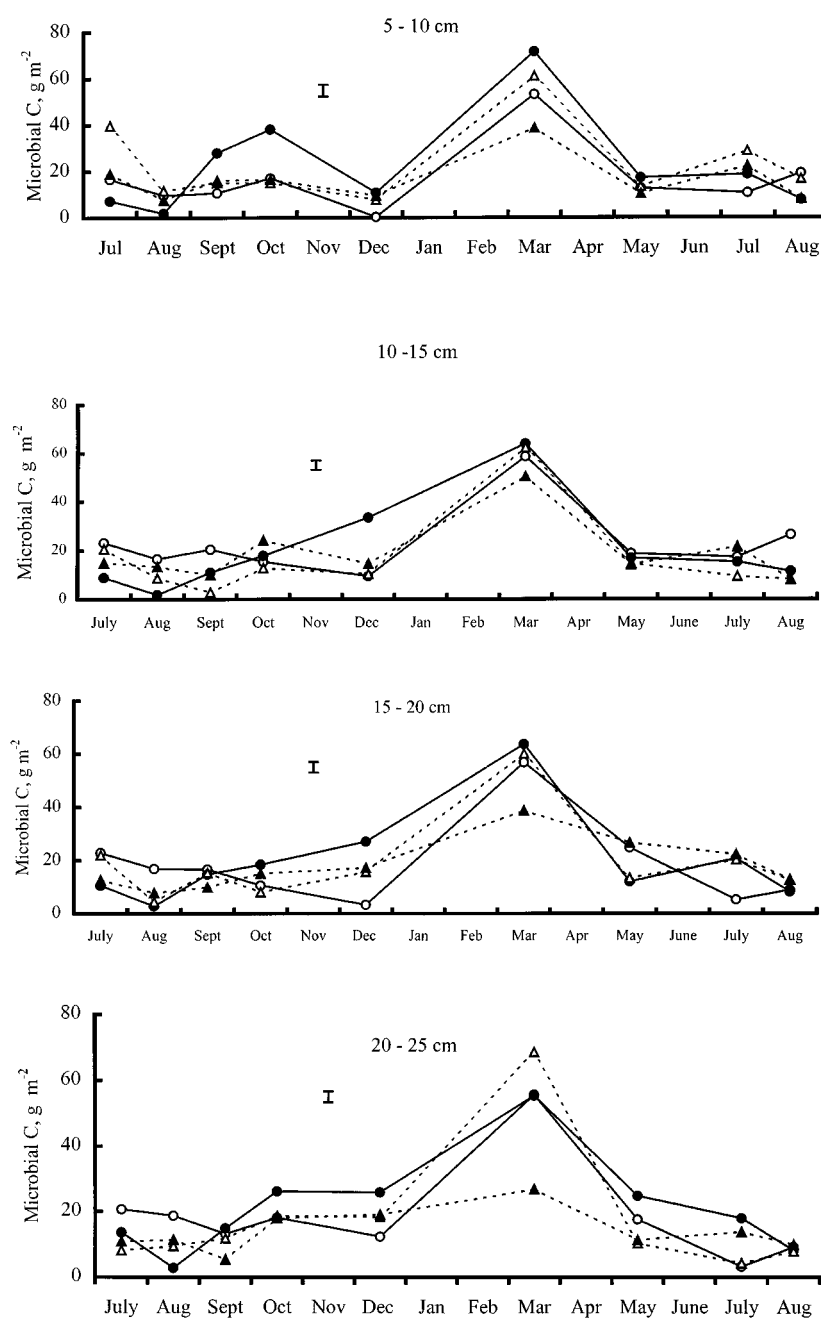


Figure 1. Contents of microbial biomass carbon (SIR), g C m<sup>-2</sup>, in control and N treated cores receiving, 0.115 g N m<sup>-2</sup> every two weeks, for *S. capillifolium* and *S. recurvum* cores, at four depths, 5-10, 10-15, 15-20 and 20-25 cm below the top of the moss. Error bars indicate the standard error of difference between means for each sample time. ○ – *S. capillifolium* control, ● – *S. capillifolium* + NH<sub>4</sub>NO<sub>3</sub>, △ – *S. recurvum* control, ▲ – *S. recurvum* + NH<sub>4</sub>NO<sub>3</sub>.

Table 2. Mean monthly amounts (cm) of rainfall and water table depth (cm) below the surface of *S. capillifolium* and *S. recurvum* on the Moidach More.

Month	Rainfall (cm)	Water table depth (cm)	
		<i>S. capillifolium</i>	<i>S. recurvum</i>
July 1994	6.5	23.6	21.5
August	5.3	27.7	25.9
September	11.4	8.6	8.4
October	4.1	7.7	3.7
November	10.7	5.3	1.7
December	2.6	3.0	0.1
January 1995	11.9	3.5	4.9
February	4.8	1.4	2.1
March	4.7	5.3	0.3
April	3.4	2.4	0.9
May	13.8	6.9	5.5
June	6.1	7.4	3.1
July	4.7	27.9	20.5
August	0.7	32.8	35.0

20–25 cm layers, there were no significant differences between the species and N treatments at different times.

#### *Microbial C (FE)*

Microbial biomass C (FE) increased sharply between August and September 1994 coinciding with the rise in the water table and decreasing aeration (Figure 2). The lowest values recorded in August were in samples from the 5–10 cm layer, where aeration was greatest. Biomass C decreased again during May, but this preceded any marked change in water table and could not be related to any other factors.

At all depths, the effect of N addition varied significantly ( $P < 0.01$ ) with time largely because during October and May microbial C values were greater in the N treated cores (Figure 2). The total microbial C content, summed for the 5–25 cm depth and averaged over the two species, for the October sample date was also significantly ( $P < 0.01$ ) greater in the N treated cores than in the control,  $125.3 \text{ g C m}^{-2}$  compared with  $50.3 \text{ g C m}^{-2}$  (sed = 11.1,  $n = 6$ ). In May, the total microbial C values averaged over the two moss species were again significantly ( $P < 0.05$ ) greater in N treated than control cores, 64.0 and  $15.8 \text{ g C m}^{-2}$  (sed = 18.8,  $n = 6$ ), respectively.



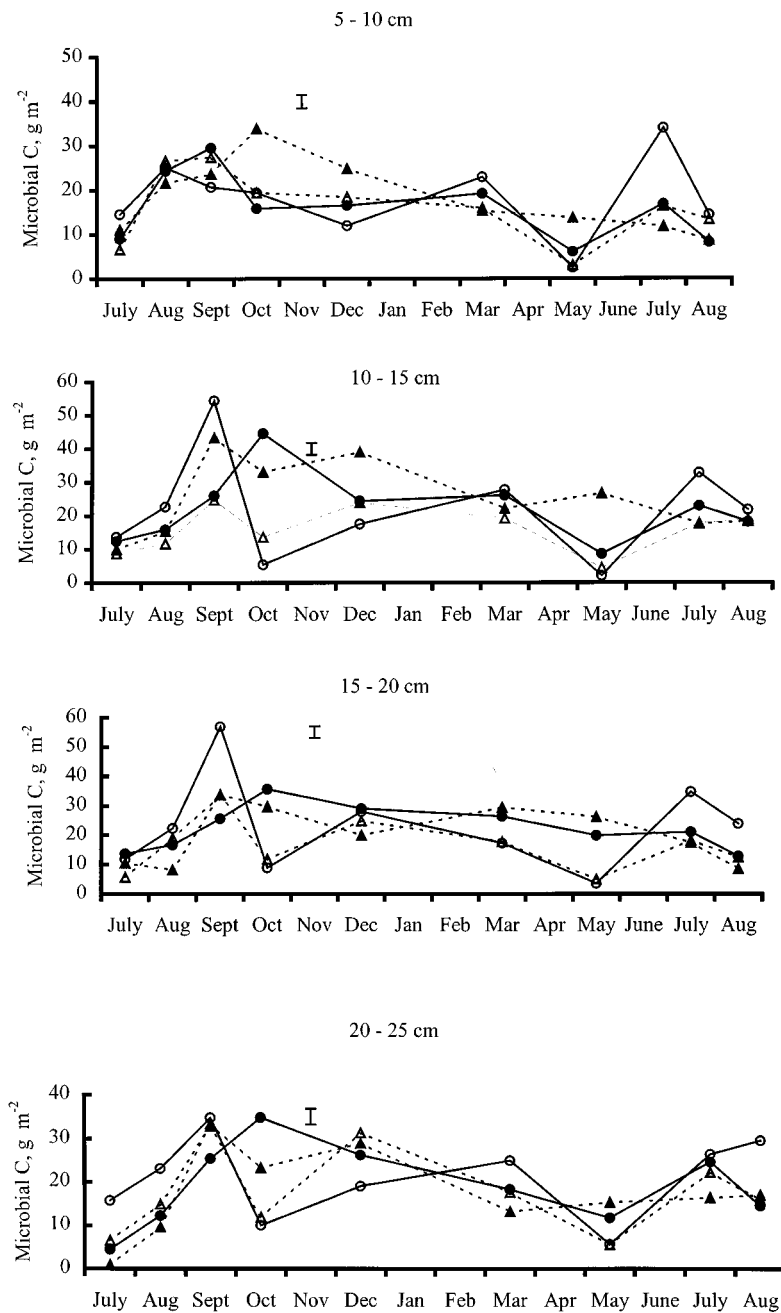


Figure 2. Contents of microbial biomass C (FE),  $\text{g C m}^{-2}$ , in control and N treated cores receiving,  $0.115 \text{ g N m}^{-2}$  every two weeks, for *S. capillifolium* and *S. recurvum* cores, at four depths, 5–10, 10–15, 15–20 and 20–25 cm below the top of the moss. Error bars indicate the standard error of difference between the means for each sample time. Symbols as in Figure 1.

Table 3. Mean microbial C values ( $\text{g C m}^{-2}$ )  $\pm$  standard error of the mean, measured by the SIR and FE methods and microbial C:N ratio based on the FE method, averaged over all sample dates, species and N treatments at four depths below the moss surface.

Depth (cm)	FE	SIR	Microbial C:N
5–10	17.3 (0.7)	19.8 (1.2)	7.6
10–15	21.3 (0.9)	19.6 (0.9)	8.3
15–20	20.5 (0.9)	18.9 (0.9)	10.6
20–25	18.6 (0.8)	17.8 (0.8)	10.4

Mean microbial C values obtained by the FE method were not very different from those determined using the SIR method when averaged over all sample dates, N treatments and species (Table 3). The patterns of change with time were different for the two methods and values obtained by the two methods were not well correlated.

#### *Microbial N (FE)*

At all depths, microbial N was greater in the N treated than in control cores during October, but not at other sample dates and this interaction between N treatment and time was significant ( $P < 0.01$ ) below 10 cm depth (Figure 3). When the values obtained during October were compared using the repeated measures analysis for the four depths, the greater microbial N in the added N treatment was significant ( $P < 0.001$ ) throughout the 5 to 25 cm depth. The total microbial N content in the cores at this time, from 5 to 25 cm, was significantly ( $P < 0.05$ ) greater in N treated than control cores, 10.4 g and 2.1  $\text{g N m}^{-2}$ , ( $\text{sed} = 2.95$ ,  $n = 6$ ), respectively. The difference in microbial N values between the control and N treated cores (8.3  $\text{g N m}^{-2}$ ) greatly exceeded the amount of N added as  $\text{NH}_4\text{NO}_3$  during the first four months (1  $\text{g N m}^{-2}$ ).

The effect of added N during October was greater in peat beneath *S. capillifolium* than under *S. recurvum* (Table 4), and corresponded with lower microbial N contents in the control cores in *S. capillifolium* than in *S. recurvum*.

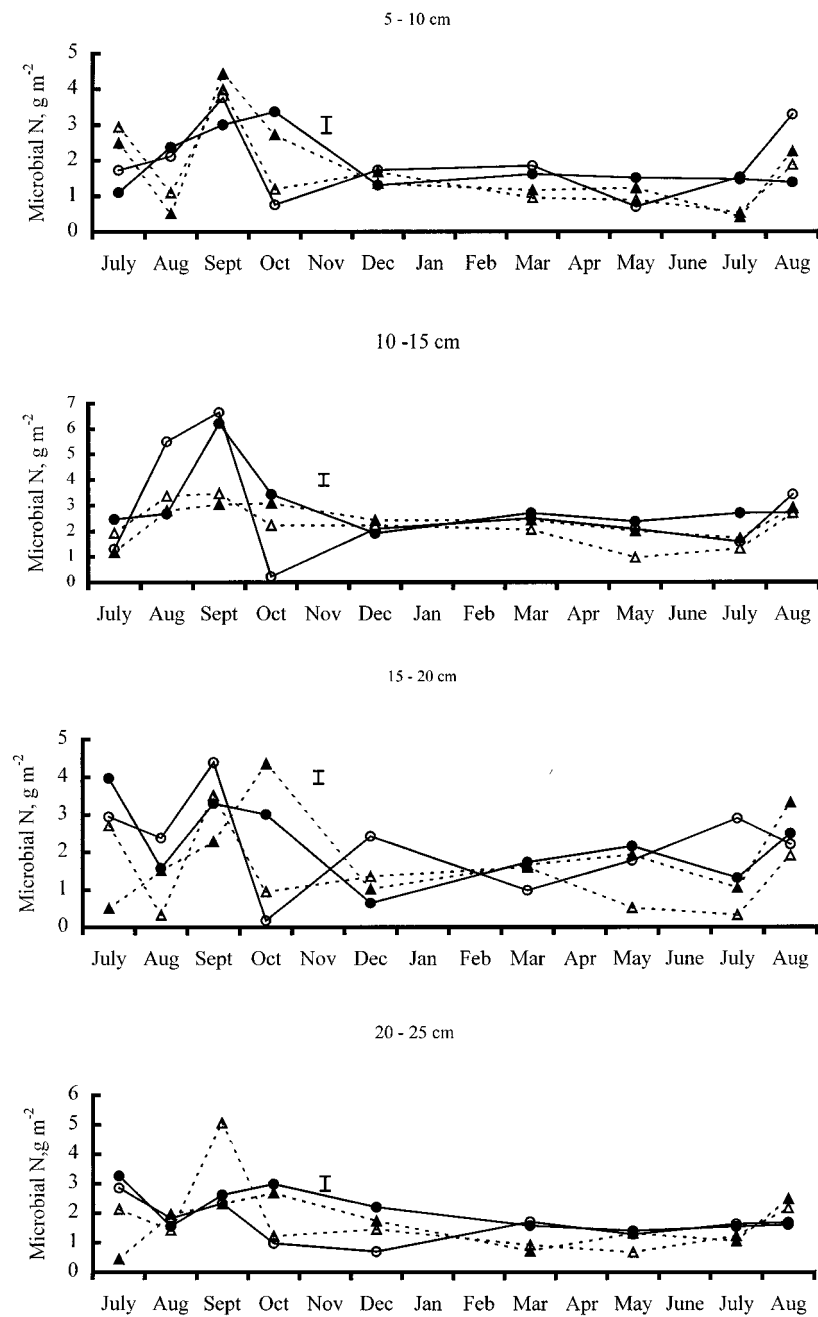


Figure 3. Contents of microbial biomass N (FE), g N m<sup>-2</sup>, in control and N treated cores receiving, 0.115 g N m<sup>-2</sup> every two weeks, for *S. capillifolium* and *S. recurvum* cores, at four depths, 5-10, 10-15, 15-20 and 20-25 cm below the top of the moss. Error bars indicate the standard error of difference between the means for each sample date. Symbols as in Figure 1.

Table 4. Microbial biomass N,  $\text{g N m}^{-2}$ , in the peat beneath *S. capillifolium* and *S. recurvum* in control and N treated cores sampled October 1994 after the application of 8 bi-weekly additions of  $0.115 \text{ g N m}^{-2}$  as  $\text{NH}_4\text{NO}_3$ . Standard error of difference between the means for the species x N interaction shown in parentheses.

Depth (cm)	<i>S. capillifolium</i>		<i>S. recurvum</i>	
	Control	+N	Control	+N
5–10	0.76	3.28	1.46	2.72 (0.2)
10–15	0.22	3.43	2.08	3.08 (0.4)
15–20	0.18	3.01	0.95	4.24 (0.7)
20–25	0.70	2.98	1.19	2.68 (0.9)
Total	1.86	12.7	5.68	12.73 (2.3)

#### Extractable organic matter (DOM)

##### DOC

The content of extractable C changed significantly ( $P < 0.001$ ) with time in the 5–20 cm depth, varying between 4 and  $7 \text{ g C m}^{-2}$ , and showed the same broad seasonal pattern with greater values during August and March than at other times at the three upper depths. Below 20 cm, the quantity of DOC did not change with time and no differences between the *Sphagnum* species or N treatments were detected between 5 and 25 cm depth.

##### DON

The amounts of DON extracted from the peat with  $0.5 \text{ M K}_2\text{SO}_4$  showed significant ( $P < 0.001$ ) seasonal variation to 25 cm depth (Figure 4). At each depth, the amount of extracted DON was greatest during July reaching the lowest values in October and December. In the 5–10 cm layer, DON amounts, averaged over all sampling dates and N treatments, were significantly ( $P < 0.01$ ) greater beneath *S. recurvum* than under *S. capillifolium*,  $3.2 \text{ g N m}^{-2}$  compared with  $1.8 \text{ g N m}^{-2}$ , respectively (sed = 0.32,  $n = 54$ ). This difference between the species varied significantly ( $P < 0.001$ ) with time and was greater during July and August 1994 than during subsequent sampling dates. During July and August 1994, the addition of N increased extractable DON beneath *S. recurvum* and decreased it under *S. capillifolium* and compared with other sample dates this effect was significant ( $P < 0.001$ ). At 10–15 cm depth,  $\text{NH}_4\text{NO}_3$  significantly ( $P < 0.01$ ) decreased the quantity of DON from  $0.7 \text{ g N m}^{-2}$  to  $0.5 \text{ g N m}^{-2}$ , averaged over time and species (sed = 0.06,  $n = 54$ ). The effect of N on DON varied with time ( $P = 0.052$ ) and was

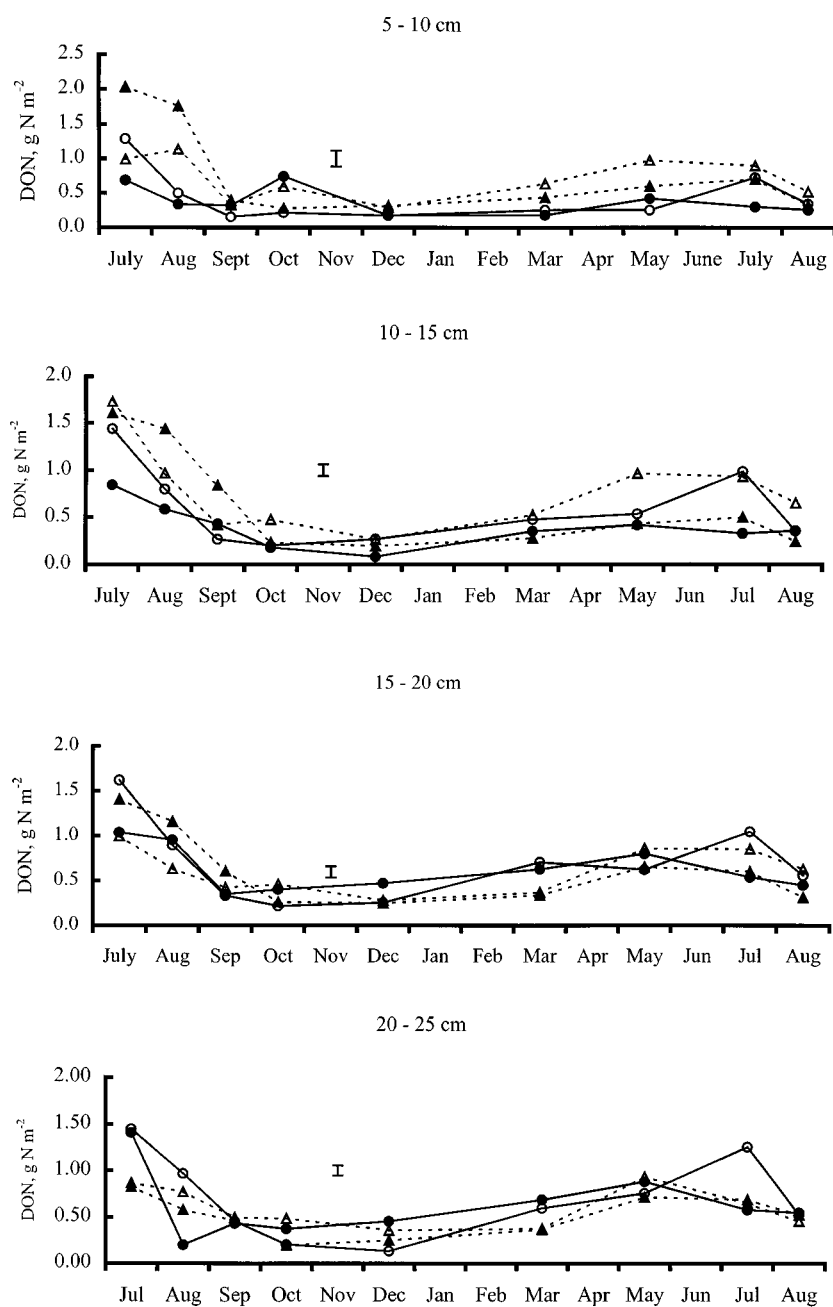


Figure 4. Contents of dissolved organic N (DON),  $\text{g N m}^{-2}$ , extracted from peat with 0.5 M  $\text{K}_2\text{SO}_4$  in control and N treated cores receiving,  $0.115 \text{ g N m}^{-2}$  every two weeks, for *S. capillifolium* and *S. recurvum* cores, our depths, 5–10, 10–15, 15–20 and 20–25 cm below the top of the moss. Error bars indicate the standard error of difference between the means for each sample date. Symbols as in Figure 1.

Table 5. Mean C:N ratios of DOM extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub>, averaged over species and N treatments. Standard error of difference between the means at each time ( $n = 12$ ) shown in parentheses.

Depth (cm)	July	Aug	Sept	Oct	Dec	March	May	July	Aug (sed)
5–10	3.9	10.0	15.6	19.5	20.2	19.3	6.2	6.4	10.7 (3.1)
10–15	3.6	8.1	13.9	22.3	32.7	15.7	5.8	6.4	10.7 (4.5)
15–20	4.3	8.7	15.5	23.0	26.8	14.2	6.1	6.5	12.3 (3.8)
20–25	4.9	10.3	13.0	26.5	27.4	13.8	6.7	6.6	11.1 (4.4)

greater during July 1995 than previous dates. Below 15 cm, DON changed significantly ( $P < 0.001$ ) with time but showed little effect of species or N addition.

#### *C:N ratio of DOM*

The C:N ratio of the extracted DOM changed significantly ( $P < 0.001$ ) with time and showed the same seasonal pattern at each depth (Table 5). The values, range 3.6 to 4.9 in July, increased in October, attaining between 20.2 and 32.7 in December before decreasing. At both 5–10 and 10–15 cm depth values were significantly ( $P < 0.01$ ) greater under *S. capillifolium*, 15.3 and 15.5, respectively, than *S. recurvum*, 9.5 (sed = 1.2,  $n = 54$ ) and 10.9 (sed = 1.8,  $n = 54$ ), respectively, when averaged over all sampling dates and N treatments. Differences between the species did not extend below 15 cm and values were not affected by N additions.

## Discussion

Tracer studies with  $^{15}\text{NH}_4^{15}\text{NO}_3$  had shown that the *Sphagnum* moss in the core accounted for an average of 72% of the added N and that little or no  $^{15}\text{N}$  could be detected in the  $\text{NH}_4^+$ , DON or microbial N pools in the peat below 5 cm depth (Williams et al. 1999). Capture of  $^{15}\text{N}$  by the moss was least during October when the water table level had risen to the surface, but the missing label could not be accounted for, indicating that the added N, if it had penetrated into the peat below 5 cm, had not entered pools measured in this study. It is quite possible that the effect of N addition on microbial biomass C and N values during October was an effect of the unaccounted N, except that the microbial N value was maintained above that of the control by almost a factor of ten times more than the amount of N added. It is more likely that the effect had been transmitted from the moss at the surface

and is associated not with the rising water table, but with the rainfall which preceded it.

The elevated microbial C values involved an additional  $75 \text{ g C m}^{-2}$  in the total microbial C value in the 5–25 cm depth. This amount of C is large compared to the average net primary production values of *Sphagnum* on peatland; reported values ranged from 40 to  $68 \text{ g C m}^{-2}$  for blanket bog (Clymo & Reddaway 1974) and *S. magellanicum* treated with  $3 \text{ g N m}^{-2} \text{ yr}^{-1}$  the previous year on Moidach More increased its net primary production by approximately  $35 \text{ g C m}^{-2}$  (Williams & Silcock 1997). No new microbial biomass appeared to have been formed and the added N maintained the biomass C and N values at a time when values were falling in the untreated cores. Wardle (1998) reviewed temporal variability in microbial C and N in a range of forest, grassland and arable soils and concluded that biomass C and N in these soils had small turnover rates and were relatively stable. The effect of the fluctuating water table on microbial biomass in peats may distinguish them from the aerated soils compared by Wardle (1998). Consequently, the effects of added N could be interpreted as delaying turnover and prolonging stability for a limited time. Indeed, specific rates of respiration decreased in the peat at Moidach More beneath *S. magellanicum* treated with  $1 \text{ g N m}^{-2} \text{ yr}^{-1}$  also signifying increased stability of the biomass (Williams & Silcock 1997). There was no evidence of increased turnover of the microbial N pool. The decline in the microbial C and N did not coincide with an increase in the size of the extractable DOC and DON pools and  $\text{NH}_4^+$  contents (Williams et al. 1999) showed no evidence of increasing as a result of ammonification.

The overall mean values obtained by the two methods of microbial C determination were very similar at the four depths. In contrast, results obtained at a single date beneath *S. magellanicum* showed greater differences between the two methods in the surface 15 cm than at lower depths (Williams & Silcock 1997). Additions of N increased both measures of biomass C during October and December, yet, the different seasonal variations in the values obtained by the two methods were marginally different, indicating that they probably measure different components of the biomass as observed by Smolander et al. (1994). The values obtained here were higher than values reported previously for organic soils in New Zealand (Sparling et al. 1990), but are in the same range obtained by Smolander et al. (1994) for a mor humus beneath Norway spruce and for highly organic tundra soils (Cheng & Virginia 1993). Williams and Silcock (1997) had shown previously increased biomass C beneath *S. magellanicum* treated with  $1 \text{ g N m}^{-2}$  as  $\text{NH}_4\text{NO}_3$  also on the Moidach More, but this result was restricted to a single time point. Our results indicate the importance of making measurements at different

times as the impact of N addition varied with time, habitat and depth in the profile.

Fluctuations of the microbial pools involved relatively large amounts of N of the order of  $2 \text{ g N m}^{-2}$ . Microbial N values in this raised bog were comparable with values equivalent to  $3.7 \text{ g N m}^{-2}$  obtained for the same depth in a blanket bog in the north of Scotland (Williams & Wheatley 1989) and  $1.5\text{--}3 \text{ g N m}^{-2}$  beneath *S. magellanicum* on Moidach More (Williams & Silcock 1997). In contrast to the profiles beneath *S. magellanicum*, where a single sampling occasion showed microbial N reaching a maximum value of  $3 \text{ g N m}^{-2}$  at 15–20 cm depth, the seasonal fluctuations were greater than the differences between the four depths.

The C:N values around 4 obtained for the extractable DOM indicated that during summer this material must be composed mainly of low molecular weight amino acids and other metabolites. Indeed results of the  $^{15}\text{N}$  labelling in this experiment showed that a small proportion of the  $\text{NH}_4\text{NO}_3$  had appeared as DON in the water surrounding the moss. Reports (Baxter et al. 1992) that inorganic N addition increases free amino acid concentrations in the moss tissues suggest that the DON released includes mainly amino acids. However, the quantities of DON measured in the moss water were a 1000-fold less than those extracted from the underlying peat and the contribution of the surface moss to the fluctuations in the DON pool in the underlying peat could only be small. Plant uptake of low molecular weight components of DOM, e.g. amino acids, has been reported in boreal soils (Nasholm et al. 1998) and uptake of amino acids by *Sphagnum* mosses has also been described (Simola 1975). This suggests that the DON pool is both a source of N for plants and microorganisms as well as a sink. The quantity of DOC did not change as much as DON and the increase in C:N ratio during the winter could be attributed mainly to the decrease in DON and not a flux of carbonaceous material.

In conclusion, additions of  $\text{NH}_4\text{NO}_3$  to cores of *S. capillifolium* and *S. recurvum* although captured mainly by the moss, had significant effects on the microbial biomass C and N values in the underlying peat. These effects did not involve the production of new biomass, but appeared to increase its stability during the autumn following increased rainfall and rising water table levels. Fluctuations in the C:N ratios of the DOM indicated that during the summer months low molecular weight amino acids contributed to the DOM pool, which became less rich in N and qualitatively different during the winter. There were no obvious relationships between the elevated levels of microbial N and C and the dynamics of C and N in the DOM pool.



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## References

- Aerts R, Wallén B & Malmer N (1992) Growth-limiting nutrients in *Sphagnum*-dominated bogs subject to low and high atmospheric nitrogen supply. *J. Ecol.* 80: 131–140
- Anderson JPE & Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soil. *Soil Biol. Biochem.* 10: 215–221
- Baxter R, Emes MJ & Lee JA (1992) Effects of an experimentally applied increase in ammonium on growth and amino-acid metabolism of *Sphagnum cuspidatum* Ehrh. ex. Hoffm. from differently polluted areas. *New Phytol.* 120: 265–274
- Bremner JM (1965) Inorganic forms of nitrogen. In: Black CA (Ed) *Methods of Soil Analysis*, Part 2. (pp 1179–1206). American Society of Agronomy, Madison, Wisconsin, U.S.A.
- Brookes PC, Landman A, Pruden G & Jenkinson D S (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* 17: 837–842
- Chapman SB (1971) A simple conductimetric respirometer for field use. *Oikos* 22: 348–353
- Cheng W & Virginia RA (1993) Measurement of microbial biomass in arctic tundra soils using fumigation-extraction and substrate-induced respiration procedures. *Soil Biol. Biochem.* 25: 135–141
- Clymo RS & Reddaway, E.J.F. (1974) Growth rate of *Sphagnum rubellum* Wils. on Pennine blanket bog. *J. Ecol.* 62: 191–196
- Crooke WM & Simpson WE (1971) Determination of ammonium in Kjeldahl digests of crops by an automated procedure. *J. Sci. Food Agric.* 22: 9–10
- Damman AWH (1988) Regulation of nitrogen removal and retention in *Sphagnum* bogs and other peatlands. *Oikos* 51: 91–305
- Daniels RE & Eddy A (1985) *Handbook of European Sphagna*. Institute of Terrestrial Ecology, Natural Environment Research Council. Cambrian News, Aberystwyth, U.K.
- Genstat 5 Committee (1993) *Genstat 5 Release 3 Reference Manual*. Oxford University Press, Oxford
- Henriksen A & Selmer-Olsen AR (1970) Automatic methods for determining nitrate and nitrite in water and soil extracts. *Analyst* 95: 514–518
- INDITE (1994) *Impacts of Nitrogen Deposition in Terrestrial Ecosystems*. Report of the United Kingdom Review Group on Impacts of Atmospheric Nitrogen. Department of the Environment, London
- Kenward MG (1987) A method of comparing profiles of repeated measurements. *Appl. Stats* 36: 296–308

- Li YH and Vitt DH (1997) Patterns of retention and utilisation of aerially deposited nitrogen in boreal peatlands. *Ecosci.* 4: 106–116
- Nasholm T, Ekblad A, Nordin A, Giesler R, Hogberg H & Hogberg P (1998) Boreal forest plants take up organic nitrogen. *Nature* 392: 914–916
- Silcock DJ & Williams BL (1995) The fate and effects of inorganic nitrogen inputs to raised bog vegetation. In: Jenkins A, Ferrier RC & Kirby C (Eds) *Ecosystem Manipulation Experiments*. Ecosystems Research Report 20 (pp 44–48). European Commission, Brussels
- Simola LK (1975) The effect of several protein amino acids and some inorganic nitrogen sources on the growth of *Sphagnum nemorum*. *Physiol. Plant.* 35: 194–199
- Smolander A, Kurka A, Kitunen V & Mälikönen E (1994) Microbial biomass C and N, and respiratory activity in soil of repeatedly limed and N- and P-fertilized Norway spruce stands. *Soil Biol. Biochem.* 26: 957–962
- Sparling GP, Feltham CW, Reynolds J, West AW & Singleton P (1990) Estimation of soil microbial C by a fumigation-extraction method: use on soils of high organic matter content, and a reassessment of the  $k_{EC}$ -factor. *Soil Biol. Biochem.* 22: 301–307
- von Post L (1929) Sveriges Geologiska Undersöknings torvinventering och några av dess hittills vunna resultat. *Svenska Mosskulturför. Tids.* 36: 1–27
- Wardle DA (1998) Controls of temporal variability of the soil microbial biomass: a global-scale synthesis. *Soil Biol. Biochem.* 30: 1627–1637
- Williams BL, Shand CA, Hill M, O'Hara C, Smith S & Young ME (1995) A procedure for the simultaneous oxidation of total soluble nitrogen and phosphorus in extracts of fresh and fumigated soils and litters. *Commun. Soil Sci. Plant Anal.* 26: 91–106
- Williams BL & Silcock DJ (1997) Nutrient and microbial changes in the peat profile beneath *Sphagnum magellanicum* in response to additions of ammonium nitrate. *J. Appl. Ecol.* 34: 961–970
- Williams BL, Silcock DJ & Young MY (1999) Seasonal dynamics of N in two *Sphagnum* moss species and the underlying peat treated with  $^{15}\text{NH}_4^{15}\text{NO}_3$ . *Biogeochem.* 45: 285–302
- Williams BL & Sparling GP (1984) Extractable nitrogen and phosphorus in relation to microbial biomass in acid organic soils. *Plant and Soil* 76: 139–148
- Williams BL & Wheatley RE (1989) Nitrogen transformations in poorly-drained reseeded blanket peat under different management systems. *Int. Peat J.* 3: 97–106
- Woodin SJ & Lee JA (1987) The fate of some components of acidic deposition in ombrotrophic mires. *Environ. Pollut.* 45: 61–72