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Implications of acidified S inputs on the fate and consequences of N deposition: results

from a field manipulation of a Sitka spruce canopy in Southern Scotland.

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This paper describes a field manipulation experiment where the effects of a simulated decline in acidified S

inputs on the fate of N on Sitka spruce growing on an organo-mineral soil were investigated, along side those of

the original treatments: ammonium nitrate, with and without sulphuric acid and sodium sulphate. Five years of

treatment, at canopy height, with up to 100 kg N and S ha-1y-1 were extended for a further 3 years, for half the

plots, while the remaining plots were deprived of N, sulphuric acid or S. Stem area increment was unresponsive,

whereas foliar N and Mg concentrations and fine roots were sensitive to the removal of N and acidity. This

recovery experiment confirmed that the presence of acidified S modifies the fate of N and suggests the reduction

in acidified S deposition will increase the bioavailability of N.

Keywords: Fine roots, foliar nutrients, litter, soil solution chemistry, stem area increment,

1. Introduction

Control measures to restrict sulphur (S) emissions were put in place in the 1980s. Between 1986 and 2002, emission reductions in the UK were dramatic, down from almost 2000 kt S to < 600 kt S [1]. Sulphur deposition in the UK has also fallen, although by proportionally less than would be expected from the fall in emissions, because of the nonlinearities in source receptor relationships for S and N compounds [1]. Not withstanding this, the ratio of S to N has gone down, and yet we know little about the consequences of these ratio changes for our forests. Particularly pertinent questions that need addressing are: to what extent has acidified S deposition modified the effects of N deposition to forests and, what changes are involved in recovery?

The implications of changes in the ratio of acidified S to N for forests have not been widely investigated in the field and remain poorly characterised. Sheppard [2], Sheppard and Crossley [3] and Sheppard et al [4,5] treated young Sitka spruce (*Picea sitchensis* Bong. Carr.), growing on an organo-mineral soil, with S and N and combinations: ammonium nitrate (NH₄NO₃), with and without sulphuric acid (H₂SO₄ at pH 2.5), and sodium sulphate (Na₂SO₄). In five years, stemwood quadrupled but the growth was heterogeneous with less than 30% being explained by the simulated anthropogenic deposition. Nitrogen additions of 48 kg N ha⁻¹ y⁻¹ did enhance stemwood increments but the 20% increase over 5 years was only just significant (P< 0.05). The inclusion of acidity with N, even at double the acid + N dose, made no difference [5]. These observations suggested that neither enhanced N nor acidified S deposition pose a potential threat to the growth of young Sitka, at least in the short-term.

However, tree growth does not appear to be overly sensitive to N deposition. Wright and Rasmussen [6] concluded that effects of N deposition were strongly dependent on site type (soil chemistry and climate) and the developmental stage of the stand. The stand reported in Sheppard et al [5] was in the exponential growth phase leading up to canopy closure and would have been expected to have a high N demand. Sigurgeirsson [7] suggests

that N inputs, similar to the single N dose (48 kg N ha⁻¹ y⁻¹) used in [5], are mostly retained by the soil and thus would be unlikely to significantly influence tree growth over the short-term. Emmett [8] also suggests that N inputs below 60 kg N ha⁻¹ y⁻¹ take several years to change growth.

Short-term experiments, <5 year minimum, cannot therefore be relied on to predict the potential impacts of enhanced acid and N deposition on tree growth, even when there are changes in foliar and soil chemistry [9]. It is also possible, when considering the combined effects of N and acidity, that the acid and N effects cancel each other out. The capacity of tree growth to buffer change should not be underestimated. Sheppard et al [5] showed no growth effect at double the acid + N dose, despite significant increases in litterfall and canopy transparency and lowered foliar P and Mg status. Innes [10] reports minimal growth effects until > 50% of the tree needles have been lost or damaged.

The issue of what to measure to assess the effect of acid deposition on N availability is complex, reflecting the temporal nature of effects, which in turn depend on each systems capacity to buffer the chemical changes and previous deposition history [11]. The Sheppard et al [5] study identified the responsiveness, rates and magnitudes, of different parts of the system to acidic S and N deposition and found that soil water N and S increased significantly as did the fine roots, which are in direct contact with the soil solution. The saprophyte community, which is coupled to litter and throughfall chemistry responded over a similar time-scale while the ectomycorrhizal community, buffered via the trees carbon supply [11], took longer. Amongst the slowest responding parts of the system studied by Sheppard et al [5] were foliar nutrient concentrations. Foliar N failed to register a significant change for annual N inputs of 48 kg N ha⁻¹ y⁻¹ over the 5 treatment years, though did show a significant albeit small increase (<20%) in response to 96 kg N ha⁻¹ y⁻¹. Response times may be related to the sizes of the soil N pools [12] but these were not quantified.

Questions concerning sustainability, how long sites can continue to buffer anthropogenic inputs, or recover if inputs decline therefore remain highly topical as so few experiments extend beyond 3-5 years, typical grant lengths. The relevance of historical S loadings on N use is still important because unlike S emissions, N emissions have not fallen in recent years, in the UK [13].

This paper reports on a three year extension of the experiment of Sheppard et al [5] which, in addition to evaluating the temporal aspects of the ecosystem response to acidified S and N additions, by maintaining the original treatments to half the plots, also examined the potential for recovery, by the removal of sulphuric acid, sodium sulphate and ammonium nitrate constituents. The aims were to:

- Evaluate above and below-ground responses to the combination of sulphuric acid and N additions to assess the influence of acidified S on N responses.
- Assess the rate of responsiveness of ecosystem recovery to the removal of acidified S,
 N or a combination of the two.

2. Methods

2.1 Site Description

Table 1 near here

The site was located within a young commercially managed Sitka spruce plantation, planted in 1986, in the Scottish Borders, 20 km S. W. of Edinburgh (290 m above sea level at latitude 55°46'N and longitude 3°18'W on an organo-mineral soil, < pH 3.0 in CaCl₂). The study area comprised 1.5 ha of trees, approximately 2 m apart on mounds formed, from the inversion of 0.7 m of peat, litter and small but variable amounts of mineral soil, when drainage furrows were created using a double moleboard plough. This intervention created considerable plot to plot variability in soil properties due to the different amounts of mineral soil turned over by the plough [2,5].

2.1 Treatments

Two plots (selected at random, irrespective of block) were maintained on the original treatment, and the other two had an element of the treatment removed to simulate reductions in emission/deposition, as shown in table 1. The maintained treatments provided 50 kg ha⁻¹ y⁻¹ and 48 kg ha⁻¹ y⁻¹ of S and N respectively, or twice those doses, at a maximum ionic strength of 1.6 or 3.2 mM. The double dose was achieved by doubling the spraying frequency over the annual spray period, between May and November. In the 'recovery' plots the N and S were withheld from the rainwater and H₂SO₄ (SAc) was withheld from the NSAcid (NSAc) and 2NSAcid (2NSAc) treatments to leave only Nr or 2Nr. The new treatment regime was implemented in 2001, 2002 and 2003 (table 1).

The amount of treatment applied per spray event was equivalent to 2mm precipitation, just sufficient to wet the canopy but not the soil. Treatments supplied an additional 10% precipitation over the year. The trees were sprayed at a pressure of 1.5 bar with droplets of between 100 and 250 μm in diameter. The galvanised steel scaffolding ($13m \times 5m$) supported the 24 full cone sprayer units.

2.2 Treatment periods and environmental parameters

The spraying schedule in relation to rainfall and soil temperature, 0-5 cm depth is given in Table 1. Treatments generally began in May preceding budburst. In 2001 and 2002 all the treatment was applied, compared to the final year when the 'drought' led to only sufficient rainfall being available to apply 75% of the treatment. Soil water collections corresponded to the start and finish of spraying, and winter no spray periods. Rainfall was based on a tipping bucket and together with soil temperature was measured about 1 mile north of the forest, across the moor. Mean soil temperatures over the spray and winter periods varied by < 1°C (table 1). Rainfall varied hugely from ~ normal in 2001 to very wet in 2002 to dry in 2003 (table 1).

2.3 Measurements

Stem area was measured annually at breast height to calculate stem area increment (SAI). For foliar chemistry, several two-year-old shoots were removed annually from the upper third of each tree in January, bulked by plot, separated into current and one year old shoots, dried, the needles separated and ground [5]. N and S were measured with a CNS analyzer (Vario-EL elemental analyzer) and P, K, Ca and Mg in a modified Kjeldahl digest and analysed in a 1% sulphuric acid on a Perkin Elmer 4300DV ICP-OES at a UKAS accredited laboratory. Litter was collected twice a year from 1m² of guttering, which was also used to collect throughfall [13] in the first year of treatment change. Soil water was collected with zerotension lysimeters [4]. One or two samples were collected for the winter period and between 2 and 4 over the treatment period. Field samples were preserved using thymol. Volumes collected decreased significantly as the trees closed canopy. Soil cores, 4 from the middle of each plot were removed, using a bulb planter, for assessment of fine roots and mycorrhizal infection and assessed at 2 depths. A separate assessment was also conducted to examine the C:N ratio of the litter. Plot soil chemistry was assessed for the organic litter layer and the A₀ horizon, 0-10 cm of peat, separately for the ridge and undisturbed area between the two lines of trees, in March 2003 on bulk samples each representing 10 cores. pH was measured using 1:2.5 vol.: vol. in deionised H₂O and 0.01M CaCl₂ on fresh soil before the soil samples were air dried, sieved, remaining fine roots removed and ground. Cation exchange and exchangeable cations, Ca, Mg, K, Na, Fe, Mn and Al were measured in 0.5M BaCl₂ and the metals analysed on a Perkin-Elmer PE4300DV ICP-OES.

2.4 Statistics

The N, S, SAc and 2SAc removal treatments were evaluated against their original treatment pairs for the 3 year period. Data were analysed using Genstat 6 for Window's, one-way ANOVA, no blocking and using plot moisture as a covariate, because of its highly significant correlation with growth over the previous 5 years [5]. Residuals were checked for normality

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and data transformed as necessary. Where the treatment effects were significant (P < 0.05) Fishers least significant difference test was used to separate the means.

3. Results

3.1 Tree growth, recovery from SAc and responses to N and timescale issues

Table 2 near here

Over the eight growing seasons of this experiment (1996-2003) annual relative SAI declined from > 60 % to < 10% as the trees, planted in 1986, reached canopy closure with an average area of 140 cm² tree⁻¹ at breast height and a predicted yield class of > 28. Treatment effects remain relatively modest (figure 1). N availability continued to exert a relatively small influence on SAI at this site, although the smaller SAI in response to N removal, indicates the additional N was beneficial. The removal of acidity and S did not significantly change growth increments (table 2). The trees, in their exponential growth phase, responded positively to N but in the presence of large amounts of acidity the N response was overridden by 'other' influences. The addition of the spray, $\sim 10 \%$ additional precipitation, to the canopy over the growing period had a significant (P< 0.05) detrimental effect on growth (table 3).

Table 3near here

Figure 1 near here

3.2 Treatment effects on needle weights

Figure2 near here

Plot to plot variation in needle weight was high and no significant treatment effects were found. The one year old needles were almost 50% heavier than current year needles (figure 2). Over the three years of recovery needle weights declined, -15%, in the control plots. For current and one year needles, the removal of acidity tended to increase needle weights.

3.3 Responses of nutrient concentrations in current and one year old needles

In this field study, the treatments were applied to the canopy, enabling potential canopy as well as below-ground interactions. The needle weight and nutrient concentration data has been presented for the recovery treatment years and for the preceding year, when the replicates received the original treatments. Differences in needle weight can influence foliar nutrient concentrations [14]. In good growing years and / or mild winters, such as 1999/2000 (pre-recovery), large amounts of non-structural carbohydrate can accumulate, serving to dilute nutrient concentrations, which are expressed per dry weight. The original and recovery treatment pairs often started at different weights or nutrient concentrations (figures 2,3). To identify trends in the recovery treatments and divergence from the original treatment, linear fits to the 4 data points have been included when the R² exceeded 0.9.

Figure3 near here

Mg concentrations, in both needle age classes, were significantly affected by the treatments (P < 0.05). In current year needles, acidity reduced Mg concentrations in proportion to dose, minus ~ 15 or 30% respectively over the 3 years relative to the control. Neither S nor N alone significantly affected Mg concentrations. Removing acidity almost restored Mg concentrations to those of the control. However, recovery was much slower in the double acid treatments (figure 3).

In one year old needles the double acid treatment significantly lowered %Mg. Removal of acidity had no effect at either dose (figure 3). N and S treatments had small positive effects on the Mg status of one year old needles, relative to the control, which was absent in the recovery treatments. The removal of S (Na₂SO₄) caused Mg concentrations to fall (figure 3).

Ca concentrations were not significantly affected by treatment (P>0.05) for either age class of needles. In both current and one year old needles Ca concentrations declined over the

treatment period by $\sim 40\%$, down from 0.25 % and 0.45% respectively. Removal of acidity slowed down the Ca decline (figure 3).

K concentrations (data not shown) were not significantly affected by treatment, but in both year classes of needles the additional wetting of the canopy lowered %K by ~ 25% below those in needles from unsprayed control trees. Mean K concentrations for all treatments were 0.28, 0.34, 0.29 and 0.33% in prerecovery and recovery years 2001, 2002 and 2003 respectively for current year needles, and 0.26, 0.27, 0.26 and 0.26 % in one year old needles. **S concentrations** (data not shown) were not significantly affected by treatment (P> 0.05). Mean concentrations for all treatments increased from year to year: 0.09, 0.1, 0.11, 0.12 %S and 0.09, 0.11, 0.12, 0.14 %S in new and one year old needles respectively.

P concentrations for all treatments showed almost no annual or treatment variability and averaged 0.12% P in current and 0.11% P in one year old needles.

N concentrations in current year needles were increased by treatments containing N by comparison with the control, significantly in recovery year 2 (P= 0.051, <0.001, 0.18 respectively) (figure 3). Removal of acidity increased foliar N concentrations. Removal of N caused % N to decrease. In 2003, foliar N concentrations were noticeably higher than previous years, in the treatments received N, especially where acid was removed from the double acid treatment. Similar but mainly none significant treatment trends were seen in one year old needles (figure 3). In year three the increase in N status in response to the removal of the double acid dose was significant with respect to the control.

3.4 Changes in litterfall

Figure 4 near here

Prior to implementing the recovery treatments there were differences (none-significant) between the treatment pairs, especially for the 2NS Acid treatment (figure 4). Between

seasons 2001 and 2003 the effects of treatment recovery on litter weights were small and non-significant. In 2000 the average litter loss was high ranging from 300 gm⁻² in most plots, and up to 750 g m² in the double acid + N treatment, but proceeded to stabilize in subsequent years as more of the plots reached canopy closure.

3.5 Effects of recovery treatments on forest floor litter accumulation, litter pH and N concentrations and fine root mass and N concentration

Tabl 4 near here

After two treatment seasons' removal of acidity was reflected in litter pH, but not significantly (table 4). The weight of the litter layer, was reduced when acid was removed, significantly so in the double acid plots (table 4). C:N ratios (table 4) were all >30, just above the critical ratio of 25-27 indicating N saturation [15]. There were no significant treatment effects on litter N (P=0.21). Litter N concentrations were >40% higher than N concentrations in the live foliage and the N treatments increased litter N by $\sim 10\%$. The removal of acidity had a positive though non-significant effect on fine root mass, whereas removing N reduced fine root mass. The +N treatments contained most fine roots. Concentrations of N in the fine roots exceeded those in the foliage by > 40%. Fine root N concentrations were not significantly affected by any treatment (table 4), but were higher in the single acid treatment when acidity was removed. Nitrogen removal did not affect fine root N concentrations.

3.6 Treatment effects on Ectomycorrhizas (ECM) fruitbody numbers and root morphotypes Table 5 near here

Fruitbody numbers were significantly lower where the original treatments contained N, except when the N was applied with acid at the single N+ acid dose (table 5), particularly those of *Lactarius rufus*. Numbers of the smaller *Inocybe* were also low, as were numbers of saprophytic fruiting bodies in these treatments (table 5). Of the saprophytic fruitbodies, *Mycena* spp. were most sensitive to acidic conditions, being absent from the acid plots (table

5). There was no significant recovery in fruitbody numbers in response to two years of acid or N removal.

Table 6 near here

Proportions of root with the *Tylospora* morphotype were greatest in the N plots, which had least *Lactarius rufus* (table 6). Depth sampling showed that proportions of *Tylospora* morphotypes were greater in the 0-5 cm layer, whereas ECMs of *L. rufus. Cortinarius* and *Inocybe* were more prevalent at depth (data not shown). The contrasting effects of the original treatments and soil depth on the occurrence of *Tylospora* and *L. rufus* morphotypes meant their distribution across the site was inversely related (*P* <0.001; r = -0.85). *Cortinarius* and *Inocybe* morphotypes were again sparsely distributed. *Cortinarius* was most sensitive to the acid treatments, being completely absent from the double acid +N plots. *Inocybe* preferred the S plots (table 6). Removal of acidity, S and N increased the production of saprophytic fruitbodies after 2 years, but not significantly. Responses to the recovery treatments were small among the ECM morphotypes and fruiting bodies of the larger sporocarp formers.

3.7 Effects of the original treatments and removal of N, S and acidity on soil chemistry Soil pH:

Table 7 near here

Soil pH governs many biological activities in the soil from microbial transformations to root growth [16]. Thus, changes in soil pH may be crucial to the vitality and sustainability of the below-ground community structure and function. Soil pH (CaCl₂) in 2003, into the third treatment season, showed no treatment effect, whereas in water pH showed significant increases due to removal of the double acid dose and N from the N treatment (table 7). pH in water indicated a significant acidifying effect of the acid and also N treatments on the peat soil so that after 7 years, acidity was increased more by adding N than by adding N + acid.

Soil water:

Figure 5 near here

Soil water Mg was unaffected by acidity in 2001 but in 2002 and 2003, was almost doubled compared to that measured in the control treatment which averaged $\sim 30\mu mol_c$ throughout, except in 2003 when it rose to $\sim 50\mu mol_c$ (figure 5). By comparison the N and S treatments produced similar Mg concentrations to the control. Highest Mg concentrations were in the acid treatments. During the winters, in the absence of treatment, Mg concentrations remained similar to or below the control for all treatments. When acidity was removed the Mg response disappeared, Mg concentrations fell well below the original treatments, and were at least 50% below the control concentrations. The effects of S and N removal on soil water Mg concentrations were minimal as neither treatments had significantly affected soil water Mg concentrations.

Soil water Ca concentrations (figure 5) mirrored the treatment responses reported for Mg (figure 5) except that the effects were all more exaggerated. Ca concentrations exceeded those of Mg. Removal of acidity caused Ca concentrations to decline to below control concentrations. Control Ca concentrations averaged $\sim 40 \mu mol_c$ all except for 2002 when they rose to $80 \mu mol_c$.

Soil water K responded differently to the original treatments by comparison with Mg and Ca (figure 5). In 2001, except for the S treatment, all other treatments had lower K concentrations than the control, which measured 11μmol_c. Over both winter periods concentrations likewise tended to be below the control values of ~8 and ~10μmol_c for 0102 and 0203 respectively. In 2002, the effects of the spray treatments were pronounced with the acid and N treatments increasing K concentrations and the S and double acid + N dose treatments reducing K concentrations to below the ~16μmol_c in the control. Removing S especially and N increased K concentrations. In 2003 only the large impact of removing S was maintained (figure 5), there was no effect of the acid removal.

Soil water AI responded similarly to Mg and Ca (figure 5). In 2001 concentrations for the acid and N treatments were similar to the control (\sim 26 μ mol_c)(figure 5). Doubling the acidity + N increased soil water AI as did removing S. Acid removal from the double acid + N treatment did lower the AI concentration but in the absence of treatment, winter, this effect disappeared. As seen with Mg and Ca during the 2002 spray season, all the treatments showed elevated concentrations, \sim 80% higher for the control (\sim 44 μ mol_c). In 2002 the single acid + N treatment increased AI which was reversed in the minus acid recovery treatment. AI concentrations were significantly lowered. N and S removal increased AI, but not significantly. After this summer peak in 2002, all AI concentrations fell back to control values or less during the winter no spray period and were barely increased during the restricted 2003 treatment season.

The **base cation (BC) to Al ratio** where BC=Mg+Ca+K, was highest in the N treatment and reduced by the removal of N, in line with the control (figure 5). The ratios were lowest in 2001/2 but were 3 fold higher in 2003. The S and double acid + N treatments had the lowest BC/Al ratios, which were not affected by the removal of S or acidity.

Sodium concentrations remained relatively constant in the control (~140, 180, 270,150, 110 μmol_c) except during the 2002 spray season when concentrations almost doubled (figure 5). The S treatment (Na₂SO₄) more than doubled soil water Na compared with the control and even in the absence of spray, Na concentrations remained elevated. Omitting Na₂SO₄ caused Na concentrations to fall. Sodium concentrations were fairly unresponsive to the N and N + acid treatments although, N removal tended to increase Na concentrations.

Figure 6 near here

Soil water pH control values measured 4, 4.1, 3.7, 4.2, 4.3 over the five periods, being significantly more acid in 2002, when all the cation concentrations were elevated (figure 6). Soil water pH was more acid than the control in all the spray treatments except the one

receiving only N. The S treatment did not significantly affect on soil water pH, during any period (figure 6). Adding N made soil water more acid but where N was removed the soil water pH was lower still. The acid + N treatments had the most acid soil water and there were none significant increases in soil water pH when acidity was removed.

Soil water NH₄ $^+$ concentrations over the three years were relatively stable in the control (~6.0, 5.5, 7.5, 10, 8 µmol_c) Adding or removing S did not affect soil water NH₄ $^+$. There was a large response to adding the double acid $^+$ N treatment ($^+$ 5 fold soil water NH₄ $^+$) whereas, the single acid $^+$ N treatment had no effect and N alone only doubled soil water NH₄ $^+$. Over the winter no spray periods, NH₄ $^+$ concentrations were all more similar to control concentrations, except where acidity was removed from the double acidity $^+$ N treatment, when concentrations remained high (figure 6). The addition of acid $^+$ N led to higher NH₄ $^+$ (significantly in 2003) than when N alone was added (figure 6).

Soil water NO₃ concentrations exceeded NH₄⁺ concentrations in the N addition treatments (N, NSAcid and 2NSAcid) but were barely measurable in the control and S treatments (<1 μmol_c figure 6). Adding N alone increased NO₃ concentrations more than N + acidity. When N was removed the NO₃ concentrations fell back to control values. Generally removal of acidity increased NO₃ concentrations (figure 6).

Soil water $SO_4^{2^-}$ concentrations varied seasonally in the control plots (~ 90, 60, 140, 60, 50 μ mol_c). There appeared to be treatment effects even where S was not added (figure 6). Treatments containing S significantly enhanced $SO_4^{2^-}$ concentrations during the spray period and there was also a small memory effect (figure 6). N additions reduced the amount of $SO_4^{2^-}$ relative to the dose (figure 6). Removal of S caused $SO_4^{2^-}$ concentrations to fall back to concentrations measured in control plots.

Soil water PO_4^{3-} concentrations behaved in a different way from all other ions and the concentrations were very low *e.g.* controls over the five measurement periods were 0.4, 0.3, 1.2, 3.3 and 1µmol_c respectively. Treatment effects varied with the measurement period, but

some generalisations were apparent. Phosphate concentrations were very low in the double acid treatment and there was negligible recovery (figure 6). Effects of single acid + N were much less pronounced. In 2002 plots treated with N or which had received N, had increased phosphate concentrations (figure 6). Adding S barely affected soluble PO₄³⁻ concentrations.

3.8 Relationships between ions in soil water:

Figure 7 near here

A range of relationships between ions were explored, and the best relationships were between SO_4^{2-} and AI^{3+} (Figure 7) for double acid + N R²= 0.9609, acid + N R² = 0.9738 and for the recovery treatments minus acid R² = 0.9767 and minus N R² = 0.9209. Ca²⁺ concentrations were also linearly related to SO_4^{2-} concentrations in the acid treatments R²= 0.9718 (double) and 0.93 (single).

3.9 Exchangeable cations

Figure 8 near here

These were only assessed once in the spring following the eighth and final year of treatment. Cation concentrations were highly variable between plots and between the ridges, where the trees were planted, and the undisturbed area between the two rows of trees. No significant treatment effects were found, either between the original or recovery treatments for either area and so the data were averaged for the ridge and middle areas. Data for the main cations, likely to affect the trees are shown in figure 8, as proportions of the CEC, \sim 185 cmol_c kg⁻¹. The exchange complex was dominated, > 50% by Al³⁺, which was increased in all the treatments. Ca²⁺ occupied 8-19%, < 10% by Mg²⁺ and <5% for the monovalent cations K⁺ and Na⁺.

4 Discussion

This recovery experiment was established to investigate how quickly different parts of a forest ecosystem (Sitka spruce), which had been treated with elevated N, S and acid + N deposition, could recover when these pollutants were removed. Some responses were quite rapid, parameters that had responded quickly to the original treatments, *e.g.* elevation of cation concentrations in the soil water appeared to be reversed equally quickly, with no enhancement once acidity was removed. By comparison most effects of the double acid + N treatment, which involved supplying pH 2.5 H₂SO₄ + NH₄NO₃, at twice the frequency and thus N and S doses as the acid + N treatment, produced significant effects that were often not, or less effectively, reversed.

4.1 Growth, N responses and factors influencing growth

Tree growth appears to be something of an enigma on this site, yield class 27 is good, yet the needle nutrient concentrations, for N, P and Ca all lie very close to the minimum values reported by Innes [10] for 30-40 year old Sitka spruce surveyed in the UK. Concentrations of soil solution Mg which was very sensitive to the treatments, was relatively abundant in the needles, possibly reflecting the maritime influence at this site [17,18]. Foliar N concentrations, although low were still 10-20% above the minimum reported values, possibly explaining the absence of a large growth response to N. However K concentrations, not unexpectedly as peat is notoriously K deficient [19], were less than half of the minimum reported by Innes [10]. By comparison with minimum nutrient concentrations for Norway spruce [10], our nutrient values still fell at the low end of values expected to restrict growth and cause deficiency symptoms, neither of which were apparent.

Thelin [20] used the ratio of N to other nutrients as an indicator of nutrient status, the higher the value the more deficient the nutrient relative to N. Our ratios for Sitka spruce, expressed as a percentage relative to N, at ~29, 10, 10, 19 for K, P, Mg and Ca respectively for current year needles fall at the very lowest end for K, quoted for Norway spruce [20]. Doubling acidity reduced the K ratio to 20, and yet growth was not obviously reduced. This

treatment also shed most needles and may have redistributed nutrient through internal recycling to meet demand, but this theory is not supported by other nutrient data.

The sprayed control trees performed poorly compared with the other treatments, particularly the no spray control, which also received no additional nutrients. Significant leaching of K with just water was measured in throughfall from all treatments [17,18]. We were unable to detect these losses in measurements of the foliar nutrient concentrations, suggesting that reporting nutrients as percent dry weight may not be representative of physiologically active nutrient pools. Sitka spruce, before canopy closure, appears to be more tolerant than Norway spruce of unfavourable nutrient concentrations / nutrient ratios, so long as N is not deficient. This experiment has highlighted the increased risk of K deficiency, from enhanced leaching, for Sitka spruce growing on organic soils under a wetter climate.

4.2 Effects of acid removal on base cations

After 8 years of treatment with acid + N the concentrations of soil water Mg and Ca were still elevated, while K concentrations were low. After removal of the acid these ion concentrations barely exceeded the control, but because Al concentrations also fell, the BC:Al ratio remained relatively stable. Values fall within the range that Sverdrup and Warfvinge [21] consider unlikely to negatively affect the growth of Norway spruce, which would appear to be more conservative than Sitka spruce with respect to its soil chemical tolerance. Hruška et al. [22] found a negative relationship between the BC/Al ratio in the organic rooting zone and defoliation however their values were < 2; our values ranged between 2 and 5. Foliar base cation concentrations reflected these falling soil solution base cation concentrations, but on this site remained sufficient. We cannot comment on how long the base cation status could be sustained, but with the maritime source of Mg, which the trees were able to take up [17], together with the potentially reducing demand of a closed canopy, it seems likely that on this site the reducing base cation concentrations as a response to falling acidity would not compromise tree growth.

Declining K availability as acidity inputs fall could reduce growth. Nitrification responded positively to falling acidity and this could increase K leaching, via the mobile anion effect. However, sulphate concentrations also fell back to below control values in the recovery treatments and could counteract the nitrification effect. Both Ca²⁺ and Al³⁺ concentrations were positively related to SO₄²⁻ concentrations, as observed by Sogn and Abrahamsen [23] in a lysimeter experiment with H₂SO₄ and NH₄NO₃. The benefit of falling Al concentrations would be offset by the concomitant fall in Ca²⁺ concentrations. In this soil there was no 'memory effect' and desorption of SO₄²⁻ over and above control concentrations, as implied by Matzner and Murach [24] suggested that when soil solution SO₄²⁻ concentrations fall to very low levels the legacy of previous S deposition may desorb, offsetting the benefits of lower S deposition. One possible explanation for minimal desorption maybe that the large C and organic ligand resource present restricted the amount of SO₄²⁻ retained [24] by this soil over the eight years of inputs.

4.3 Effects of acid removal on N uptake and availability

The presence of acidity has had contrasting effects on N uptake by the trees. At the canopy level, NO₃ uptake was stimulated by the presence of acidity, H⁺ ions, via co-transport to maintain electroneutrality, whereas the uptake of NH₄ ions fell in the presence of acidity, out competed by H⁺ for uptake sites [17,18]. Supplying N with acidity negated its stimulatory effect on fine roots, so restricting the potential of the trees to take up the additional N via the roots [5]. Fine root growth responded positively to the significant increase in soil pH (H₂O) assessed after 2 years, when acidity was removed. These changes led to a noticeable increase in foliar N status. These results strongly suggest that as the proportion of acid to N deposition declines, N uptake will increase in response to the improvement in fine root growth. Mycorrhizas were less affected by acidity *per se* and we have already seen that N additions alone can reduce their diversity [25]. Whether the increased potential for N uptake by spruce represents an improvement in the *status quo* will depend on the N status of the ecosystem.

The removal of acidity also influenced the availability of N in the soil. On this acid peat, the removal of acidity from the double acid treatment led to higher soil solution NO₃ concentrations. Ammonium concentrations appeared to go in the opposite direction, implying increased nitrification in response to lower acidity [26]. Adding N with acid enhanced soil water NO₃⁻ concentrations by less than half as much as N and even when the acid was removed soil water NO₃⁻ concentrations remained below those in the N only treatment, again implying that the acidity had reduced nitrification. Removing acidity had variable, seasonally dependent effects, probably linked to the microbial nitrifying community, (nitrification was not assessed). Killham [27] suggests between year variation in the scale of change in soluble N reflects the balance between soil moisture and temperature effects on nitrification. Nitrification is also sensitive to allelopathy, so the effect of removing acidity may not just be direct, but may also involve effects on other microorganisms which may include soil fauna, fungi and heterotrophic bacteria [27]. Equally NH₄⁺ may not be the only N source, nitrifying fungi can use organic N which is much more abundant (> 10 fold) than the inorganic ions, even where the treatment includes mineral N. The acidity effects on N availability in this acid peat soil appear to be mediated through nitrification and a high degree of pH sensitivity amongst the soil microbial community.

4.4 Fate of applied N

Increases in foliar N status and stemwood growth, relative to the control trees, were relatively modest on this site, suggesting the extra N (400 and 800 kg N ha⁻¹) was not stored above ground. Large pools of N were measured in the fine roots and litter, almost 50% higher than N concentrations in the foliage, but neither the N nor acidity had significantly enhanced root N concentrations. Ectomycorrhizal fungi can sequester N in osmiophilic vacuolar bodies in the fungal mantles that enclose mycorrhizal roots, which tend to be more common following N additions [28]. No difference in % N was found, but there were large differences in root mass in response to N, probably diluting the N concentration. Acidity restricted fine root growth and N uptake but root mass was substantially increased by N without acidity. N additions also increased litter, especially when added with acid. On this site 25% of the additional N, was sequestered in the litter layer. There was no effect of the single acid dose on the amount of N sequestered. The double acid + N treatment sequestered about the same proportion of its mineral N input explaining the higher soil solution N concentrations.

These observations suggest that acidity increases the amount of N that will leach and be lost from the system. The C:N ratio which ranges from 30 to 37 still exceeds the critical ratio below which NO₃⁻ leakage is predicted [29]. However, our results suggest that when N is deposited with acidity the system will be more likely to leak N at a higher C:N ratio.

4.5 N effects below ground

The significance of increasing N concentrations, and acidity, with respect to effects on fine roots and mycorrhizas has been widely debated [30]. In this very acid soil fine root mass was highly sensitive to the effects of acidity and N inputs. Removing acidity significantly increased fine root mass whereas removing N reduced the mass of fine roots. There were no significant changes in mycorrhizas in response to N or acid removal. Nitrogen additions significantly increased the proportion of non-mycorrhizal tips. None of the treatments affected live root percentages. Our results appear to contradict those discussed by Matzner and Murach [30] who report negative effects of increasing N inputs on fine roots. Our results indicate Sitka spruce roots respond positively to N but negatively to acid, but that N does compromise mycorrhizas. For a more detailed discussion of the effects of N deposition on mycorrhizas see Sheppard and Wallander [31].

5 Conclusions

A decline in acidity will affect the bioavailability of N through several mechanisms: improved fine root growth *ie.* increased uptake surface and improved conditions for nitrification. Base cation concentrations will also be reduced but the impact on tree growth will be site and species dependent. Effects on N and base cations were quite rapid. Where acid inputs have been high, recovery may be slow and significant amounts of N deposition may be lost through leaching. As observed previously the soil solution is very responsive to N and acid inputs, unlike tree growth. Foliar nutrient concentrations appear to be more responsive to the removal of acidity and N than their addition.

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Figure legends:

Figure 1 Effects of 3 years of recovery treatments: removing acidity (SAc), S, double acidity (2SAc) and N, applied to a 15 year old Sitka spruce canopy growing in Deepsyke forest in the Scottish borders, implemented in 2001, on stem relative area increment (SRAI) % at 1.3m (n=2, + n=4 adjusted for covariate plot moisture).

Figure 2 Weights (g) of 100 current (new) and one year old (old) needles collected in January from a 15 year old Sitka spruce canopy growing in Deepsyke forest in the Scottish borders. Mean values are given for the pre recovery treatment year for the paired plots and the three years of recovery treatments: removing SAc (NSAr), S (Sr), 2SAc (2NSAr) and N (Nr). Where the trends corresponded to linear fits $R^2 > 0.9$ the line has been included.

Figure 3 Mg, Ca and N concentrations (% dwt) in current and one year old needles collected in January from a 15 year old Sitka spruce canopy growing in Deepsyke forest in the Scottish borders. Mean values are given for the pre recovery treatment year for the paired plots and the three years of recovery treatments: removing SAc (NSAr), S (Sr), 2SAc (2NSAr) and N (Nr). Where the trends corresponded to linear fits $R^2 > 0.9$ the line has been included.

Figure 4 Weights of litter (g m⁻²) in paired plots taken from a 15 year old Sitka spruce canopy growing in Deepsyke forest in the Scottish Borders. The pairs received the original treatment up to the end of 2000. The recovery treatments, removing SAc, S, 2SAc and N, were implemented in 2001 (dashed lines).

Figure 5 Cation concentrations (μ mol_c l⁻¹) of Mg, Ca, Al, K, BC to Al ratio and Na in soil water collected by zero tension lysimeters, (bulk of 10 per plot) during the time the recovery treatments (NSAc-SAc, 2NSAc-2SAc, S-S and N-N) were implemented, 2001-2003. The original treatments were NSACID, 2NSACID, S and N.

Figure 6 H⁺, NH₄-N, NO₃-N, SO₄-S and PO₄-P concentrations (μ mol_c l⁻¹) in soil water collected by zero tension lysimeters, (bulk of 10 per plot) during the time the recovery treatments (NSAc-SAc, 2NSAc-2SAc, S-S and N-N) were implemented, 2001-2003. The original treatments were NSACID, 2NSACID, S and N. (Control values omitted for clarity, given in text).

Figure 7 Relationships between soil water SO_4^{2-} concentrations and the concentrations of Al^{3+} and Ca^{2+} for original and recovery treatments for the five spray and no spray sampling periods. The relationships are shown for the different treatments, SO_4^{2-} versus Al^{3+} , R^2 for 2NSAc = 0.9609, NSAc = 0.9738, -NSAc = 0.9767, S = 0.9209; for SO_4^{2-} versus Ca^{2+} , R^2 for 2NSAc = 0.97, NSAc = 0.93.

Figure 8 Proportion (%) of the cation exchange capacity, below the litter layer, occupied by Ca, Mg, Al, Na, and K after 3 years of recovery treatments and 8 years of the original treatments, data for the middle and ridge have been combined. There were no significant treatment effects.

Table 1 Dates of spray treatment periods between 2001 and 2003 when the 'recovery' treatments were applied. Rainfall (mm) amounts during the winter with no treatment and over the spray periods. In 2003 only 25% of the treatment was applied due to insufficient rainfall. Recovery treatments implemented in 2001: (2 plots per treatment, 4 wet and dry control plots). N inputs were 48 or 96 kg N ha⁻¹ y⁻¹ and S were 48 or 96 kg S ha⁻¹ y⁻¹.

	2001	2002	2003 ~
Spray start	22 May	8 May	13 May
Spray finish	29	18	20
	November	October	November
Rain Jan. to start spray (mm)	310	427	146
Rain over the spray treatment period (mm)	521	883	411
Mean soil temp Jan. to start of spraying (°C)	3.4	4.5	3.9
Mean soil temp. over the spray treatment	10.0	10.1	10.7
period (°C)			

Phase 1		Phase II	
NSAcid (NI	$H_4NO_3+H_2SO_4$	NSAcid pH 2.	5
		N-SAc	removal of S and acidity (-SAc)
2NSAcid 2*	$(NH_4NO_3+H_2SO_4)$	2NSAcid pH 2	2.5
		2N-2SAc rem	oval of S and acidity (-2SAc)
N only	NH ₄ NO ₃	N only	
		N - N remove	al of N to rainwater only
S only	Na ₂ SO ₄	S only	
		S-S remove	al of S to rainwater only
Control	rainwater	Control	no change
No spray		No spray	no change

Table 2 Effects of Recovery treatments, removing SAc, S, 2SAc and N, applied to a 15 year old Sitka spruce canopy growing in Deepsyke forest in the Scottish Borders, implemented in 2001, on absolute growth area increments (cm²) adjusted for covariate (n=2). The ratio of treatment means pre and post recovery are provided for comparative purposes. (†At this time both pairs received the same treatment). Probabilities for treatment (Fpr) and covariate (Fpr_{cov}) effects are also included.

	Increment after 5 yrs	Ratio	Increment post 3 yrs	Ratio
	prerecovery †		recovery	
NSAc	81.3		36.4	
-SAc	81.1	1.00	37.2	0.98
s -s	67.2		29.7	
-S	67.2 64.5	1.04	28.1	1.06
2NSAc	77.2		32.5	
-2SAc	77.1	1.0	33.2	0.98
N	82.1		41.6	
-N	78.2	1.05	33.8	1.23
F pr	0.36		0.62	
F pr F pr _{cov}	0.36 < 0.01		<0.001	
			1 1 1	1

Table 3 Effect of 8 years treatment with 48-50 kg N/S ha⁻¹ y⁻¹ or 96-100 kg N/S ha⁻¹ y⁻¹ with or without acidity on relative area increment RAI (%) or actual stem area at 1.6m (cm²) (n=2 or 4, adjusted for the covariate plot moisture). Values followed by the same letter are not

significantly different.

	RAI 1996-2003	Area 1996-2003
	%	cm ²
NSAcid	652 a	138.5
S	595 a	124.4
2NSAcid	600 a	132.3
N	546 ab	137.1
Control +	464 b	114.3
Dry control +	606 a	141.2
F pr treat	0.032*	0.164
LSD	141	32.2
CV%	10.2	9.8
	I	

Table 4. Forest floor properties and fine root distribution and N chemistry in the original and recovery treatments, sampled in November 2002 after 2 treatment seasons. 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	Litter	Litter	Litter	Fine roots	Fine roots
	pН	g m ²	N%	C:N	g m ²	%N
				ratio		
NSAcid	3.91 ab	1500 ab	1.62	33.1	164	1.55
N-SAc	4.0 b	1120 b	1.63	32.5	363	1.65
\mathbf{S}	4.17 bc	510 с	1.44	36.9	83	1.51
Min S	4.24 c	682.c	1.4	37.1	54	1.67
2NSAcid	3.79 a	1733 b	1.57	34.2	191	1.56
2N-SAc	3.86 a	1177 a	1.68	31.9	257	1.58
N	4.05 bc	1215 b	1.7	30.8	318	1.67
Min N	4.12 bc	1030 b	1.66	31.7	190	1.68
Control	4.21 bc	757c	1.55	33.8	116	1.52
No Spray	4.15 bc	857c	1.45	35.8	83	1.65
P value	0.012	0.01	0.21	0.39	0.24	0.89
LSD	na	509	0.27	6.9	271	0.3

Table 5 Effects of the **Original treatments** on fruitbody (FB) numbers and species diversity assessed in autumn 2002, n=2.

	NSAcid	S	2NS	N	Control	No	P value
			Acid			spray	
Total ECM FB	238bc	322ab	94c	79c	386ab	611a	0.005
Tylospora	21.2	20.6	16.5	24.4	20.8	20.7	0.064
fibrillosa							
Lactarius rufus	199abc	256ab	77bc	30c	337a	556a	0.021
Cortinarius spp.	3.4	16.3	0	8.7	20.8	24.0	0.170
Inocybe spp.	0.51bc	2.34a	0.16c	1.40abc	1.66ab	2.33a	0.016
Laccaria spp.	12.0	6.7	0.2	4.8	2.0	0	0.175
ECM diversity	1.87	3.40	1.62	2.38	3.04	3.31	0.051
Total sapro. FB	129a	112a	30b	79a	136a	116a	0.005
Marasmius	122	68	25	59	99	98	0.142
androsaceus							
Mycena spp.	0c	11.6ab	0.2c	6.6b	22.6a	16.8a	<0.001
Galerina sp.	6.8	32.4	4.8	13.1	14.3	1.4	0.945
Saprophytic	1.00cd	1.94ab	0.81d	1.42bcd	1.75abc	2.40a	0.013
diversity							

Letters indicate LSD between means in the same row if P < 0.05. Log(n+1) transformations were performed on all data except diversity measurements. Means have been adjusted for the covariate.

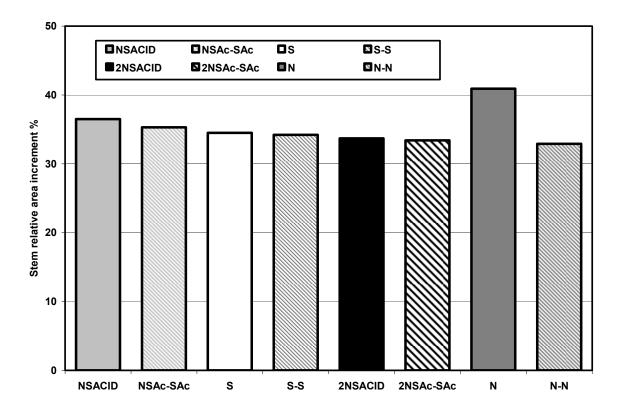
Table 6 Effect of the **original treatments** on litter depth, root growth and proportions of ECM morphotypes sampled from the central undisturbed area between the two rows of trees.

	NS	S	2NS	N	control	No	Proba
	Acid		Acid			spray	bility
Litter depth (cm)	1.13b	1.03b	2.42a	1.13b	0.56c	1.15b	< 0.001
Total root tips	3351ab	3888ab	3725ab	5963a	3065bc	1547c	0.009
Fine root dry wt (mg)	831a	687a	683a	796a	548ab	306b	0.010
% live roots	23.6	24.2	25.2	19.9	21.9	22.9	0.914
% non-mycorrhizal	24.1b	18.8bcd	43.2a	21.2bc	13.5cd	9.1d	< 0.001
tips							
% Tylospora	45.6ab	30.0b	40.0ab	59.1a	25.1bc	12.7c	0.002
% Lactarius rufus	24.4c	37.4bc	16.8c	14.6c	52.8ab	72.8a	0.001
% Cortinarius	5.2	6.8	0	0.6	7.9	5.6	0.158
% Inocybe	0.72b	6.68a	0b	3.07b	0.95b	0b	0.001

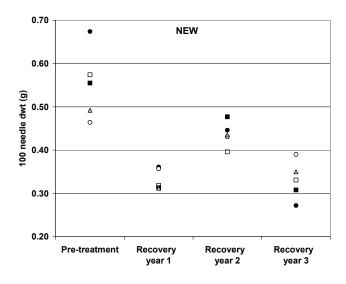
Means are adjusted using soil moisture (%) as a covariate. Letters indicate LSD between means in the same row if P < 0.05. Square root transformations were performed on root tips and root dry weight, and arcsine transformations on percentages.

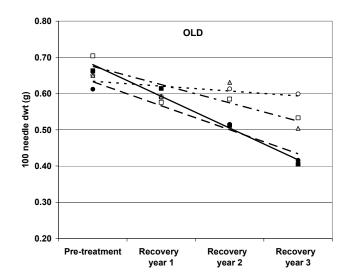
Table 7 Soil pH measured in June 2003 (5 cores/plot from the upper 10cm) measured in $CaCl_2$ (10⁻²M) and water. Values followed by the same letter in each column are not significantly different at P<0.05. Paired effects of recovery are shown in **bold**.

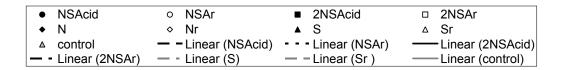
	pH in CaCl ₂	pH in H ₂ O
NS Acid	2.98	3.84 b
N-Ac	2.88	3.91 b
S	3.01	4.0 b
-S	2.96	3.94 b
2NSAc	2.88	3.69 a
2N-Ac	3.02	4.0 b
N	2.93	3.67 a
-N	2.92	3.84 b
Control	2.92	4.02 b
No spray	3.00	4.05 b
P value	0.26	0.011

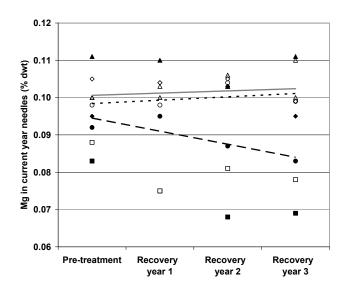


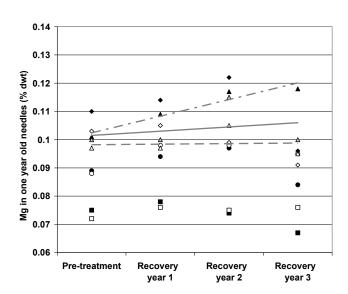
 NSAcid 	NSAr	2NSAcid	□ 2NSAr
◆ N	♦ Nr	▲ S	△ Sr
△ control	– Linear (NSAcid)	Linear (NSAr)	Linear (2NSAcid)
- Linear (2NSAr)	— - Linear (S)	— - Linear (Sr)	Linear (control)

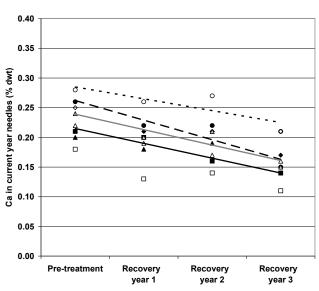


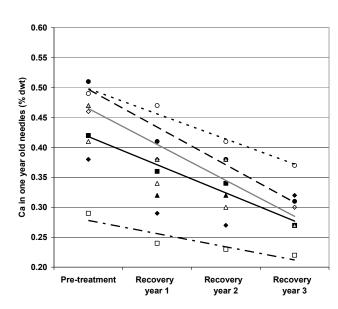


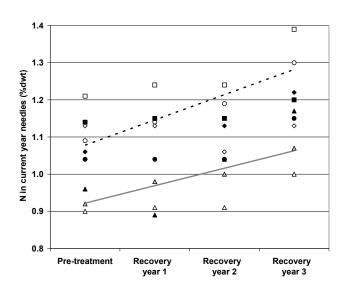


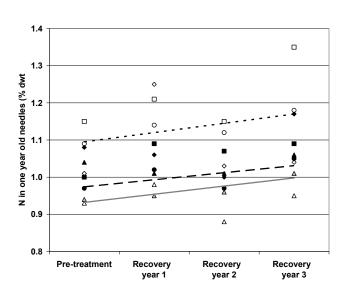


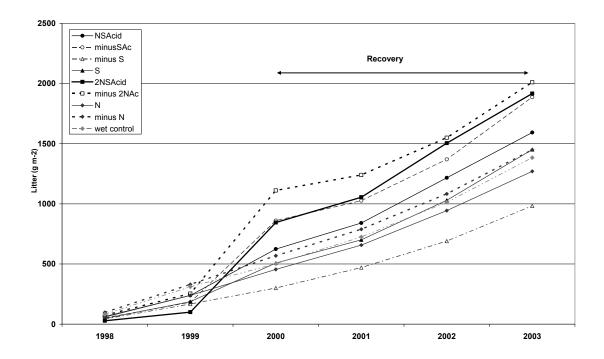




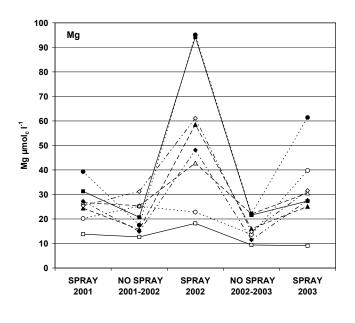


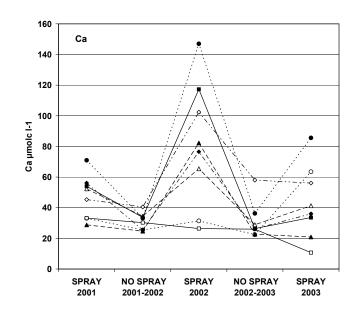


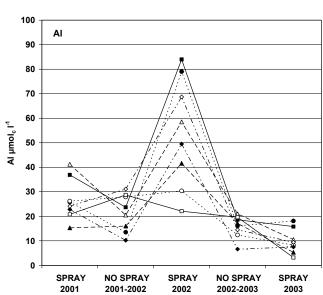


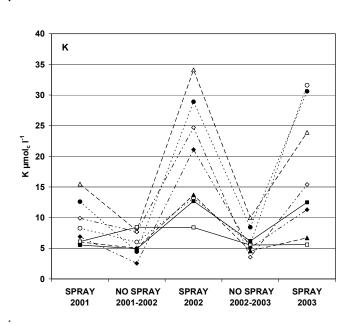


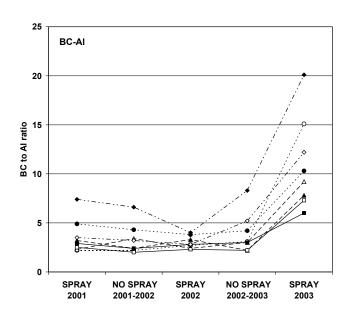
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→ 2NSACID	2NSAc-SAc◆-	N	-

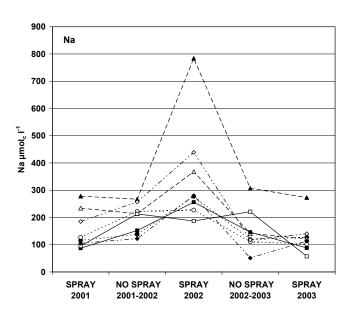












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