

Interim Report June 2003

The effects of polluted cloud water
on a Sitka spruce plantation (Deepsyke)
Phase II: potential for recovery

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SUMMARY

- Significant trends in soil solution chemistry and foliar chemistry are still apparent in year two of 'recovery' phase.
- Removing sulphuric acid from the treatments can be detected in the soil solution, foliar chemistry, and forest floor pH and nutrient content. Mg^{2+} availability decreased in the soil water but concentration increased in needles. Soil solution NO_3^- increased and increased N concentration was observed in the needles. Litter loss appears to be declining. Soil pH (H_2O) has increased.
- Where soil pH was ca. 3.7 (in H_2O) removing the H_2SO_4 has increased soil pH and stimulated nitrification.
- If the presence of NO_3^- in soil water is an indicator of N saturation both 2N and the 2NSAcid treatments are saturated even though C:N ratio in the forest floor exceeds 30, above the critical level of 23-27.
- In the winter, with no treatment, NO_3^- concentrations in soil were much lower but in the 2N treatment were still 'leaking' NO_3^- .
- Ammonium concentrations are slower to respond than NO_3^- but even they are increasing in the 2N treatment.
- Additions of SO_4^{2-} increased the availability of phosphate, whereas the counter ion Na^+ reduced K^+ concentrations in the soil solution.
- Growth at the site is strongly affected by soil moisture and this has been used as a covariate across plots. No treatment effects are detectable but there is still a positive N response.
- Foliar N concentrations at ca. 1.1% N are on the low side for Sitka spruce.
- Foliar N concentrations are responding to changes in soil water chemistry, and apparently to the increase in NO_3^- .
- Fine root growth has responded positively to the reduction in forest floor acidity and increased in response to N. Most of the roots occur in the litter layer.
- Approximately 75% of the fine roots are infected with ecto-mycorrhizal fungi (ECM); there was no effect of treatment on live root number.
- Fine root N content was ca. 50% greater than foliar N content in all treatments – suggesting that ECMs may be storing N.
- *Lactarius rufus*, an intermediate stage fungus, is now increasing; *Tylospora fibrillosa*, an early stage fungus, is declining.
- The litter N concentration is also 50% higher than the current year foliage in all treatments, indicating significant immobilization.
- N additions have increased N immobilization in the litter and fine roots by increasing their amount, rather than by affecting their N concentration.

Phase II - Background

In 2001 a new treatment regime was implemented which removed S and acidity from the NSAcid and 2NSAcid treatments, removed N from the N treatment, and S from the S treatment – the new treatment regime has been running in 2 plots in parallel with the original treatment (2 plots). Monitoring has continued in the Control and No Spray plots, which have remained the same. Apart from the mycorrhizal studies, all the data have been analysed with plot moisture as a covariate, because of its highly significant effect on growth.

Tree Growth – (Table 1)

At this site growth continues to be good (Yield Class > 28) but the differences in plot moisture are still exerting a greater absolute influence than the treatments. All analyses for growth and foliar K show a highly significant effect of plot moisture. Before the recovery treatments were introduced, significant treatment effects were only detected after 5 years. In this recovery phase, no significant treatment effects have been found either in year 1 or year 2, and relative increases are very similar across all plots. Actual stem areas, while not significantly different between the recovery treatments, do show a positive 8-10% response to N. The double dose 2N/2NS Acid treatments have **not** increased stem area in response to dose. It seems likely that, at this stage of forest growth, canopy closure leading to competition between adjacent trees may be confounding treatment effects.

Table 1. Effects of treatment on stem area (cm²) at 1.3m (DBH) and annual relative increments (%) adjusted for plot moisture

	Area 00	Area 01	Area 02	Rel. % inc. 99-00	Rel. % inc. 00- 01	Rel. % inc. 01- 02
NSAcid	99.4	115.1	126.7	19	16	10
N-Acid	101.0	116.4	129.0	18	15	10
S	85.0	98.0	107.1	20	15	9
-S	78.9	89.5	99.0	18	13	10
2NSAc	98.1	109.1	119.8	20	13	10
2N-Ac	97.3	110.5	121.3	17	11	10
N	104.0	118.4	131.8	20	14	12
-N	99.3	111.9	123.6	16	12	10
Control	91	102	111	17	12	9
No spray	101	117	128	19	18	9
Probability of effect	0.25	0.27	0.33	0.54	0.33	0.95
LSD	22.0	25.7	31.1	5	4	4
Covariate.	***	***	***	NS	**	.085

Foliar nutrition

Weights of 100 needles taken in January each year show that needle weights were exceptionally high in the 2000 growing season, prior to the change to a recovery scenario, *ca.* 30% larger than in 2001 and *ca.* 20% larger than in 2002. In 2000 there were no significant differences between the paired treatments for N content. Current year needle N concentrations in 2002 were significantly affected by removing acidity and N. In both the single and double NS Acid treatments, N concentrations increased with the removal of the acid. However, this result partly reflects the change in method for N measurements. Significant differences were not apparent from the Kjeldahl digest, but only where the new CN analyses were used. With the latter method there is no loss of volatilised products. This suggests some of the N in the non-acid treatment was stored as NO_3^- .

Table 2. Percent changes in foliar N concentrations (2002 January, CN analyser) in response to recovery treatments in current year (C) and one-year-old (C+1) needles.

* indicates significant at $P=0.05$

Needle age Element	C Total N	C Mg	C+1 P
N-Acid vs. NSAcid #	+14% *	+20%*	-0.5%
-S vs. S	-12%*	0	-14%
2N-Acid vs. 2NSAcid #	+ 8%*	+16%	+ 0.6%
-N vs. N	-6%	+5%	+5%
Overall mean % dwt	1.1	0.11	0.10

2NSAcid and NSAcid Mg concentrations were *significantly* ($P<0.05$) 35 and 25% lower than the Control foliage; 2NSAcid had *significantly* ($P<0.05$) 25% less P in its one-year old foliage than the Controls.

P concentrations in current-year foliage did not respond to the recovery treatments at the 95% significance level. Significant changes were observed in foliar Mg concentrations, in response to the second year of recovery were significantly higher with the removal of H_2SO_4 in the single acid treatment. Mg concentrations were within the deficiency category for the 2NSAcid treatment. Only P concentrations responded to the treatment in one-year-old foliage and there was no recovery effect

Litterfall (Figure 1)

Litter accumulation continues to be highest in the treatments receiving N. There was a very large amount of litter produced between April 2000 and April 2001, following a growth surge in the 1999 growing season in the 2NSAcid and NSAcid treatments. By contrast, the Control, No Spray, N-only and S-only treatments have shown a more gradual increase in litterfall. The acid response suggests that nutrient demand in these treatments is met either by nutrient remobilisation and/or via litterfall. Where the acidity has been removed (dashed lines Fig. 1) there has been a relative reduction in litterfall (solid line approaching dashed line). Removal of N and S do not appear to have influenced the amount of litterfall.

Accumulating litter over 5 years (1-3 pre recovery)
 mean of 2 plots year 5 not adjusted for covariate

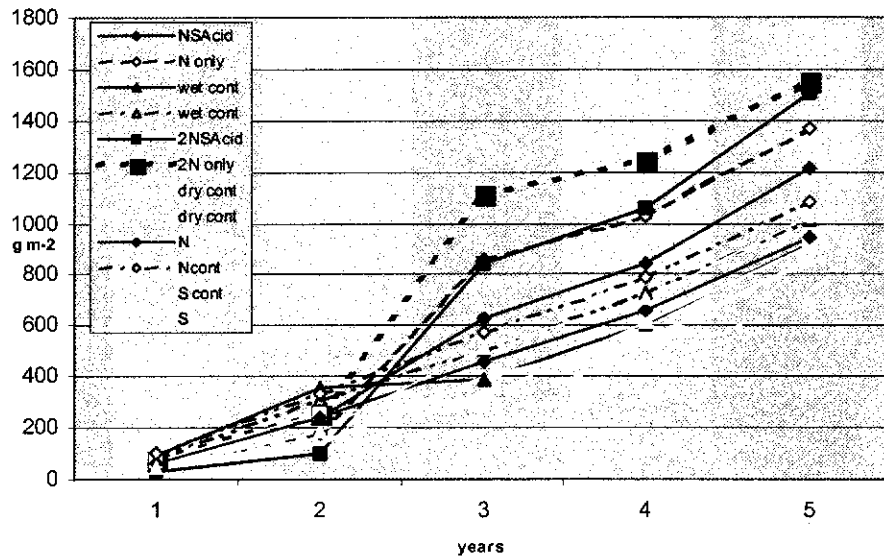


Figure 1. Cumulative amounts of litter from each of the 12 treatments

Forest floor – Table 3

Litter pH reflected the acid dose to the chambers, and although there were signs of recovery, these were not statistically significant. There was a + 15% increase in litter N for those treatments supplying N compared with the Control. There was no dose response to 2N treatments. C:N ratios were all >30, above the critical ratio of 25-27 indicating N saturation (Gundersen & Rasmussen., 1990). Fine roots removed from the litter showed no treatment effects with respect to N content or weight/m²/plot. The most fine roots were collected in the N-only treatments i.e. N, 2N (recovery) and N (N-Acid recovery). Although not statistically significant (+30-50%) this proliferation of roots in response to N is consistent with the below-ground mycorrhizal studies on root tips. Trees receiving additional N invest more C in the roots, probably to enhance uptake of other nutrients to support an N-driven growth response. The N content of these fine roots did not reflect the N treatments, but was 50% higher than foliar N concentrations, possibly lending some support to Aber et al's (1998) hypothesis that mycorrhiza may store N. Although there was no overall treatment response to N for the forest floor N concentration the amount immobilised in the litter and fine roots would be considerably enhanced in the N treatments due to the N stimulation of litter production.

Soil pH (June 2003; Table 4) measured in CaCl₂ showed no treatment effect, whereas in water the effects of removing the acid from the double dose NSAcid treatment significantly increased the pH, as did removing N from the N treatment. pH in water indicated a significant acidifying effect of NSAcid and also N on the peat soil. This is the first time that acidification associated with NH₄NO₃ treatment has been measured in these plots.

Table 3. Forest floor and fine root (FR) chemistry in recovery treatments, sampled in November 2002. Values followed by the same letter in each column are not significant at $P=0.05$. Paired effects of recovery are shown in **bold**.

	Litter pH	Litter gm ²	Litter C%	Litter N%	C:N ratio	Fine roots gm ⁻²	Fine roots N%
NSAcid	3.91 ab	1500 ab	53.6	1.62	33.1	164	1.55
N-Acid	4.0 b	1120 b	52.8	1.63	32.5	363	1.65
S	4.17 bc	510 c	52.4	1.44	36.9	83	1.51
-S	4.24 c	682.c	51.6	1.4	37.1	54	1.67
2NSAcid	3.79 a	1177 b	53.6	1.57	34.2	191	1.56
2N-Acid	3.86 a	1733 a	53.7	1.68	31.9	257	1.58
N	4.05 bc	1215 b	52.6	1.7	30.8	318	1.67
-N	4.12 bc	1030 b	52.6	1.66	31.7	190	1.68
Control	4.21 bc	757c	52.4	1.55	33.8	116	1.52
No Spray	4.15 bc	857c	51.9	1.45	35.8	83	1.65
Probability	0.012	0.01	0.19	0.21	0.39	0.24	0.89
LSD	na	509	1.7	0.27	6.9	271	0.3
CV %	20	19	1.4	7	8	5.5	10

Table 4. Soil pH measured in June 2003 (5 cores/plot from the upper 10cm) measured in CaCl_2 (10^{-2}M) and water. Values followed by the same letter in each column are not significant at $P=0.05$. Paired effects of recovery are shown in **bold**.

	pH in CaCl_2	pH in H_2O
NS Acid	2.98	3.84 a
N-Ac	2.88	3.91 a
S	3.01	4.0 b
-S	2.96	3.94 b
2NSAc	2.88	3.69 a
2N-Ac	3.02	4.0 b
N	2.93	3.67 a
-N	2.92	3.84 b
Control	2.92	4.02 b
No spray	3.00	4.05 b
Probability	0.26	0.011

Forest floor communities

So far there have been no responses from the mosses or higher plants to the recovery treatments. Re-colonization is driven by light *i.e.* 'holes' in the canopy and the absence of litter. The 2NSAcid plots have some *Plagiothecium undulatum* and *Mnium spp.*, and in the absence of acid (2N) there is some *Eurynchium praelongum*. In the NSAcid plots, *P. undulatum*, *E. praelongum* and some *Rhytidiadelphus squarrosa* could be found. At the present time it is not possible to separate the direct effects of treatment chemistry from the indirect effects of litter depth and light. However, there appears to be re-colonization of some of the acid chambers. The moss species identified are in abundance around the plots and are returning as forest floor gets more light. The species present are known to be N tolerant.

Soil water chemistry

During the first summer with the new treatment (2001), soil water was sampled on 5 occasions using zero-tension lysimeters. Treatment means were calculated for the numbers of collections with or without treatment (summer or winter respectively; Table 5) and adjusted using soil moisture as a covariate. As observed in Phase I the soil water is highly sensitive to removal of the H_2SO_4 from the former NSAcid plots. Although acidity was not affected, there was a significant reduction in S and Ca, and almost significant in Mg concentrations. There were no effects on N. Where the double dose of acidity was changed to 2N, base cation (Mg) concentrations fell significantly along with H^+ , but NO_3^- concentrations were significantly increased, though not at the expense of NH_4^+ . P concentrations did appear to respond to the treatments but the level of between-plot variability masked significant differences – reducing acidity increased P concentrations. Removing N also increased P concentrations. S concentrations were very sensitive to inputs. P concentrations were low in the 2NSAcid treatments, probably explaining the low foliar P concentrations. During the following summer (2002) the effects on base cations and acidity were reinforced. Ca, Mg and H^+ were significantly reduced in response to the removal of H_2SO_4 . Overall ion concentrations were considerably higher, especially for Mg, H^+ and SO_4^{2-} during 2002. NO_3^- concentrations were again higher in the 2N treatment compared with the 2NSAcid. There was a large significant effect of removing N on NO_3^- concentrations but less so for NH_4^+ . Both NO_3^- and NH_4^+ concentrations were higher than in 2001, suggesting the capacity of the soil sink and tree uptake was declining relative to N inputs. The pH of the soil water was most acid during the summer collections, possibly reflecting nitrification pulses.

Over the winter period, with no treatment, base cations were similar in all treatments. pH still showed treatment effects. In the acid treatments, NH_4^+ and NO_3^- were lower than in the corresponding N recovery treatments, and were especially enhanced in the 2N treatments. P concentrations were lower than in the summer. S concentrations showed no treatment effect, with similar concentrations to the controls. The N concentrations in winter soil water were quite high given the absence of inputs, further evidence of N leakage.

**Table 5a . Soil water chemistry. Concentrations in $\mu\text{mol}_c \text{ l}^{-1}$
Summer 2001. Values shown in bold show significant effects of recovery.**

	Al	Ca	Fe	H	K	Mg	NH ₄	NO ₃	P	S
NSAcid	26	71	6	203	12.6	39	8.6	12.5	.42	229
N-Acid	26	33	34	196	8.3	20	7.7	2.5	.97	97
S	15	29	23	100	6	24	0.2	0	.55	249
-S	41	52	32	95	15.5	27	4.6	0	.88	113
2NSAcid	37	54	4	289	5.5	31	31.2	29.0	.07	336
2N-Acid	21	33	7	203	6.1	14	26.0	81.0	.59	81
N	23	56	0	46	6.9	27	14.2	24.9	.04	54
-N	24	45	28	97	9.9	25	6.4	9.0	.62	98
Control	23	51	12	103	9	34	8	<0.62	.39	96
No Spray	32	52	11	81	12	32	4	<0.66	.4	84
Probability	.539	0.096	.062	<0.001	.03	.148	.447	.023	.191	.013*
LSD	30	28	26	49	5.7	17	35.5	42	.79	131
CV%	47	25	65	13	26	27	118	90	63	34
Cov			Sig	Sig					Sig	

**Table 5b. Soil water chemistry. Concentrations in $\mu\text{mol}_c \text{ l}^{-1}$
Summer 2002. Values shown in bold show significant effects of recovery.**

	Al	Ca	Fe	H	K	Mg	NH ₄	NO ₃	P	S
NSAcid	79	147	7	522	28.9	95	16.1	24.9	.37	729
N-	30	31	47	266	13.2	23	8.8	26	1.05	110
S	42	82	28	341	13.7	59	-1.8	-18	.3	787
-S	58	65	54	277	34.1	43	5.5	-2	.79	162
2 NSAcid	84	117	10	773	12.7	94	37.1	66	-18	802
2 N	22	26	9	251	8.4	18	23.7	120	.05	87
N	49	76	-2	216	21.1	48	23.9	99	2.67	81
-N	69	102	23	422	24.7	61	8.4	7	4.09	197
Control	38	79	32	230	15	48	13.2	1.2	1.17	151
No Spray	37	64	25	158	18	46	5.2	0.3	1.18	120
Probability	.218	.02	.226	.006	.141	.005	.101	.054	.307	<.001
LSD	56	63	49	223	20.1	33	25.2	85	3.84	179
CV%	43	32	93	24	42	24	68	86	153	20
Cov			Sig	Sig						

Table 5c. Soil water chemistry. Concentrations in $\mu\text{mol}_e \text{l}^{-1}$
Winter 2001/2002. Values shown in bold show significant effects of recovery.

	Al	Ca	Fe	H	K	Mg	NH ₄	NO ₃	P	S
NS Acid	14	33	4	90	4.4	17	2.8	.8	.10	72
N-	28	32	32	154	6.0	25	11.8	4.9	.22	93
S	16	25	23	86	5.0	16	6.9	.4	.16	84
-S	20	34	17	66	7.9	25	5.7	.8	.14	56
2 NS Acid	24	34	7	160	4.9	21	6.0	7.4	.28	126
2 N	29	30	14	179	8.4	13	36.9	14.6	.24	141
N	10	26	-4	22	2.6	15	-1.0	2.7	.09	11
-N	31	40	17	101	7.7	31	4.9	1.9	.21	62
Control	19	34	13	84	5.0	31	6.5	1.11	.29	58
No Spray	26	42	7	64	10.3	40	4.1	1.06	.24	59
Probability	.516	.85	.048	.151	.162	.244	.542	<.001	.35	.282
LSD	26	26	18	49	4.6	16.7	41	4.2	.19	105
CV%	50	34	53	44	32	33	183	41	45	53
Cov			Sig	Sig	Sig					

Ectomycorrhizas and their fruiting bodies (sporocarps)

There were no significant changes between the recovery pairs so that for comparisons with data for 2000 the data are discussed with respect to the original treatments.

Fruit bodies (Table 6)

Most fruit bodies were found in the Control or No Spray plots, with least in the 2NSAcid plots. This was the case for both ECM fruit bodies and those of the saprophytes. *Mycena* spp. were also less common than in 2000, and their fruit body production was particularly low in the 2NS Acid treatment.

Roots and root tip colonisation (Table 7 and Figure 2)

As the forest matures, the proportion of roots colonized by *Lactarius rufus*, an intermediate stage fungus, has increased at the expense of *Tylospora fibrillosa*. This could reflect an increase in the availability of carbohydrates, or a change in their partitioning with age. Numbers of *L. rufus* were significantly lower in both the 2NS Acid and N treatments, reflecting their higher soil water N levels. Other mycorrhizas (*Cortinarius*, *Inocybe* and *Laccaria*) were again sparse. Litter depth showed a dose response to N, suggesting that the increase in litter is a growth response to N, reinforcing other observations. While not significantly greater, there was still a tendency for more root tips in the N treatment without acidity. But, in contrast to many published studies that saw reduced C partitioning to the roots in response to N, in this soil the opposite was true; fine-root growth was increased by N.

Soil depth (Table 8) There were more than 4 times as many root tips in the upper litter layer compared to the more decomposed, consolidated peat. *Tylospora* tended to favour the surface litter whereas *L. rufus* could be found lower down the soil profile.

Table 6. Treatment effects on total numbers of fruit bodies (FB) in the plots. Values in the same row followed by the same letter are not significantly different.

Original treatment	NS Acid	S	2NS Acid	N	Control	No spray	P value
Total FB	366bc	410abc	122d	219cd	500ab	712a	<0.001
Total ECM FB	237c	300bc	93e	137d	365b	597a	0.004
<i>Lactarius rufus</i>	198	228	76	105	311	538	0.084
<i>Inocybe</i> spp.	1.8cd	23.5a	0.3d	7.3bcd	6.8abc	12.0ab	0.023
<i>Laccaria</i> spp.	12.0	7.8	0.3	2.0	3.0	0.3	0.408
<i>Cortinarius</i> spp.	3.5bc	19.5ab	0c	0.3c	23.8a	26.0a	0.007
<i>Tylospora fibrillosa</i>	21.3	21.0	16.5	23.3	21.3	21.0	0.308
Total sapro. FB	129a	111ab	29c	82b	135a	115a	0.009
<i>Marasmius androsaceus</i>	122	61	24	76	93	94	0.109
<i>Mycena</i> spp.	0c	12.3ab	0.3c	4.7b	23.3a	17.3a	<0.001
<i>Galerina</i> spp.	7.0	37.0	5.0	1.0	18.5	4.2	0.471

Table 7. Effects of N, S and acidity on litter depth, root growth and ECM colonisation.

Values in the same row followed by the same letter are not significantly different.

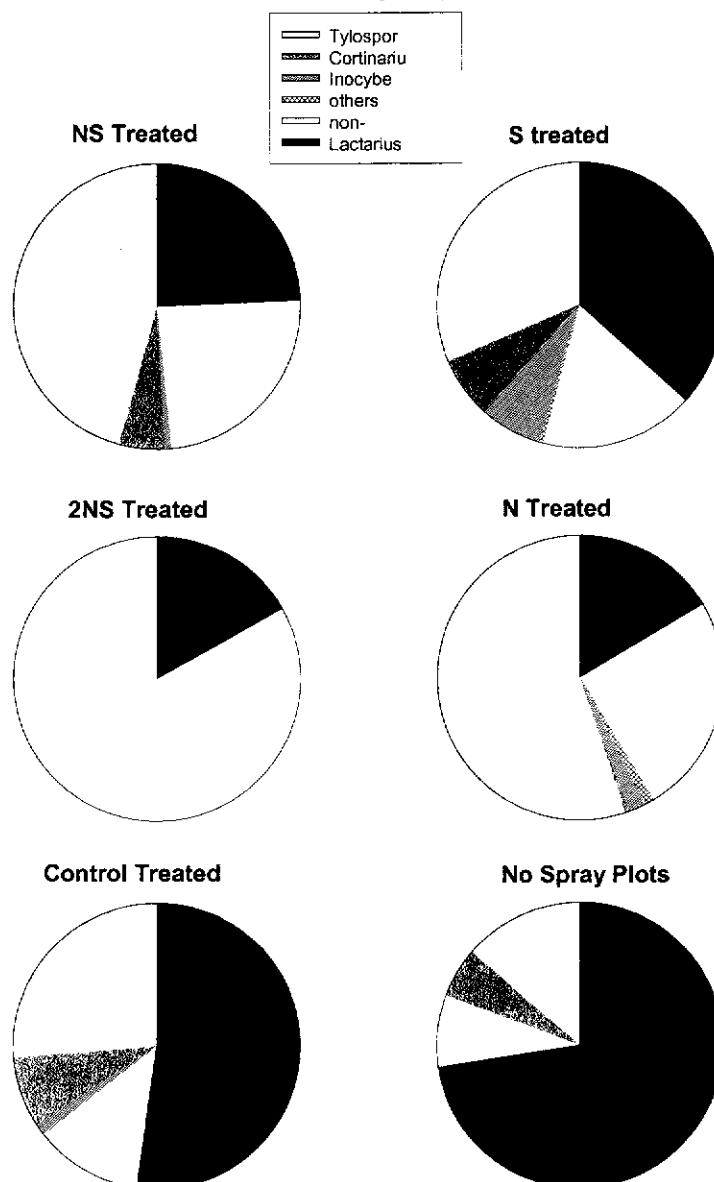
	NS Acid	S	2NS Acid	N	Control	No spray	P value
Litter depth (cm)	1.12b	0.99b	2.42a	1.23b	0.52c	1.13b	<0.001
Total root tips	3353a	3936a	3727a	5837a	3109ab	1577b	0.023
Fine root dry wt (mg)	829a	640ab	681ab	919a	505bc	276c	0.005
% live roots	23.6	23.8	25.2	21.1	21.5	22.6	0.915
% non-mycorrhizal tips	24.0b	17.4bc	43.1a	25.0b	12.1c	8.1c	<0.001
% <i>Tylospora</i>	45.7ab	31.5b	40.1ab	55.1a	26.4bc	13.6c	0.007
% <i>Lactarius rufus</i>	24.4c	36.7bc	16.8c	16.4c	52.2ab	72.4a	0.005
% <i>Cortinarius</i>	5.2	6.9	0	0.5	7.9	5.6	na
% <i>Inocybe</i>	0.7	7.1	0	1.9	1.4	0.1	na
% <i>Laccaria</i>	0	0.4	0	0	0	0	na
% <i>Cenococcum</i>	0	0	0	1.0	0	0.1	na

Table 8. Effect of sampling depth on root growth and proportions of ECM morphotypes.

Values in the same row followed by the same letter are not significantly different.

	Sampling depth		
	Upper (0-5 cm)	Lower (5-10 cm)	<i>P</i> value
Total root tips	5828a	1352b	<0.001
Fine root dry wt (mg)	1057a	226b	<0.001
% live roots	28.3a	17.6b	<0.001
% non-mycorrhizal tips	19.9	23.4	0.618
% <i>Tylospora</i>	40.7	30.1	0.079
% <i>Lactarius rufus</i>	29.7	43.3	0.070
% <i>Cortinarius</i>	8.2	0.5	na
% <i>Inocybe</i>	1.3	2.5	na

Figure 2. Proportions of ectomycorrhizal morphotypes and non-mycorrhizal root in soil cores removed from plots sprayed with different pollutants



Collaborative studies

Bayreuth University, Germany (BITOK). As part of a PhD examining changes in the distribution of aphids and leaf surface micro-organisms, Eva Muhlenberg spent 12 weeks at CEH Edinburgh. This work confirmed the earlier observations (Stadler et al 2001) that aphids show a much greater preference for trees that received NSAcid. Treatment with S and N alone had very similar levels of infestation to control trees and 2-3 times fewer aphids than NSAcid needles. Molecular techniques were able to separate different populations on these plots by comparison with the N or S plots or controls.

Aberdeen University have a postdoctoral fellow and PhD student examining the microbial communities at Deepsyke supported by the NERC GANE thematic programme. Results from the PDRA's work on the diversity of ammonia oxidising bacteria (AOB) based on the amplification of ammonia oxidiser specific DNA sequences isolated from the upper 10cm of the peat have shown:

- Changes in the denaturing gradient gel electrophoresis (DGGE) derived from 16S-rDNA sequences and *amoA* sequences indicative of change in the AOB community in response to the 2NSAcid treatments but not at the lower dose.
- At high N and S deposition *Nitrosomonas* cluster 7 increased at the expense of another previously dominant *Nitrosomonas* sequence (between cluster 5 and cluster 6).
- Detection of phylogenetic clusters of AOB and of treatment effects was dependent on the primer set used.
- Populations according to their DGGE pattern of 16r DNA sequences at Deepsyke, an acid peat, were significantly different from those at Pwylpeiran an upland grassland site.

Further studies

As forests mature, growth of individual trees becomes more and more sensitive to the size of the adjacent trees. Thus at Deepsyke an increasing proportion of tree growth will be determined by factors other than treatments. Therefore we recommend changing the emphasis to monitoring effects below-ground, which have been particularly sensitive to treatments, in response to stopping the treatments after 2003.

1. Restrict vernier measurements of DBH to bi-monthly Mar-Oct (4) to see if there is any 'memory' effect.
2. Maintain collections of soil water every 2-3 months (4) to address how quickly base cations, N and S respond to a large reduction in supply.
3. Maintain annual foliage sampling to see if the increases in soil water N can be observed in the trees.
4. Maintain studies of mycorrhizal fruiting and infection to identify which changes are due to chronology and which are in response to N/acidity – 28 days every 2 years+ 2 d/yr for 4 assessments of fruiting bodies.
5. Maintain litter collections (2/yr).
6. Identify where the inputs of N have gone – a detailed sampling of plot horizons is required for total N analysis.
7. Collaborate with modellers and undertake measurements as identified.

References:

Aber *et al* 1998 N saturation in temperate forest ecosystems. *Biosci.* 48 921-934. Gundersen P & Rasmussen L 1990 Nitrification in forest soils: effects of N deposition on soil acidification and Al release. *Rev. Environ. Contam. Toxicol* 13 1-45. Stadler B, Muller T, Sheppard LJ & Crossley A 2001 Effects of *Elatobium abietinum* on nutrient fluxes in Sitka spruce canopies receiving elevated N and S deposition. *Agric & For Entom* 3 251-261.