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C and N mineralization and earthworm populations in a Norway spruce forest at Hasslöv (SW Sweden), 25 years after liming

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Abstract

During the last decades of the 20th century, acid rain affected many areas in Europe and Northern America. Soil acidification was considered a large problem for forest ecosystems, because it was expected that tree growth would be hampered by low pH, nutrient deficiencies and high concentrations of free Al. Furthermore low soil pH can change the soil fauna and soil microbial biomass. High acidity has also been shown to reduce C and N mineralization. Liming was expected to be a good measure against soil acidification. The present study assesses C and N mineralization and the earthworm community in the Hasslöv forest (SW Sweden; Norway spruce), 25 years after liming with a low dose of $CaCO_3$ (1.75 t ha^{-1}) and a low, medium and high dose (1.55, 3.45 and 8.75 t ha^{-1}) of dolomitic lime.

Soil pH correlated to the lime dose. Liming with medium and high doses of dolomitic lime increased C mineralization rates in the FH layer and the upper mineral soil. Liming did not increase N mineralization rates, though there was an increase in nitrification in the soil with the highest dose of lime. The C and N pool were lower in the heavily limed soil and to a lesser extent in the medium limed soil, compared to the control soil, mostly due to a reduction in the organic layer. Furthermore earthworm populations were larger with increasing doses of lime. The dominant species, *Dendrobaena octaëdra*, was present in all treatments whereas three other species were only found in the highest doses.

When extrapolated to the field, both C and N mineralization expressed on an area basis were lower in soils with the highest lime treatment than in the control, and N mineralization was also lower in the medium limed soil. This can be explained by the fact that the C and N pools had been markedly reduced and that the increases in mineralization rate per gram soil could not make up for this reduction.

In conclusion, addition of lime has long-lasting effects that can be seen as higher pH, higher C and N mineralization rates, lower soil pools of C and N and higher abundances of earthworms still after 25 years from the liming event. We recommend that as a measure against acidity liming should only be used with great caution.

Contents

1 Introduction

1.1 Acidification

Over the last three decades of the 20th century, and to a lesser degree up to this day, acid rain (atmospheric deposition of nitrogen, sulphur and protons) affected many areas in Europe and North America (e.g. Prietzel *et al.*, 2006). N in the acid rain came both from excessive fertilization and, in the form of nitric oxides, from industrial pollution and heavy traffic. Sulfur dioxide was present in industrial emissions and remains of fuel combustion. In the atmosphere nitric oxide is converted to nitric acid and sulfur dioxide to sulphuric acid. Deposition of these acid substances can lower soil pH dramatically.

Acid rain caused many forests in e.g. Czech Republic, Poland and Germany to suffer severe decline through the devastating effect of sulphuric acid on the foliage (Murach and Ulrich, 1988; Kandler and Innes, 1995). Acidification of the soil was also considered a major problem for forests. Though pH itself has a marginal impact on tree growth, lowered soil pH due to acid rain can affect the soil system in various ways and can be an indirect threat to the forest. Aluminum is more water soluble at low pH (Lundström *et al.*, 2003). Too much of free Al^{3+} affects plants negatively, particularly young individuals, by disrupting cell processes in the root tips and so preventing root growth (Rangel *et al.*, 2009). This happens especially when the concentration of Ca is low. Although trees like Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* Karst.) and birch (*Betula spp.*) are themselves not much affected, high concentrations of Al^{3+} can still be problematic. When Al^{3+} covers the root tips, it can lessen nutrient uptake by the tree and so theoretically decrease growth or health. However, a review by Nyberg et al. (2001), covering int.al. 80 forest sites in southern Sweden did not show a correlation between soil acidification and forest growth. In fact, soil acidification can even have beneficial effects on tree growth because increases in N deposition can act as a fertilizer for the trees (Tamm and Wiklander, 1980; Tveite, 1980). This has stimulated for example the growth of Scots pine in Germany and southern Scandinavia (Prietzel *et al.*, 2006) and in general forest growth in Europe in the era of acid rain was greater than ever before (Kauppi *et al.*, 1992; Kandler and Innes, 1995).

Lowered pH (e.g. 4.5 → 4) can cause changes in soil biota (e.g. Bååth *et al.*, 1980; Persson, 1989; Persson and Wiren, 1993), including dramatic declines of earthworm populations (Ammer and Makeschin, 1994), which are important ecosystem engineers. Changes in soil biota can be either due to direct effects of acidity, like in the case of earthworms, or to indirect effects like changed competitive relations, for example between earthworms and enchytraids (Persson, 2002).

Experimental acidification studies (both in lab and field) and field sampling studies have shown that soil acidification has a definite influence on soil processes like mineralization (Box 1). This is closely related to the sensitivity of the soil microbial biomass to pH. Most types are pH sensitive and acidification can decrease their activity substantially. In this way acidification can either stimulate (e.g. Tamm, 1977), but usually depress (e.g. Klein *et al.*, 1984) N mineralization. It can also reduce carbon mineralization on short (> 2 yr) and middle-long (3-15 yr) term (e.g.

Bryant *et al.*, 1979; Bååth *et al.*, 1980; Klein *et al.*, 1984), even though pH has recovered (Persson and Wiren, 1993). Experimental N deposition (Högberg *et al.*, 2006) and experimental acidification are not always representative ways to investigate the effects of acidification on natural soils: acid application usually involves higher doses over a short period of time, which can make the effect shift from suppression to stimulation of N mineralization, through different reactions of the microbial community (Klein *et al.*, 1984; Novick *et al.*, 1984). Tamm et al. (1977) suggested that high N mineralization after application of acids could depend on a mortality of parts of the soil bacterial community, after which these are mineralized, including their N content. This would mean that increased N mineralization is a short-term effect and will not last over a long time (Aber *et al.*, 1982).

Box 1. C and N mineralization.

Mineralization refers to the chemical process of conversion of an element from an organic to an inorganic form. In the present study, only C and N mineralization are considered. C mineralization is performed by the microbial biomass in the soil who consumes organic materials for synthesis of their cell components; they release the superfluous CO_2 through respiration.
N mineralization refers usually to two processes: ammonification and nitrification (Figure 1). Ammonification is the conversion of organic N to ammonium (NH_4^+), performed by the microbial biomass. Nitrification is the conversion of ammonium to nitrite (NO_2) and subsequently to nitrate (NO_3^-). This can be performed either autotrophically or heterotrophically. Total (net) N mineralization is the increase in ammonium and nitrate over time. It is measured as ammonification + nitrification because the product of the one step (ammonium) is the substrate of the next step. Although ammonification is the 'real' N mineralization, only the sum of the products of both processes gives a good measure of N mineralization.
CO_2 is a gas and can therefore be measured in a gas chromotograph. Since neither NH_4^+ nor NO_3^- are gases, they have to be extracted from the soil material and measured in a liquid. Ammonium causes soil acidification when taken up by roots. Nitrate in itself does not cause acidification, but nitrification does. Since nitrate is weakly bound to surfaces, it can easily wash out with rain water and eutrophicate the ground water. The protons produced in nitrification wash out too and cause acidification (Berg, 1996).

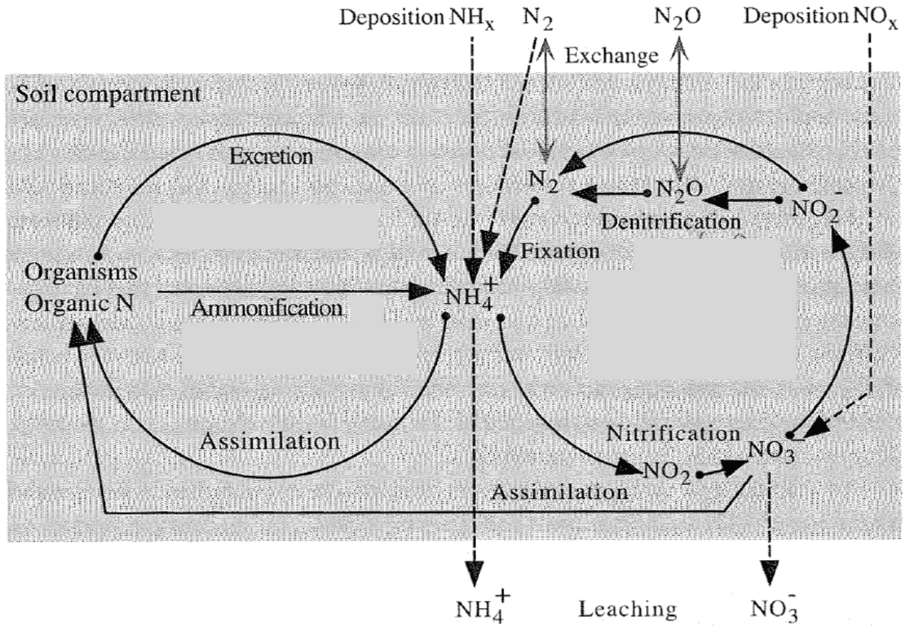


Figure 1. The N cycle in a temperate coniferous forest ecosystem. (Picture taken from Berg (1996).)

1.2 Liming

Liming (Box 2) has often been used as a measure to counteract acidification and prevent forest damage. For example, between 1907 and 1986 a total of about 150 trials were conducted in Sweden and Finland (Staaf *et al.*, 1996), and also in Central Europe different kinds of trials were set up (e.g. Kreutzer, 1995; Huber *et al.*, 2006; Kunes *et al.*, 2007). In Northern Europe, the motive was not in the first place to counter forest damage, because the massive 'Waldsterben' of Central Europe was not observed there. Liming was used as a measure against observed acidification of surface water and soil. For an overview of forest liming trials in Scandinavia and Central Europe and their results, see the overview by Lundström et al. (2003). Liming increases pH and base saturation (Matzner, 1985; Kreutzer and Göttlein, 1991). Increased pH in turn affects soil biota, including earthworms (Söderström, 1984; Persson, 1988; Kreutzer, 1995; Persson, 2002) and reduces the concentration of free Al (e.g. Marschner and Wilczynski, 1991). Especially a pH increase from 4 to 5 is important for the binding of Al to soil surface (Hargrove and Thomas, 1981).

Box 2. Liming practice.

Liming as a measure to elevate soil pH is both used in forestry (mostly Europe) and agriculture (mostly USA). In many forest trials in Europe ground dolomitic limestone is used e.g. (Kreutzer, 1995; Borken and Brumme, 1997), while laboratory experiments often take pure $CaCO_3$ powder e.g. (Persson, 1989). Dolomitic lime, which is a mixture of $CaCO_3$ and $MgCO_3$, is especially suitable to keep the calcium/magnesium balance in the soil. Sandy soils can be very low on magnesium. In agriculture either regular limestone, dolomitic limestone, burned lime, hydrated lime or more local materials (slag, marl, wood ash) can be used (Plaster, 1992).

Reduced concentrations of free Al^{3+} have a potential to stimulate tree growth. However, liming does not seem outstandingly beneficial in this respect. It can improve mineral nutrition (Kulhavy *et al.*, 2009) and increase growth (Andersson *et al.*, 1996) of Norway spruce stands, but liming with doses of 3-15 t ha^{-1} might not affect stem increment in Scots pine stands, though it increases colonization by ectomychorrhizal fungi (ECM) (Borja and Nilsen, 2009). Increase of ECM colonization was remarkable in this case because the soil was not poor in N. Liming can also decrease tree growth, for example with 10-16% in Norway spruce stands (Derome *et al.*, 1986; Kreutzer, 1995). Liming has been shown to cause a redistribution of the fine root biomass. Kreutzer (1995) shows that 7-8 years after liming with 4 t ha^{-1} the biomass of fine roots (< 1.5 mm) in the top mineral soil (0-5 and 5-10 cm) was decreased while the amount per cm soil in the humus layer increased dramatically (about 1,7 fold). This could have been due to increased mineralization rates (see below) which makes it beneficial for trees to expand the root network in the humus layer. On the other hand, decline of the humus layer, which often occurs in limed soils (e.g. Kreutzer, 1995), reduces the space for these roots to grow. The absolute amount of fine root biomass might thus decrease.

The pH increase brought about by liming treatment stimulates the activity of the soil microbial community (e.g. Bååth and Arnebrant, 1994; Persson *et al.*, 1995), which can increase more than 2-fold in the organic layers (Anderson, 1998). Higher numbers of decomposing microorganisms result in higher mineralization rates (Persson, 1989). C mineralization is stimulated on the short (< 1 yr) (Adams and Cornforth, 1973; Persson, 1989) or medium-long term (± 3-15 yr) (Lohm *et al.*, 1984; Nilsson *et al.*, 2001). This is also due to higher pH, which increases the availability of soluble C sources in the soil (Salonius, 1972; Foster *et al.*, 1980; Söderström *et al.*, 1983). Effects of liming may sometimes not be visible on the short term (Borken and Brumme, 1997). The effect on C mineralization on the long term (>20 yr) is unclear. After 37-42 years, lime application can both have increased and decreased C mineralization (Persson *et al.*, 1995), but liming studies of that duration are very rare. C pools in limed soils tend to decrease e.g. (Kreutzer, 1995; Persson *et al.*, 1995; Anderson, 1998; Lundström *et al.*, 2003), which is in agreement with higher C mineralization for a longer period of time. The rate of decrease is not always in a value corresponding to C mineralization (e.g. Nilsson *et al.*, 2001; Lundström *et al.*, 2003). This could be caused by a higher influx of easily decomposable matter in the form of more undergrowth which is stimulated by more available nutrients.

Furthermore, liming can increase (Sahrawat *et al.*, 1985; Borken and Brumme, 1997; Duliere *et al.*, 1999; Nyberg *et al.*, 2001; Lundström *et al.*, 2003) or decrease N mineralization (Nommik, 1968), at least on a relatively short term (< 5 yr). Nömmik (1979) observed that this is, at least in mor humus, related to the C:N ratio of the soil: if this is below 30, N mineralization is stimulated by liming, but if the C:N ratio is above 30, then it is suppressed (Nommik, 1979). Persson *et al.* (1989) suggested that this was due to the response of the microbial biomass. Because the C:N ratio of soil microbial biomass is low, a treatment that stimulates the microbial biomass might cause N immobilization if the C:N ratio of the microbial substrate is high. The biomass simply needs almost all N for its own growth. On the long term the effect of liming seems to change. Though the soil C:N ratio was 22 (in unlimed plots), Persson et al. (1995) found that net N mineralization rate had decreased 37-42 years after liming. Potential nitrification increased (Persson *et al.*, 1995). N pools, like C pools, tend to decrease after lime treatment. If this is to a smaller degree than the decrease of the C pool, C:N ratios increase (e.g. Marschner and

Wilczynski, 1991; Persson *et al.*, 1995; Anderson, 1998). Reduction in C and N pools mostly takes place in the humus layer. This layer can decrease with as much as 23% in 7 years (Kreutzer, 1995). Increased C and N mineralization due to liming can cause a massive loss of C and N from the forest ecosystem, if trees do not respond with better growth (Persson *et al.*, 1995; Lundström *et al.*, 2003). However, C and N pools can increase under a very low dose of lime (2 t ha^{-1}) (Derome, 1990).

Because of the observed increase in N mineralization, an old concern was that liming would cause an enormous increase in nitrate leaching in soils with a high N content. Fortunately, these concerns have not proven true. Harmfully high nitrate leaching after liming has only been observed in a few cases and is very dependent on site characteristics (Kreutzer, 1995). This means that one cannot consider it a general feature of liming practice. However, it remains a risk of liming of nutrient rich soils.

1.3 Earthworms

Increased pH can cause changes in soil biota. Earthworms are important soil organisms. They are classified as ‘ecosystem engineers’, which means that they have effects on their environment, either biotic or abiotic, that go beyond their own body size and life span (Jones *et al.*, 1994; Jouquet *et al.*, 2006). Earthworms influence soil structure by creating burrows, by bringing litter into the soil, fragmenting it and mixing it with humus and mineral soil, by homogenizing the soil, and by the ejection of casts (Brown *et al.*, 2000). Especially epigeic (litter dwellers) and anecic (soil dwellers that move vertically through the soil profile) species bring a large amount of litter into the soil. Litter fragmentation and soil mixing increase the soil surface for decomposing bacteria to attach to and so increase decomposition. Earthworm casts contribute to the availability of N (NH_4^+-N and NO_3^--N), P, and K (Mariani *et al.*, 2007). In pot experiments, Makeschin (1980) studied the influence of several species of earthworms on nutrient availability. Of the tested earthworm species, only *Eisenia foetida* had a (positive) effect on the availability of potassium and phosphorus. Two of the other species increased the ammonium content in the soil, while a third species additionally increased nitrification (Makeschin, 1980). Annual N turnover by earthworms under field conditions is estimated at about 14 kg ha^{-1} in grassland soils (Anderson, 1983). Through their activity in the soil, earthworms can influence plant growth. The influence of earthworms on their environment can be large-scale, changing landscapes and vegetation (e.g. Jouquet *et al.*, 2006).

Few earthworm species are tolerant to low pH (Makeschin, 1997). Naturally or artificially increasing soil pH is therefore beneficial for earthworms (Ammer and Makeschin, 1994), though there is little known about long-term effects. Liming with 4.4 t ha^{-1}, which increased pH from 4 to slightly over 5, has been shown to increase population density of *Aporrectodea trapezoides* to 6.3 times that in unlimed plots (Chan and Mead, 2003). Persson (2002) showed that liming with 1.55, 3.45 and 8.75 t ha^{-1} dramatically increased pH which, is still visible after 6 years (from 4 in control plots to 4.5, 5.2 and 6.0, respectively) and stimulated earthworm populations even after 15 years (Persson, 2002). These last measurements were done at the same site as the present study is based on (see section 2. Materials and methods). Because earthworms are large stimulants of soil processes, liming can also in this way influence mineralization rates.

1.4 Goals and expectations

Spruce and pine are the main forest vegetation in Scandinavia, where many forests are production forests and thus have an economic value. Therefore it is important to keep track of the soil status (C and N pools, nutritional value, pH) of experimental forests that are present. In this study, we assess the current status of the Hasslöv experimental forest. The experiment, which was started by researchers of the Swedish University of Agricultural Sciences in Uppsala, Sweden, is a long-time (now 25 years) liming experiment in a Norway spruce forest.
The main goals of the present study were:

1. To study the differences in C and N pools and mineralization rates between control plots and plots with different application doses of lime, in a mature Norway spruce stand 25 years after liming;
2. To investigate species composition and abundance of the earthworm community as an important example of the reaction of soil fauna on liming;
3. To discuss if the changed mineralization rates in combination with possibly reduced C and N pools might have future negative effects.

We hypothesized that liming should increase soil pH. Since the liming treatment was performed 25 years before sampling, we expected that the effects had reached the mineral soil. Redistribution in fine root biomass from mineral soil to FH layer should have occurred. Based on earlier studies of C and N pool changes, we hypothesized that liming would have decreased N mineralization. We hypothesized that this had also become the case for C mineralization. The question if higher mineralization rates have had a negative effect on stand production had to be discussed in relation to general features of the soil. Furthermore we hypothesized that earthworms would be more abundant and the population would be more diverse with higher lime application.

A smaller C and N pool in combination with increased C and N mineralization rates in the past (Andersson *et al.*, 1994; Nilsson *et al.*, 2001) should have resulted in net loss of C and N from the ecosystem, a trend that already started in Hasslöv in the earlier years (Nilsson *et al.*, 2001). However, since we expected that mineralization rates were lower in limed plots compared to unlimed plots, the additional loss of C and N would have been coming to an end. The conclusion about the value of liming as a measure against soil acidity was dependent on all factors listed above. All effects should be stronger with higher lime application. If certain features of limed plots were different than would be expected, based on former research, then this was most likely due to site characteristics.

2 Materials & Methods

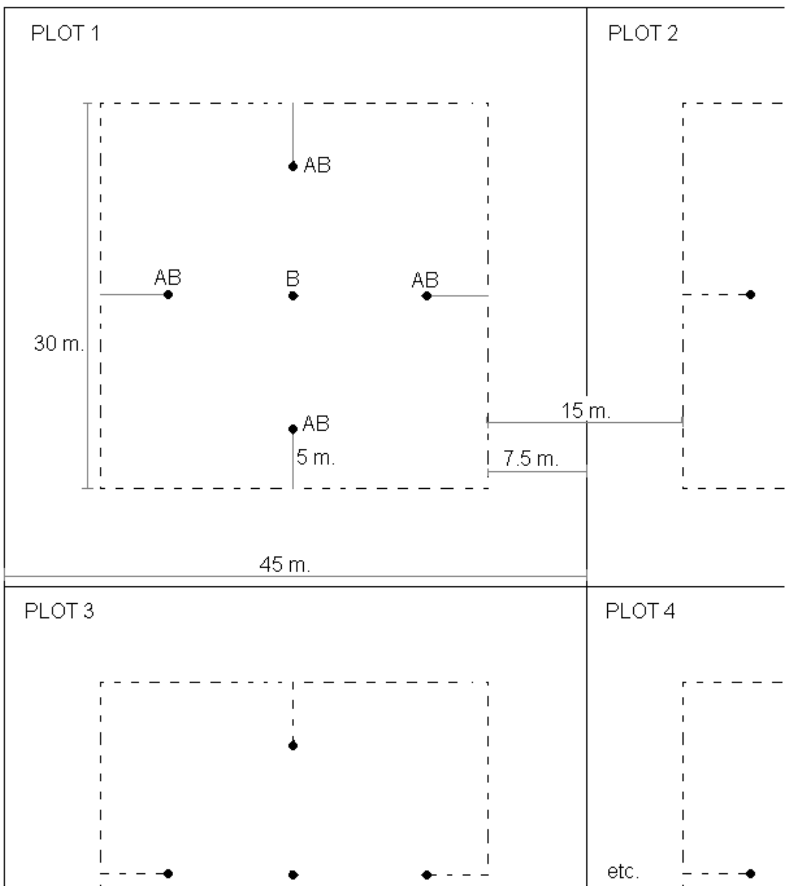
2.1 Site description

All samples were taken in the Hasslöv experimental forest in south-west Sweden. The Hasslöv site (56º24’N, 13º00’E, 185 m above sea level) is situated on a ridge in a larger stand of Norway spruce that was planted in 1949 on former *Calluna* heathland. This area is located about 10 km from the sea in the province of Halland, near Laholm. The site set-up is a randomised block design with four replicated blocks (see Appendix 1). Each block contains 5 treatment plots: control (no treatment), low dose $CaCO_3$ (1.75 t ha^{-1}), and low, medium and high doses (1.55, 3.45 and 8.75 t ha^{-1}, referred to as D1, D2 and D3, respectively) of dolomitic lime (see Box 2). This basic design implies that n = 4. The liming took place in November 1984. Each plot is 45 x 45 in size with a net plot of 30 x 30 m.

2.2 Field sampling

Samples were taken on April 7 and 8, 2009. Samples were taken as close as possible to the places in the plot described in Figure 2, but rocks and stumps were avoided. To collect earthworms, five samples within each plot (all blocks and treatments) were taken from both the LF (litter + fragmentation) and HA (humus + upper cm mineral soil) layer, adding up to 200 samples in total. From the middle of all four sides of the net plot, we moved 5 m into the plot and took a sample, plus a sample in the center of the plot (Figure 2). Both layers were sampled with a quadratic 100 cm^2 frame. Sampling in former research has shown that the taken number of samples is sufficient because there are often significant differences between treatments (Persson, pers. comm.).

For the mineralization study, samples were only taken in the control plots and the medium (D2) and high (D3) dose of dolomitic lime. Four samples were taken within each plot (in all blocks), one next to each earthworm sampling location, except in the center of the plot. Material was taken from the following layers: L (litter), FH (fragmentation + humus), and 0-5 cm, 5-10 cm, 10-20 cm and 20-30 cm mineral soil. The L layer and FH layer were sampled with a cylindrical 250 cm^2 frame. The 0-5 cm and 5-10 cm mineral soil was sampled with a quadratic 100 cm^2 frame, which means that 500 cm^3 material was obtained per layer per sample. The 10-20 cm and 20-30 cm mineral soil was sampled with a 15.9 cm^2 round corer, which gives 159 cm^3 material per layer per sampling. The samples were bulked to one composite sample per plot and soil layer to obtain mean values for each soil layer of the plot, giving a total of 72 samples.



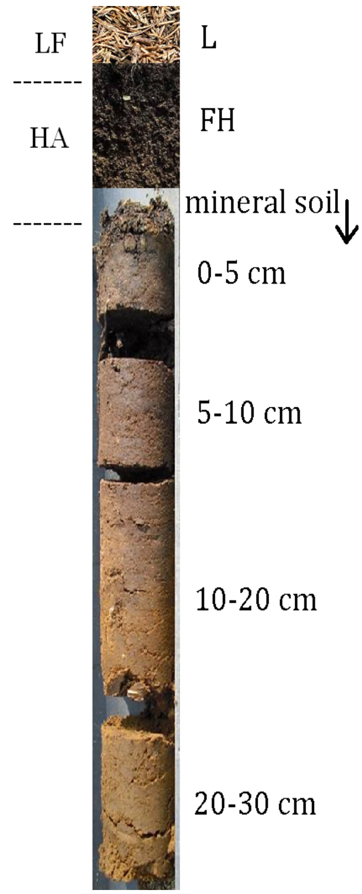


Figure 2a. Graphic representation of the plots in Hasslöv. Samples for mineralization experiments were taken at A in the control, D2 and D3 plots in every block. Samples were pooled per plot and per soil layer. Samples for earthworm extraction were taken at B, in all five treatments in every block. These were not pooled.

Figure 2b. The soil profile. Samples for earthworm extraction were taken from the litter + fragmentation (LF) and the humus + upper cm mineral soil (HA) layer. Samples for the mineralization study were taken from the litter (L) layer, fragmentation + humus (FH) layer, and from the 0-5, 5-10, 10-20 and 20-30 cm mineral soil.

2.3 Earthworm extraction

The size of the earthworm populations was estimated by Tullgren funnel extraction. Samples were stored at 5 °C prior to earthworm extraction. Storage time varied from 1 day or 2 days for the first 60 samples to 16 or 17 days for the last 20. The extracted earthworms were stored in 70% ethanol. For determination the key provided by Århus Naturhistorisk Museum was used (Andersen, 1997).

2.4 Soil property determinations

Before starting the incubation studies, fresh and dry weight, pH and C:N ratio of the soil were measured. After determining fresh weight, soil samples were sieved. The litter and FH layer were passed through a 5-mm sieve. Mineral soil was sieved through a 2-mm sieve. Living plant material was collected from the litter samples and fine roots (< 2 mm) were collected from FH and 0-5 cm and 5-10 cm mineral soil samples. Fresh weight / dry weight ratios were determined at 105 °C. Subsamples of the fresh soil were taken and dried at 105 °C. Afterwards this soil was grinded and C and N concentrations were determined with a CarloErba NA1500 N/C/Sulphur Analyzer. For the pH (H_2O) measurement 35 ml of distilled water was added to 5, 8 or 30 g (fw) soil (for the L, FH and mineral soil layers, respectively). The mixture was shaken for 2 hours and allowed to air overnight. pH was then measured directly in the slurry with an inoLab WTW electrode. Dry weight and pH were also measured on the incubated samples after termination of the incubation experiment.

2.5 C and N mineralization experiments

From each of the 72 bulked soil samples, a subsample (6, 16 and 100 g dw for L, FH and mineral soil, respectively) was taken for the incubation study. C mineralization was determined by gas chromatography (Hewlett Packard 5890, H. P. Company, Avondale, PA, USA) as described in Persson et al. (2000). The soil incubation lasted for 33 days. This relatively short period was chosen because C mineralization rates are by far highest and so most typical for the field in the first 2-3 weeks of incubation; after a decrease of 10-50% of the initial speed the mineralization rates stabilise (Persson *et al.*, 2000a). Destilled water was added to the samples every two weeks to keep the water content at ± 60% WHC throughout the incubation period. CO_2 measurements were performed once a week, four times in total. C mineralization was calculated according to Persson *et al.* (1989), Persson and Wirén (1993) and Persson *et al.* (2000a) and was expressed per gram of organic C per day.

N mineralization was determined by measuring inorganic N content (NH_4^+ and NO_3^-) in the original (sieved) samples and by destructive sampling of the incubated samples at the end of the incubation period. Inorganic N was extracted by adding 100 ml KCl to ± 5 g (L layer), ± 10 g (FH layer), or ± 21 g soil (mineral soil layers). The mixture was shaken for 1 hour and afterwards filtered and stored at -20 °C prior to analysis. NH_4^+ and NO_3^- concentrations were measured in a FIA STAR 5010 analyser. N mineralization rates were calculated according to (Persson *et al.*, 2000b) and expressed in microgram per gram of organic C per day.

Extrapolation to the field is only possible if the mineralization rate as measured in the laboratory is corrected for changing temperature and moisture over the year. For Hasslöv we used the correction factor of the nearby experimental site Skogaby (Persson *et al.*, 2000a), which is 0.349 for both C and N. Field mineralization rate estimates are expressed in gram per m^2 per year.

2.6 Statistics & Analysis

Data on C:N ratios, pH, and C and N content (N=72 in each test) and the fine root biomass (N=36) were analyzed with the Mixed Procedure in SAS statistical software (version 9.1 for Windows), which tested for treatment effects, soil layer effects and interactions of treatment and soil layer ($p<0.05$). Block effects are excluded by the test prior to further analysis. The mixed procedure subsequently makes pairwise comparisons on the least square means with a t-test. After testing the C:N ratios of all layers separately, we found that there were no significant differences between any layers in the mineral soil. The chance of obtaining significant results is higher when the number of layers is reduced. Therefore we took the average C:N ratio per treatment for the mineral soil and tested again for significant differences, this time on three layers (L, FH, mineral soil, so N=36). Data on C mineralization, net N mineralization, ammonification and nitrification were tested for treatment effects for each layer separately (N=12), using the GLM Procedure in SAS. This test subsequently makes pairwise comparisons between treatments. The GLM procedure was also used to test all whole-plot data (N=12) and the total number of earthworms (N=20) for treatment effects. It is important to note that SAS calculated statistical standard errors from the whole data set and that these are thus the same for every treatment.

Additionally, using SAS we performed a Pearson correlation analysis and a regression analysis on the relation between soil pH and total earthworm numbers per m^2 (N=20). For this pH values from a survey in March 2007 were used, because then all treatments were included in pH measurements, and in the current survey only soil pH in control plots and the medium and high dose of dolomitic lime were measured.

3 Results

3.1 pH

pH (H_2O) was measured at the start (Table 1) and at the end of the mineralization experiment (data not shown). All pH values decreased with 0.1-0.4 between day 0 and day 33, except the control L average which remained 4.6.

Values in control and D2 soils differ between soil layers, whereas in D3 soils they are similar. Significant differences were found for soil layer (df=5, F=33.60, $p<0.0001$), treatment (df=2, F=41.01, $p<0.0001$), and for the interaction between these (df=10, F=6.58, $p<0.0001$). Overall, liming increased soil pH. In each layer except L and 20-30 cm mineral soil, all treatments differed significantly from each other. Between the layers in D3 plots there was only a significant difference between L and FH, and between L and 10-20 cm mineral soil (both $p<0.05$).

layer	0	D2	D3
L	4.6^a	4.8^a	5.1^b
FH	3.9^a	4.3^b	5.0^c
0-5 cm	3.7^a	4.0^b	5.0^c
5-10 cm	3.8^a	4.2^b	5.0^c
10-20 cm	4.0^a	4.4^b	4.9^c
20-30 cm	4.3^a	4.6^a	5.0^b

Table 1. Average pH per soil layer and treatment at the start of the experiment. Treatments were 0 t ha^{-1} (0) 3.45 t ha^{-1} (D2) and 8.75 t ha^{-1} (D3) of dolomitic lime. Values at the end of the experiment were about 0.2 units lower then at the start, except in five litter samples where values remained the same. Values with a different letter within the same layer are significantly different ($p<0.05$).

	0		D2		D3	
	C:N	$C_{min}:N_{min}$	C:N	$C_{min}:N_{min}$	C:N	$C_{min}:N_{min}$
L	28	34	28	50	28	46
FH	24	13	22	13	21	16
0-5 cm	19	12	19	18	19	17
5-10 cm	18	8	19	13	18	14
10-20 cm	18	9	19	12	19	12
20-30 cm	19	11	20	15	19	14

Table 2. The average C:N ratio and $C_{min}:N_{min}$ ratio per treatment and per soil layer. Treatments were 3.45 t ha^{-1} (D2) and 8.75 t ha^{-1} (D3) of dolomitic lime. Only in the FH layer a significant difference in C:N ratio between the treatments was found.

3.2 C and N pools and C:N and $C_{min}:N_{min}$ ratio

Both total C and total N pools tended to decrease under lime treatment. The total C pool decreased insignificantly (df=2, F=1.79, p=0.22) from 14.4 to 12 kg C m^{-2} (17%) between 0 and D3 plots (Figure 3). Pairwise comparisons revealed that the difference was due to the FH layer, which contained significantly less C for each higher lime treatment ($p<0.001$). The pool in this layer decreased from 4.5 to 3.2 to 1.6 kg C m^{-2}. In the other layers, no significant differences between the treatments were found.

Total N pools decreased insignificantly (df=2, F=0.57, p=0.58) from 707 to 636 g N m^{-2} (10%) between 0 and D3 plots. Again the main difference was in the FH layer, where N content differed significantly between all treatments ($p<0.05$). The N pool in this layer was 185, 141 and 74g N m^{-2} for control, D2 and D3 plots, respectively. This corresponds with the weight of the FH layer, which is heavily reduced under influence of treatment (df=2, F=10.64, $p<0.01$) (data not shown). No significant differences were found in any of the other soil layers.

C:N ratios differed significantly between treatments (df=2, F=5.66, p=0.01) and soil layers (df=2, F=102.48, $p<0.0001$). The difference between treatments is due to the values in the FH layer (Table 2). Only in this layer the C:N ratio has decreased under influence of lime treatment: from 24 (control) to 22 (D2) ($p<0.05$) and 21 (D3) ($p<0.01$). C:N ratios decreased significantly with increasing depth (from L to FH to mineral soil) for each treatment (all $p<0.01$). Values between layers in the mineral soil did not differ from each other. The ratio of C mineralization to N mineralization (mineralization rates are discussed in sections 3.3 and 3.4) in the L layer of each treatment is higher than the corresponding C:N ratio, indicating that less N is mineralized than would be expected judging from the substrate. The C_{min}:N_{min} ratio in all other soil layers of each treatment is lower than the C:N ratio, showing that more N is mineralized than expected. The differences between the C_{min}:N_{min} ratios in the L layer are larger than in the other layers.

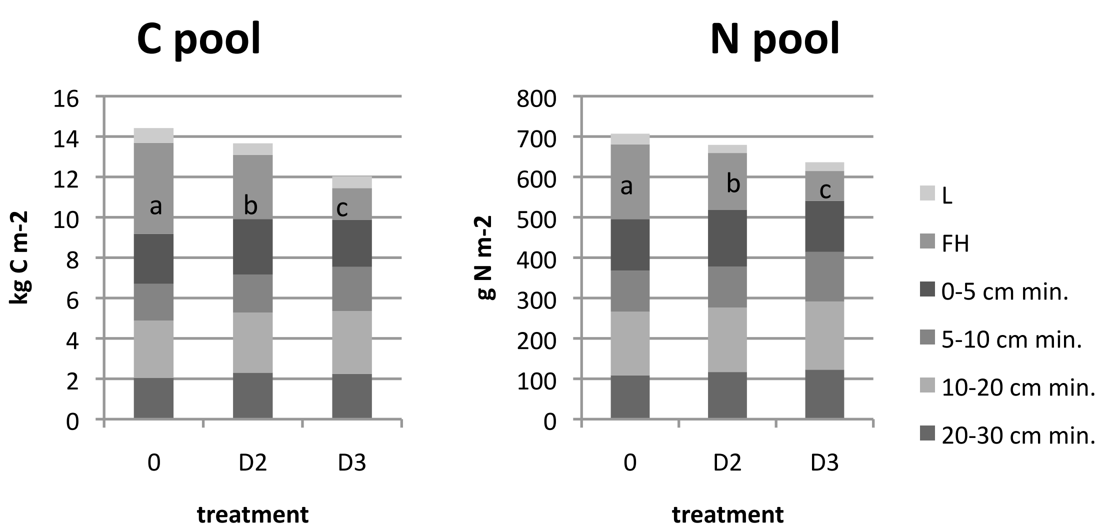


Figure 3. The average C and N content of the soil per treatment and per soil layer, expressed in gram dry weight per square meter. Treatments were 0 t ha-1 (0) 3.45 t ha-1 (D2) and 8.75 t ha-1 (D3) of dolomitic lime. Different letters in the FH layer indicate a significant difference in C content of that layer ($p<0.05$). No differences were found in the other layers.

3.3 C mineralization

For all treatments, C mineralization rate per gram of C decreased with increasing depth, though the differences between the deeper mineral soil layers were only minimal (Table 3). The C mineralization rate in the L layer was 10-15-fold higher than in the 0-5 cm mineral soil layer and 27-37-fold higher than in the 20-30 cm layer. Over the incubation period, the mineralization rate decreased (data not shown). This effect was most pronounced in the L layers and got less strong with increasing depth. C mineralization rates in the FH layer and 0-5 cm mineral soil were significantly higher in D3 plots than in D2 or control plots. These were also the only layers in which treatment made an overall significant difference (df=2, F=13.70 or 5.24, p<0.01 or 0.05, respectively). In the other layers, no significant differences were found, though there is a trend towards a higher C mineralization rate in the 5-10 cm mineral soil of D3 plots (p=0.09).

When extrapolated to the field, the mean C mineralization was estimated to be about 300 g m^{-2} y^{-1} (Figure 4). D3 plots tended to have a slightly lower mineralization, but no significant differences between treatments were found.

layer	0	D2	D3
L	1202^a	1622^a	1540^a
FH	200^a	262^a	393^b
0-5 cm	106^a	109^a	168^b
5-10 cm	70^a	86^a	104^a
10-20 cm	57^a	63^a	63^a
20-30 cm	44^a	44^a	49^a

Table 3. Mean C mineralization rate (µg C gC^{-1} day^{-1}) over a period of 33 days for each soil layer and treatment (see Materials and methods). Values were calculated from laboratory incubations at 15°C and about 60% WHC. Treatments were 0 t ha-1 (0) 3.45 t ha-1 (D2) and 8.75 t ha-1 (D3) of dolomitic lime. Values with a different letter within the same layer are significantly different (p<0.05).

3.4 N mineralization

The total inorganic N content of the soil in Hasslöv was approximately 3.1, 2.5 or 1.8 g m^{-2} for control, D2 and D3 soils, respectively (Figure 5). The difference between control and D3 was statistically significant (p<0.05), but there was no overall significance between the treatments (df=2, F=3.65, p=0.09). The NO_3^- pool was not significantly different between any of the treatments, but treatment made a significant difference (N= 12, df=2, F=7.95, p<0.05) for the amount of NH_4^+. Both D2 and D3 plots contained significantly less ammonium, compared to the control (p<0.05).

Net N mineralization rate in the L layer seemed to decrease with higher lime treatment, and seemed to increase in the FH layer, but neither of these were significant. In the 0-5 and 5-10 cm mineral soil significant differences were found: net N mineralization rate in D2 plots significantly lower than the control and D3 (Figure 5). Overall treatment did not make a difference in these layers. In the deeper mineral soil layers there were no significant differences either.

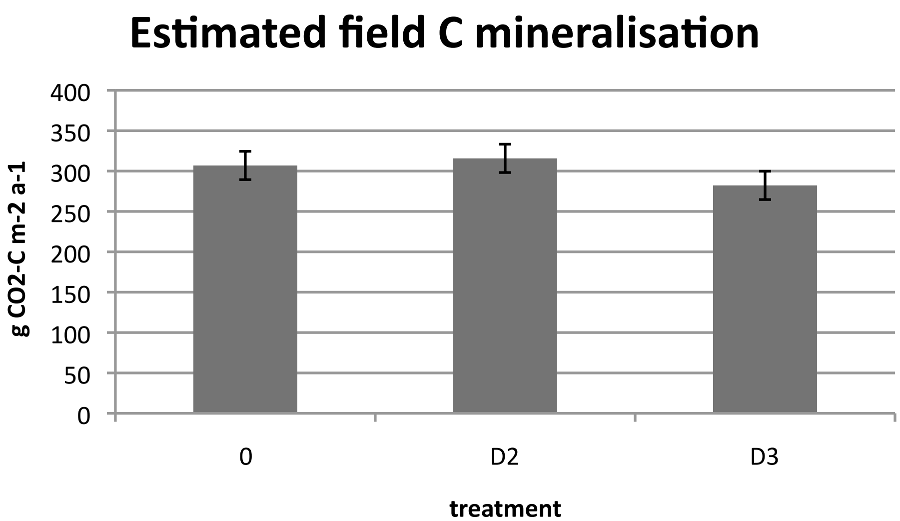


Figure 4. Mean total C mineralization of the soil under each treatment. Values are calculated from 33 days of laboratory incubation at 15°C and about 60% WHC, and corrected using the yearly correction factor from Skogaby, Sweden. Error bars depict one SE. Treatments were 0 t ha-1 (0) 3.45 t ha-1 (D2) and 8.75 t ha-1 (D3) of dolomitic lime.

The ammonification rate was significantly affected by treatment in each layer (in all cases df=2, $p<0.05$) except the 0-5 and 10-20 cm mineral soil. In the organic layers ammonification is lower in D3 plots, but in the mineral soil there is no obvious pattern. Nitrification rates were significantly affected by treatment in all layers (in all cases df=2, $p<0.05$) except the L layer and the 10-20 cm mineral soil. In the FH layer and the 0-5 cm mineral soil the nitrification rate is higher in D3 plots compared to the other treatments, but in the other mineral soil layers it is lower in the D2 plots than in D3 or control.

Extrapolated to the field, there was a trend towards decreasing net N mineralization under influence of lime treatment (N=12, df=2, F=4.12, p=0.075) (Figure 7). Whereas the rate was about 20 g N m^{-2} y^{-1} in control plots, it decreased to about 17 and 15 g N m^{-2} y^{-1} in D2 and D3 plots, respectively. The difference between control and D3 was significant ($p<0.05$). Ammonification was significantly affected by treatment (df=2, F=7.18, $p<0.05$) because it has largely decreased in the D3 soils compared to both control and D2 ($p<0.05$). Nitrification was not significantly affected by lime treatment.

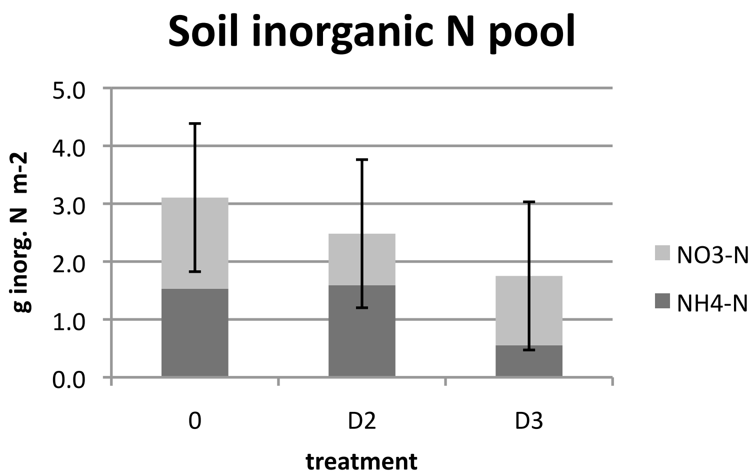


Figure 5. Soil inorganic N pools at the start of the experiment, expressed in gram per square meter. Treatments were 0 t ha-1 (0) 3.45 t ha-1 (D2) and 8.75 t ha-1 (D3) of dolomitic lime. Error bars depict one SE of the total inorganic N pool.

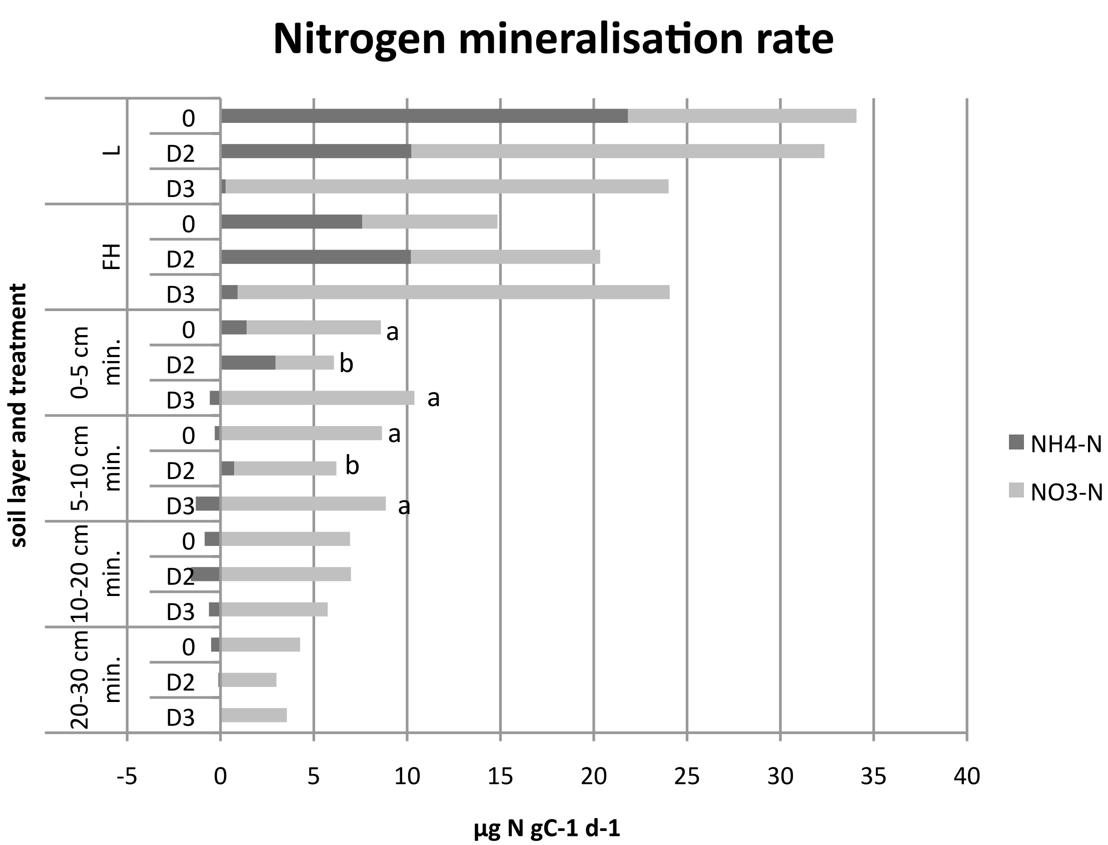


Figure 6. Mean N mineralization rate in µg N gC-1 d-1 per soil layer and per treatment, subdivided by ammonification and nitrification rate. Values are calculated from laboratory incubations at 15°C and about 60% WHC. Treatments were 0 t ha-1 (0) 3.45 t ha-1 (D2) and 8.75 t ha-1 (D3) of dolomitic lime. Bars with a different letter within the same layer are significantly different for net N mineralization rate ($p<0.05$). In the other layers no differences between net N mineralization rate were found

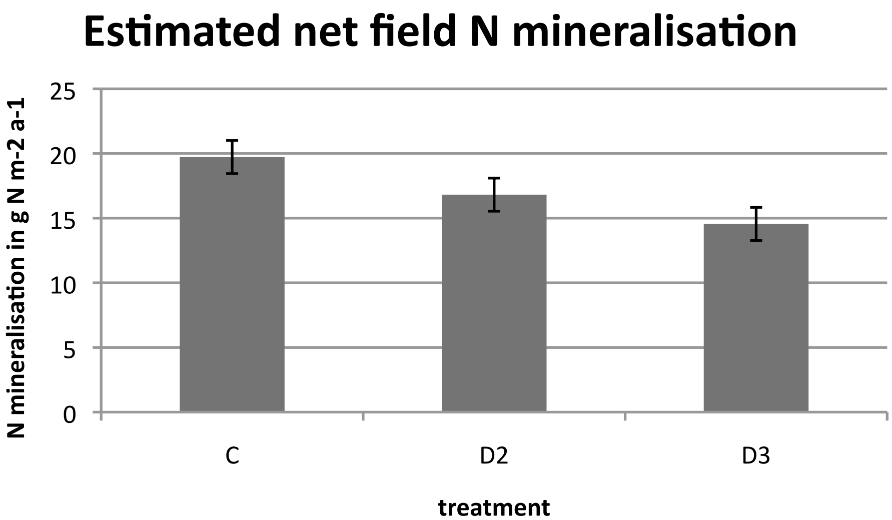


Figure 7. Mean net N mineralization of the soil under each treatment, calculated as the sum of each soil layer, per treatment. Values are calculated from 33 days of laboratory incubation at 15°C and about 60% WHC, and corrected using the yearly correction factor from Skogaby, Sweden. Error bars depict one SE. Treatments were 0 t ha-1 (0) 3.45 t ha-1 (D2) and 8.75 t ha-1 (D3) of dolomitic lime.

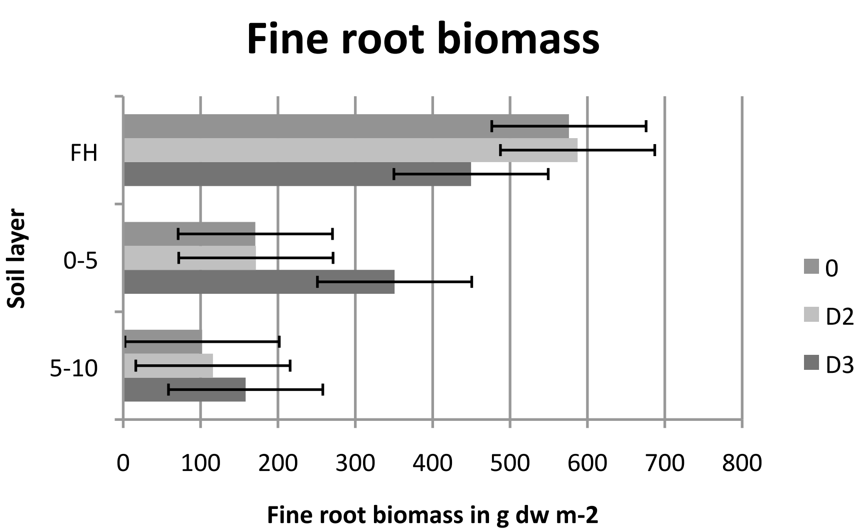


Figure 8. Mean total amount of fine root biomass in g dry weight m^{-2}, per soil layer and per treatment. The error bars indicate one SE. Treatments were 0 t ha-1 (0) 3.45 t ha-1 (D2) and 8.75 t ha-1 (D3) of dolomitic lime. There were no significant differences between the treatments.

3.5 Ground vegetation and fine root biomass

Vegetation biomass was about the same for control plots and D2 plots: on average 312- 315 g dw m^{-2}. In D3 plots the vegetation biomass was slightly higher: about 350 g dw m^{-2}. However, the variation in data was very large, values ranging from 48 to 862 g dw m^{-2} (data not shown). These values were both found in D3 treatment plots. No significant differences were found between the treatments (N=12, df=2, F=0.05, p=0.95).

Liming affected the distribution of fine root biomass between the FH layer and the first 10 cm mineral soil. Total biomass was similar for each treatment: about 850-960 g dw m^{-2}. The amount of root biomass was different between the soil layers (df=2, F=28.48, $p<0.0001$). No difference in the distribution of this biomass was found between control and D2 plots, but there was a trend towards redistribution of the biomass in D3 plots (Figure 8). The amount in the 0-5 cm layer increased from about 170 to 350 g dw m^{-2} (df=24.4, t=-1.80, p=0.085), while the amount in the FH layer decreased from 576-587 to about 450 g dw m^{-2} (df=24.4, t=1.38, p=0.18).

3.6 Earthworms

The lumbricids were represented by four species: *Aporrectodea caliginosa, Dendrobaena octaëdra, Dendrodrilus rubidus* and *Lumbricus rubellus* (Figure 9). The total number of earthworms differed per treatment (df=4, F= 6.17, $p<0.01$), which was due to the difference between the D3 treatment and the other treatments. The most abundant species in the samples from Hasslöv was *D. octaëdra* (78 individuals). This species was found in every treatment, though in very different numbers. Most were found in the D3 treatment samples; after that in D2, D1, CaCO3, and control. Treatment as a factor did not give overall significant results for *D. octaëdra*. The difference between D3 and D2 was not significant (p=0.11), but the differences between D3 and each other treatment were ($p<0.05$). The other species of earthworms (*A. caliginosa, D. rubidus, L. rubellus*) were not found for all treatments and in much lower numbers (3, 1, and 11, respectively) than *D. octaëdra*.

Regression analysis revealed a relation between between pH and total number of earthworms (N=20, R^2=0.39, $p<0.01$). When the analysis was performed on the average for each treatment, the relation was stronger (N=5, R^2=0.85, $p<0.05$). Pearson’s correlation analysis showed a significant relation between pH and the number of *D. octaëdra* (N=20, Rho=0.50, $p<0.05$). Figure 10 shows that soils with a relatively high pH (4.3 – 4.5) could be inhabited by both high and low numbers of earthworms, but soils with a low pH did never contain high numbers of earthworms.

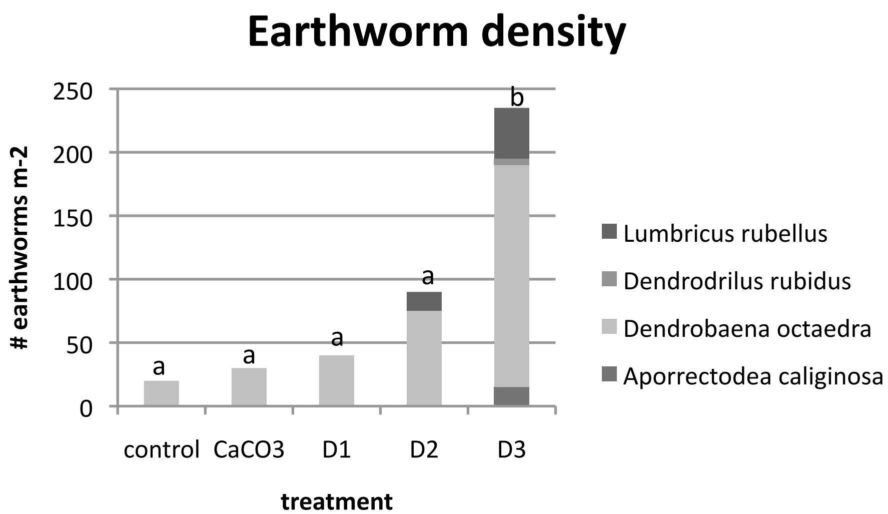


Figure 9. This figure shows the mean densities of Lumbricidae under different treatments at Hasslöv, 25 years after liming. Treatments were 1.75 t ha^{-1} $CaCO_3$, and 1.55 t ha^{-1} (D1), 3.45 t ha^{-1} (D2) and 8.75 t ha^{-1} (D3) dolomitic lime. pH values in the FH-layer were 3.9 (control), 4.3 (D2) and 5.0 (D3). Different letters indicate a significantly different total number of earthworms.

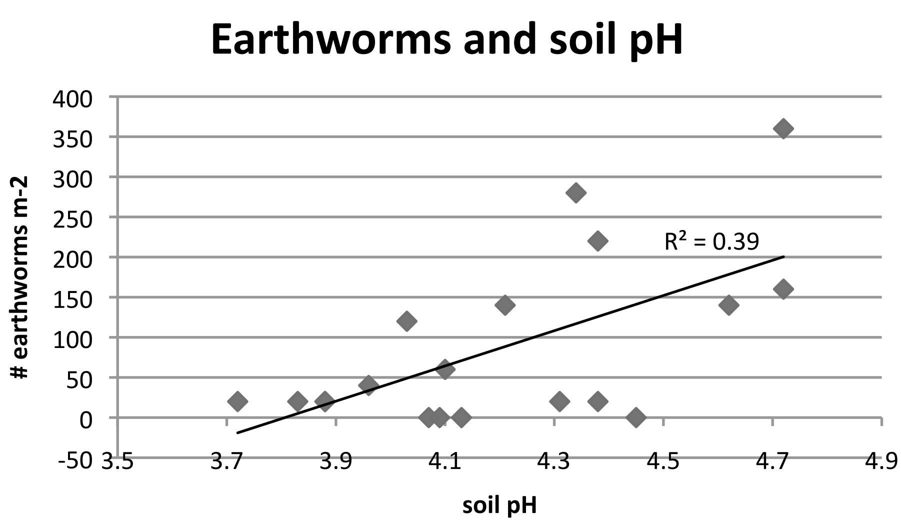


Figure 10. Correlation between soil pH and total number of earthworms found these soils. pH values used are measured in the FH layer in March 2007. pH values can be obtained in either of the five treatments: 0 t ha^{-1} (control), 1.75 t ha^{-1} $CaCO_3$, and 1.55 t ha^{-1} (D1), 3.45 t ha^{-1} (D2) and 8.75 t ha^{-1} (D3) dolomitic lime.

4 Discussion

To gain more insight in the effects of liming treatment on the long term, we studied the experimental Norway spruce forest at Hasslöv, 25 years after liming. The objectives of the present study were to study the influence of liming on C and N pools and mineralization rates, and to discuss if this might have future effects. Additionally we studied the earthworm population as an important example of the faunal community of the soil.

		pH (H_2O)			
treatment	layer	2009 (current study)	2007 (unpublished data)	1994 (Nilsson *et al.*, 2001)	1991 (Andersson and Valeur, 1994) *
0 (0 t $ha^{-1)}$	L	4.6	4.4	4.9	
	FH	3.9	3.8	4.3	4.2
	0-5 cm	3.7		4.1	
	5-10 cm	3.8	3.8	4.2	
	10-20 cm	4.0	4.1	4.3	
	20-30 cm	4.3		4.4	
D2 (3.45 t ha^{-1})	L	4.8	4.4		
	FH	4.3	4.3		5.3
	0-5 cm	4.0			
	5-10 cm	4.2	4.1		
	10-20 cm	4.4	4.3		
	20-30 cm	4.6			
D3 (8.75 t ha^{-1})	L	5.1	4.9	4.5	
	FH	5.0	4.6	5.9	5.7
	0-5 cm	5.0		5.2	
	5-10 cm	5.0	4.5	4.8	
	10-20 cm	4.9	4.5	4.8	
	20-30 cm	5.0		5	

Table 4. pH values from the present study are accompanied by values from 2007, 1994 and 2001. Treatments were 0 t ha-1 (0) 3.45 t ha-1 (D2) and 8.75 t ha-1 (D3) of dolomitic lime.
*Data obtained from leachates in an incubation column experiment. The values depicted here are the start values of the experiment.

4.1 Effects of liming on pH

In accordance with our expectations, pH markedly increased under lime treatment, and the higher the dose of lime applied, the larger the effect on pH. The pH level of D3 plots showed very little changes over the soil profile, whereas the 0 and D2 plots had a markedly lower pH in the FH layer and 0-10 cm mineral soil. The similar values in all soil layers in D3 plots show that lime has evened out over the soil profile. Data from other studies performed at the same site show that soil pH has changed over the years (Table 4). The pH values in D3 plots were higher in 1991 and 1994 than in 2009, but also a lot more variable (4.5 – 5.9). It is known that vertical penetration of lime material in the soil is rather slow (Hallbäcken and Popovic, 1985); stabilizing of pH values could therefore be expected to happen only after decades. Values in control plots and D2 plots are similar to those in 2007, but in D3 plots the current values are higher. pH values in control plots seem to be decreasing over time, compared to 1994, perhaps due to natural acidification of the forest by tree growth.

4.2 C pools and mineralization rates

The Hasslöv forest is a first generation forest, which means that the humus layer is still building up. The total soil C pool before liming (1984) was around 11 kg C m^{-2} (Persson, pers. comm.). After 25 years it had increased to 14.4, 13.7 and 12 kg C m^{-2} for control, D2 and D3 plots, respectively. In the control plots, a large part of this was stored in the humus layer, whereas this layer was heavily reduced in size in D3 and to a lesser degree in D2 plots. A small part of the C (and N) may be allocated to the mineral soil due to earthworm activity. However, the fact that the C pool in control plots were markedly larger than in D3 (and D2) plots means that, over the last 25 years, around 0.1 kg C m^{-2}, or 1000 kg C ha^{-1} more could have been built up in the soil of D3 plots if they had not received lime treatment. Roughly said, the equivalent of 1.5 m^3 crude oil ha^{-1} y^{-1} has not been incorporated into the soil and must have gone somewhere else. A part of this is incorporated into the trees, because volumetric stem growth in Hasslöv increased from 18 to 22 m^3 ha^{-1} between 1984 and 1994 in D3 plots (Andersson *et al.*, 1996). However, the rest of the C has probably been emitted as CO_2 which is in view of current and coming climate problems an alarming conclusion.

The C mineralization rate per gram C in the FH layer was only 16-26% of that in the L layer. The FH layer contains easily decomposable material but it is possible that the microbial biomass was limited by something else than C or N. In the FH layer and the 0-5 cm mineral soil the mean mineralization rate was significantly higher in D3 than in D2 and control plots. Liming up to pH 5 (D3) in the FH layer seemed enough to increase C mineralization significantly. On the contrary, Andersson et al. (1994) found that respiration was not higher in humus soil with pH 5-5.5 (liming with 3.45 t ha^{-1} (D2), in Hasslöv plots).

Explanations for the higher C mineralization rates in D3 plots, compared to control plots, are mutually related. Higher pH stimulates the microbial biomass e.g. (Bååth and Arnebrant, 1994; Persson *et al.*, 1995), which mineralizes more C. Furthermore, higher pH makes organic C more soluble, which gives decomposing organisms an easier accessible substrate. Usually Ca binds soluble C and counteracts this effect (Persson, pers. comm.), but with the high applications used in liming the pH effect of Ca apparently dominates. The fact that the C mineralization rate in the D3 plots was higher in the upper 5 cm of the mineral soil can additionally be explained by earthworm activity. The earthworms brought fresh humus into the upper mineral soil, which was subsequently mineralized. This effect was not visible in the net N mineralization rates in the mineral soil: here D3 and control plots had a similar mineralization rate.

Our study shows that liming also stimulated C mineralization on the long term, which Persson *et al.* (1989) were still questioning. C mineralization per gram C was higher especially in the organic layers of limed plots compared to control plots, but the differences between limed plots and control plots have become smaller. In 1991 respiration in D3 plots was more than 5-fold higher than in the control plots (Andersson *et al.*, 1994), while we now measured a 2-fold difference. This is important in view of both loss of organic matter from the forest and also in terms of CO_2 emissions. Because C pools have become markedly smaller in the organic soil, total mineralization per m^2 forest had declined slightly (but not significantly with n=4) in limed plots. In D3 plots the mineralization is 0.34 t ha^{-1} y^{-1} less than in D2 plots and 0.25 t ha^{-1} y^{-1} less than in control plots. This is a large difference compared to the 0.8 t ha^{-1} y^{-1} *increase* in D3 plots that was measured 10-14 years after liming (Nilsson *et al.*, 2001). Since the net forest ecosystem exchange in Sweden is -1.9 to +0.9 t C ha^{-1} y^{-1} (Valentini *et al.*, 2000), an increase due to liming of 0.8 t ha^{-1} y^{-1} led Lundström (2003) to stress that such an increase is alarming, especially in view of climate change.

4.3 N pools and mineralization rates

The total N pool in the Hasslöv plots was proportional to the amount of lime added. It was lowest in the D3 plots, mostly because the FH layer was heavily reduced by many years of high N mineralization. The inorganic N content in the soil is usually very low. However, the content in Hasslöv was 3.1, 2.5 or 1.8 g m^{-2} for the control, D2 and D3 soils, respectively. Though content and input are strictly not comparable, the corresponding 31, 25 or 18 kg ha^{-1} are the same order of magnitude as heavy N deposition. This grade of deposition was for example found in many sites in the NIPHYS/CANIF project in the mid-90s, including at Skogaby (16.4 kg N ha^{-1} y^{-1}) (Persson *et al.*, 2000c).

Since all values in the FH layer stay under 30, according to Nömmik's suggestion (1979) (see section 1.2), liming should stimulate N mineralization rates. Our results followed that pattern partly: in the FH layer the N mineralization rate tends to be highest in the D3 plots, which had the lowest C:N ratio (21) – so the highest relative N content. The D2 plots had the next highest mineralization rate (C:N = 22) and the lowest mean rate was found in the control plots (C:N = 24). However, N mineralization rates in the L layer were lower in D3 plots than in the control, though this was not significant. Liming seemed to suppress the net N mineralization rate in the L layer, which has a C:N ratio of 28, though the rates (24-34 µg N gC^{-1} day^{-1}) were still high. Apparently the N was easily available in the control soil and the D2 soil. This could have been due to a higher amount of arginin in the needles, which is a way in which trees can store excess N that they cannot use. This can be the case if there is a lack of other nutrients (Ericsson *et al.*, 1995). Differences in net N mineralization rates in the 0-10 mineral soil are most likely not related to treatment, since rates in control and D3 plots are similar, but lower in D2 plots. Neither can this be explained by earthworm activity, since the number of earthworms in the D3 plots is significantly higher than in both control and D2 plots.

Net ammonification rates in the D3 soil were very low, while the net nitrification rate was high. Since both reactions are part of the same chain, the ammonification rate was also high, but this was masked by the nitrifying bacteria. The amount of ammonium is usually not the restricting factor for nitrification (Rudebeck, 2000), even though it is the substrate of the nitrifiers. Nitrification is usually higher at higher pH (Rudebeck, 2000), and it is very likely that this also the determining factor for nitrification rate in the present study, at least in the organic layers. Under low pH and low nutrient presence in the field nitrifiers are very weak competitors (e.g. Zak *et al.*, 1990; Verhagen *et al.*, 1995; Rudebeck, 2000) compared to roots and mycorrhizal fungi, which are the stronger competitors and take up most of the ammonium. However, since this study was performed on sieved materials, root uptake was did not occur and nitrifiers could use their substrate ammonium without competition.

Over time, the differences between N mineralization rates in limed and control plots seem to decrease. In the present study, we found an insignificant difference of about 1.6-fold in the FH layer, whereas N mineralization in 1991 was still around 3-fold higher in humus from D3 plots than in control plots (Andersson *et al.*, 1994). That is important to notice this in view of loss of organic matter from the forest. Possibly, the decline in N mineralization rates in limed plots, and also the apparent decline in C mineralization rates, is due to a slow evening-out and slight decrease of the soil pH in these plots. Field N mineralization on an area basis was markedly lower with higher lime treatment, which can be explained by the fact that the N pools, like the C pools, have decreased significantly under influence of liming.

Though the difference between N mineralization rates in limed and control plots seemed to decrease and the total mineralization has become smaller in limed plots, net N mineralization was still high. In control plots we estimated the net N mineralization to be around 200 kg ha^{-1} y^{-1}, which was higher than in 1990 (120 kg ha^{-1} y^{-1}) (Persson, pers. comm.). Trees in limed plots used to show more growth than those in control plots (Andersson *et al.*, 1996) and incorporated a part of the N in their canopy. Nowadays, however, the forest is mature and needs only nutrients to change its foliage, making the system more N saturated. There is a high risk of nitrate leaching when the forest will be cut.

4.4 C:N ratios and Cmin:Nmin ratios

In the present study, we did not observe any changes in C:N ratio (Table 2), apart from the decline in the FH layer. The ratios have not changed since 1994 (Nilsson *et al.*, 2001). In general, C:N ratios in Hasslöv are very high for coniferous forests in Scandinavia. At other places ratios can be up to 62 in litter and 44 in humus (Jädraås, Scots pine, (Persson and Wiren, 1993)), or about 26 in the mineral soil (Fexboda, Norway spruce, (Persson and Wiren, 1993)).

The C:N ratio can be used as a short-cut information of nitrogen status, and changes in C:N can depend on non-parallel losses of C or N, either proportionally higher losses of C in relation to N or vice versa. We found a lower C:N with increasing doses of lime only in the FH layer. To understand these changes, we compared C:N in the substrate with the estimates of C_{min} to N_{min}. The C_{min}:N_{min} ratio gives information about the non-proportional mineralization of either C or N, if compared to the C:N ratio of the substrate. In the litter layer, the measured C_{min}: N_{min} ratio was higher than the C:N ratio, for every treatment (Table 2). This indicates that a part of the potentially produced inorganic N was taken up by, for example, the microbial biomass. That would mean that this was expanding. This effect was a lot stronger for the limed plots (50 and 46) than for the control plots (34), though the C:N ratio was the same. The microbial biomass could expand in the less acidic soil that is treated with lime. In all other soil layers the C_{min}: N_{min} ratio was lower than the C:N ratio of the substrate, indicating that a N-rich source might have been mineralized. This source could be the mycorrhizal fungi that were present in the sample and have died during the incubation period. The decline in C:N ratio with higher lime application in the FH layer did not match with the fact that N mineralization was relatively high in this layer (C_{min}: N_{min} =16 in D3 plots but 13 in control and D2 plots). This should cause a higher soil C:N ratio for higher lime application. Since the ratio was lower, this could mean that an external input with high N content was mineralized.

4.5 Ground vegetation and fine root biomass

Liming did not seem to influence the ground vegetation at Hasslöv 25 years after liming. There were no significant differences between the treatments and both the highest and lowest value found were found in D3 plots. Therefore we assume that local differences between the plots caused the large variation in our data. For example, in the plot with the highest amount of vegetation biomass the canopy had been opened by a storm, allowing more sunlight to reach the forest floor. The pH increase between control plots and plots treated with the high lime dose was only 0.5 units (4.6 to 5.1) and this difference was probably too small to cause large changes in vegetation growth.

Changes in the fine root biomass, finally, were indirectly related to treatment. They were not pH related, but seemed to follow the distribution of organic matter over the soil profile at least to some extent. In D3 soils there was a shift from the FH layer, which was heavily reduced, to the 0-5 cm mineral soil, a layer that was slightly increased. This phenomenon had not happened the D2 plots, which also experienced a reduction in FH-layer thickness. Since we do not have data on absolute FH layer thickness, we do not know if the amount of root biomass per cm soil increased in this layer. The reason that Kreutzer (2005) found an increased amount of roots per cm soil thickness in the FH layer might very well be because of the increased mineralization rates, which made it beneficial for the trees to expand the root network in this layer. It could be that the total amount of roots in the FH layer decreased too in that study, because of the reduction of this layer. However, Kreutzer (2005) also described that the amount of fine root biomass in the upper 10 cm mineral soil decreased under influence of liming, while we found either a decrease (D3) or no difference (treatment with 3.45 t ha^{-1} (D2), comparable with Kreutzer’s 4 t ha^{-1}).

4.6 Earthworm populations

It is known that different types of earthworm extraction give different results and that not all methods are equally effective. For example, hand-sorting is considered a necessary method to accurately study earthworm population dynamics (Jimenez *et al.*, 2006). Tullgren funnel extraction is just one of the methods and it does not give an overview of the entire earthworm population of a given sample; it mainly extracts the smaller types. Edwards (1991) gave a detailed overview of all methods available, including Tullgren funnels, to gather or extract soil macrofauna. In his conclusions he states that funnel extraction is often inadequate for earthworms and that specialized techniques should be used (Edwards, 1991). However, Makeschin (1997) states that heat funnel extraction is suitable to extract endogeic and epigeic earthworms and gives relatively little mortality and damage. Furthermore Smith *et al.* (2008) did not find significant differences for the number or size distribution of earthworms in their evaluation of the efficiency of handsorting, Berlese-Tullgren funnels, and Winkler bag methods. They found that in smaller cores (15x15 cm) more damaged earthworms were recorded than in larger cores (25x25 cm). In the present study 10x10 cm frames were used for sampling. This may have resulted in even more damaged earthworms. That in turn might have affected our results in terms of a lower number of earthworms than present in the field. It is however important to notice that the goal of this study is to compare different treatments. Since Tullgren funnel extraction was used for all samples, the results are comparable. Therefore we considered the use of Tullgren funnels in this study adequate.

Furthermore it is disputable that we had to use pH data from old measurements (2007) instead of the most recent values. Above we showed that these were not necessarily the same as the current pH (Table 3). However, in our situation that was the best option, since we did not measure soil pH of the 1.75 t ha^{-1} $CaCO_3$ or the 1.55 t ha^{-1} dolomitic lime (D1) treatment in the current survey. Thereby the values in the FH are comparable with exception of that in the D3 treatment, which was 0.4 units lower in 2007. And since in we found that in general more t ha^{-1} of lime treatment gives a higher pH and also more earthworms, we considered the regression with older pH values in this case sufficient.

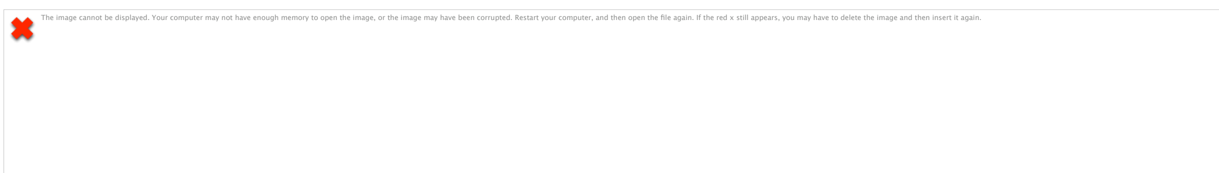
In the present study, we found an earthworm population with *D. octaëdra* as the dominant species and low numbers of *A. caliginosa, D. rubidus* and *L. rubellus*. Compared to 1984-1999, only *A. caliginosa* is a newly found species. The rest of the lumbricid representation is consistent compared to older measurements (Persson, 2002). Our results showed that liming can affect the soil community even 25 years after treatment. It would be interesting to know if this stimulating effect persists in the future if soil pH slowly starts to decrease again due to washout of the lime material. If pH had been raised even higher than now, it is possible that *D. octaëdra* will be outcompeted by other earthworm species. So far, that is not yet the case. The number of *D. octaëdra* is still higher in D3 than in D2 plots, though other earthworm species have appeared.

High numbers of earthworms, like we found in D3 and to a lesser extent in D2 plots, stimulate breakdown of organic matter and increase mineralization rates of both C and N. The invasion of European earthworm species in temperate and boreal forests of North America shows us the effects that a large earthworm population can have on the organic soil layers (Frelich *et al.*, 2006). The high number of earthworms in Hasslöv is very likely to have contributed to the reduction of the organic layers by causing high mineralization rates during the first decades after liming.

5 Conclusions and recommendations

Liming has counteracted soil acidity and stimulated the earthworm populations in the Hasslöv experiment. In those respects, liming was a successful treatment. Further, tree growth in Hasslöv has slightly increased under influence of liming (Andersson *et al.*, 1996). However, decades of increased mineralization rates in limed plots have reduced C and N pools markedly. A large part of this organic material is probably lost from the forest. Most of the lost C contributes to the greenhouse effect in the form of CO_2. Since the Hasslöv forest seemed N saturated, there is a high risk of nitrate leaching once the forest will be cut.

The results indicate that liming as a measure against soil acidity should be used with great caution. If a higher soil pH is of high priority, low/medium doses of lime should be used. In the present study, 3.45 t ha^{-1} has less adverse effects than 8.75 t ha^{-1}. Liming with 2 t ha^{-1} has been shown to be able to increase pH and C and N pools on the long term (Derome, 1990), but it probably does not stimulate C and N mineralization. Whether or not the beneficial effects of liming outweigh the negative effects, has to be decided individually for each case.



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7 References

- Aber, J.D., Hendrey, G.R., Botkin, D.B., Francis, A.J., Melillo, J.M., 1982. Potential effects of acid precipitation on soil nitrogen and productivity of forest ecosystems. Water Air and Soil Pollution 18, 405-412.
- Adams, S.N., Cornforth, I.S., 1973. Some short-term effects of lime and fertilizers on a Sitka spruce plantation. II. Laboratory studies on litter decomposition and nitrogen mineralization. Forestry 46, 39-47.
- Ammer, S., Makeschin, F., 1994. Effects of simulated acid precipitation and liming on earthworm fauna (Lumbricidae, Oligochaeta) and humus type in a mature stand of Norway spruce (Hoglwald experiment). Forstwiss. Cent.bl. 113, 70-85.
- Andersen, C., 1997. Regnorme. Naturhistorisk Museum Århus, Århus.
- Anderson, N.C., 1983. Nitrogen turnover by earthworms in arable plots treated with farmyard manure and slurry. In: Satchell, J.E. (Ed.), Earthworm ecology: From Darwin to vermiculture. Chapmann and Hall, London, pp. 189-196.
- Anderson, T.H., 1998. The influence of acid irrigation and liming on the soil microbial biomass in a Norway spruce (Picea abies [L.] K.) stand. Plant and Soil 199, 117-122.
- Andersson, F., Hallbäcken, L., Popovic, B., 1996. Kalkning och trädtillväxt. In: Staaf, H., Persson, T., Bertills, U. (Eds.), Skogsmarkskalkning, Resultat och slutsatser från Naturvårdsverkets försöksverksamhet, Report 4559. Swedish Environmental Protection Agency, pp. 122-133.
- Andersson, S., Valeur, I., Nilsson, I., 1994. Influence of lime on soil respiration, leaching of DOC, and C/S relationships in the mor humus of a haplic podsol. Environment International 20, 81-88.
- Bååth, E., Arnebrant, K., 1994. Growth-rate and response of bacterial communities to pH in limed and ash treated forest soils. Soil Biol. Biochem. 26, 995-1001.
- Bååth, E., Berg, B., Lohm, U., Lundgren, B., Lundkvist, H., Rosswall, T., Söderström, B., Wiren, A., 1980. Effects of experimental acidification and liming on soil organisms and decomposition in a Scots pine forest. Pedobiologia 20, 85-100.
- Berg, M., 1996. Decomposition, nutrient flow and food web dynamics in a stratified pine forest soil. In: Verhoef, H.A. (Ed.), Department of Animal Ecology, Faculty of Biology. VU University, Amsterdam, p. 310.
- Borja, I., Nilsen, P., 2009. Long term effect of liming and fertilization on ectomycorrhizal colonization and tree growth in old Scots pine (Pinus sylvestris L.) stands. Plant and Soil 314, 109-119.
- Borken, W., Brumme, R., 1997. Liming practice in temperate forest ecosystems and the effects on CO2, N2O and CH4 fluxes. Soil Use and Management 13, 251-257.
- Brown, G.G., Barois, I., Lavelle, P., 2000. Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. Eur. J. Soil Biol. 36, 177-198.
- Bryant, R.D., Gordy, E.A., Laishley, E.J., 1979. Effect of soil acidification on the soil microflora. Water Air and Soil Pollution 11, 437-445.
- Chan, K.Y., Mead, J.A., 2003. Soil acidity limits colonisation by Aporrectodea trapezoides, an exotic earthworm. Pedobiologia 47, 225-229.
- Derome, J., 1990. Effects of forest liming on the nutrient status of podzolic soils in Finland. Water Air and Soil Pollution 54, 337-350.
- Derome, J., Kukkola, M., Mälkönen, E., 1986. In. National Swedish Environmental Protection Board, p. 1.

- Duliere, J.F., Carnol, M., Dalem, S., Remacle, J., Malaisse, F., 1999. Impact of dolomite lime on the ground vegetation and on potential net N transformations in Norway spruce (Picea abies (L.) Karst.) and sessile oak (Quercus petraea (Matt.) Lieb.) stands in the Belgian Ardenne. Ann. For. Sci. 56, 361-370.
- Edwards, C.A., 1991. The assessment of populations of soil-inhabiting invertebrates. Agric. Ecosyst. Environ. 34, 145-176.
- Ericsson, A., Walheim, M., Nordén, L.G., Näsholm, T., 1995. Concentrations of Mineral Nutrients and Arginine in Needles of Picea Abies Trees from Different Areas in Southern Sweden in Relation to Nitrogen Deposition and Humus Form. Ecological Bulletins 44, 147-157.
- Foster, N.W., Beauchamp, E.G., Corke, C.T., 1980. Microbial activity in a Pinus banksiana lamb forest floor amended with nitrogen and carbon. Canadian Journal of Soil Science 60, 199-209.
- Frelich, L.E., Hale, C.M., Scheu, S., Holdsworth, A.R., Heneghan, L., Bohlen, P.J., Reich, P.B., 2006. Earthworm invasion into previously earthworm-free temperate and boreal forests. Biological Invasions 8, 1235-1245.
- Hallbäcken, L., Popovic, B., 1985. Markkemiska effekter av skogsmarkskalkning. Revision av skogliga kalkningsförsök. In. National Swedish Environmental Protection Board, p. 1.
- Hargrove, W.L., Thomas, G.W., 1981. Effect of organic matter on exchangeable aluminum and plant growth in acid soils. ASA Special Publication, 151-165.
- Högberg, P., Fan, H., Quist, M., Binkley, D., Tamm, C.O., 2006. Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. Global Change Biology 12, 489-499.
- Huber, C., Weis, W., Gottlein, A., 2006. Tree nutrition of Norway spruce as modified by liming and experimental acidification at the Hoglwald site, Germany, from 1982 to 2004. Ann. For. Sci. 63, 861-869.
- Jimenez, J.J., Lavelle, P., Decaens, T., 2006. The efficiency of soil hand-sorting in assessing the abundance and biomass of earthworm communities. Its usefulness in population dynamics and cohort analysis studies. Eur. J. Soil Biol. 42, S225-S230.
- Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. Oikos 69, 373-386.
- Jouquet, P., Dauber, J., Lagerlöf, J., Lavelle, P., Lepage, M., 2006. Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops. Appl. Soil Ecol. 32, 153-164.
- Kandler, O., Innes, J.L., 1995. Air pollution and forest decline in Central Europe. Environmental Pollution 90, 171-180.
- Kauppi, P.E., Mielikainen, K., Kuusela, K., 1992. Biomass and carbon budget of European forests, 1971 to 1990. Science 256, 70-74.
- Klein, T.M., Novick, N.J., Kreitinger, J.P., Alexander, M., 1984. Simultaneous inhibition of carbon and nitrogen mineralizaton in a forest soil by simulated acid precipitation. Bulletin of Environmental Contamination and Toxicology 32, 698-703.
- Kreutzer, K., 1995. Effects of forest liming on soil processes. Plant and Soil 168-169, 447-470.
- Kreutzer, K., Göttlein, A., 1991. The Hoglwald ecosystem research project: studies on the effect of acid rain and liming in an old Norway spruce stand. Forstwissenschaftliche Forschungen.
- Kulhavy, J., Markova, I., Drapelova, I., Truparova, S., 2009. The effect of liming on the mineral nutrition of the mountain Norway spruce (Picea abies L.) forest. Journal of Forest Science (Prague) 55, 1-8.
- Kunes, I., Balcar, V., Zahradnik, D., 2007. Influence of a planting hole application of dolomitic limestone powder and basalt grit on the growth of Carpathian birch (Betula carpatica W. et K.) and soil chemistry in the air-polluted Jizerske hory Mts. Journal of Forest Science (Prague) 53, 505-515.

- Lohm, U., Larsson, K., Nommik, H., 1984. Acidification and liming of coniferous forest soil - long-term effects on turnover rates of carbon and nitrogen during an incubation experiment. Soil Biol. Biochem. 16, 343-346.
- Lundström, U.S., Bain, D.C., Taylor, A.F.S., Van Hees, P.A.W., 2003. Effects of Acidification and its Mitigation with Lime and Wood Ash on Forest Soil Processes: A Review. Water Air and Soil Pollution: Focus 3, 5-28.
- Makeschin, F., 1980. EinfluB von Regenwurmern (Lumbricidae, Oligochaeta) auf den Boden sowie auf Ertrag und Inhaltsstoffe von Nutzpflanzen. In. Universitat GieBen.
- Makeschin, F., 1997. Earthworms (Lumbricidae: Oligochaeta): important promoters of soil development and soil fertility. In: Benckiser, G. (Ed.), Fauna in soil ecosystems: Recycling processes, nutrient fluxes, and agricultural production. Marcel Dekker, inc., New York, pp. 173-223.
- Mariani, L., Jimenez, J.J., Asakawa, N., Thomas, R.J., Decaens, T., 2007. What happens to earthworm casts in the soil? A field study of carbon and nitrogen dynamics in Neotropical savannahs. Soil Biol. Biochem. 39, 757-767.
- Marschner, B., Wilczynski, A.W., 1991. The effect of liming on quantity and chemical composition of soil organic matter in a pine forest in Berlin, Germany. Plant and Soil 137, 229-236.
- Matzner, E., 1985. Effects of fertilizer and lime treatment on the turnover and distribution of elements in two forest ecosystems in the Solling region. Allgemeine Forstzeitschrift, 1143-1147.
- Murach, D., Ulrich, B., 1988. Destabilization of forest ecosystems by acid deposition. GeoJournal 17, 253-260.
- Nilsson, S.I., Andersson, S., Valeur, I., Persson, T., Bergholm, J., Wiren, A., 2001. Influence of dolomite lime on leaching and storage of C, N and S in a Spodosol under Norway spruce (Picea abies (L.) Karst.). Forest Ecology and Management 146, 55-73.
- Nommik, H., 1968. Nitrogen mineralization and turnover in Norway Spruce (Picea abies (L.) Karst.) raw humus as influenced by liming. In, 9th international Congress on Soil Sciences, Adelaide.
- Nommik, H., 1979. The future role of liming in forestry. Kungl. Skogs- och Lantbruksakademiens Tidskrift, 31-37.
- Novick, N.J., Klein, T.M., Alexander, M., 1984. Effects of simulated acid precipitation on nitrogen mineralization and nitrification in forest soils. Water Air and Soil Pollution: Focus 23, 317-330.
- Nyberg, L., Lumdström, U., Söderberg, U., Danielsson, R., van Hees, P., 2001. Is there no impact of soil acidification on coniferous needle composition and tree growth? Water Air and Soil Pollution: Focus 1, 242-263.
- Persson, T., 1988. Liming, soil biology and nitrogen mineralization. Kungl. Skogs- och Lantbruksakademiens Tidskrift, 37-46.
- Persson, T., 1989. Role of soil animals in C and N mineralization. Plant and Soil 115, 241-245.
- Persson, T., 2002. Long-term effects of forest liming on Enchytraeidae and Lumbricidae in a spruce stand in southern Sweden. In, 4th International Symposium on Enchytraeidae. Natura Jutlandica Occasional Papers from Natural History Museum, Århus, Denmark, Mols Laboratory, Denmark.
- Persson, T., Karlsson, P.S., Seyferth, U., Sjoberg, R.M., Rudebeck, A., Schulze, E.D., 2000a. Carbon mineralization in European forest soils. Ecological Studies, 257-275.
- Persson, T., Rudebeck, A., Jussy, J.H., Colin-Belgrand, M., Prieme, A., Dambrine, E., Karlsson, P.S., Sjoberg, R.M., 2000b. Soil nitrogen turnover: Mineralization, nitrification and denitrification in European forest soils. Ecological Studies, 297-331.
- Persson, T., Rudebeck, A., Wiren, A., 1995. Pools and fluxes of carbon and nitrogen in 40-year-old forest liming experiments in southern Sweden. Water Air and Soil Pollution 85, 901-906.

- Persson, T., van Oene, H., Harrison, A.F., Karlsson, P.S., Bauer, G.A., Cerny, J., Couteaux, M.-M., Dambrine, E., Hogberg, P., Kjoller, A., Matteucci, G., Rudebeck, A., Schulze, E.D., Paces, T., 2000c. Experimental sites in the NIPHYS/CANIF project. Ecological Studies 142, 14-46.
- Persson, T., Wiren, A., 1993. Effects of experimental acidification on C and N mineralization in forest soils. Agric. Ecosyst. Environ. 47, 159-174.
- Plaster, E.J., 1992. Soil science and management. Delmar Publishers Inc., Albany, New York.
- Prietzel, J., Stetter, U., Klemmt, H.J., Rehfuess, K.E., 2006. Recent carbon and nitrogen accumulation and acidification in soils of two Scots pine ecosystems in Southern Germany. Plant and Soil 289, 153-170.
- Rangel, A.F., Rao, I.M., Horst, W.J., 2009. Intracellular distribution and binding state of aluminum in root apices of two common bean (Phaseolus vulgaris) genotypes in relation to Al toxicity. Physiologia Plantarum 135, 162-173.
- Rudebeck, A., 2000. Factors affecting nitrification in European forest soils. In, Department of Ecology and Environmental Research. Swedish University of Agricultural Sciences, Uppsala, p. 29 (excl. Appendices).
- Sahrawat, K.L., Keeney, D.R., Adams, S.S., 1985. Rate of aerobic nitrogen transformations in 6 acid climax forest soils and the effects of phosphorus and CaCO3. Forest Science 31, 680-684.
- Salonius, P.O., 1972. Microbiological response to fertilizer treatment in organic forest soils. Soil Science 114, 12-&.
- Smith, J., Potts, S., Eggleton, P., 2008. Evaluating the efficiency of sampling methods in assessing soil macrofauna communities in arable systems. Eur. J. Soil Biol. 44, 271-276.
- Söderström, B., 1984. In. National Swedish Environment Protection Board, p. 1.
- Söderström, B., Baath, E., Lundgren, B., 1983. Decrease in soil microbial activity and biomass owing to nitrogen amendments. Canadian Journal of Microbiology 29, 1500-1506.
- Staaf, H., Persson, T., Bertills, U., 1996. Skogsmarkskalkning - resultat och slutsatser från Naturvårdsverkets försöksverksamhet. - Naturvårdsverket Rapport 4559. In.
- Tamm, C.O., 1977. Acid precipitation and forest soils. Water Air and Soil Pollution 7, 367-369.
- Tamm, C.O., Wiklander, G., 1980. Ecological impact of acid precipitation. In: Drablos, D., Tollan, A. (Eds.), International Conference Sandefjord, SNSF project, Norway, p. 188.
- Tveite, B., 1980. Ecological impact of acid precipitation. In: Drablos, D., Tollan, A. (Eds.), International Conference Sandefjord, SNSF project, Norway, p. 204.
- Valentini, R., Matteucci, G., Dolman, A.J., Schulze, E.D., Rebmann, C., Moors, E.J., Granier, A., Gross, P., Jensen, N.O., Pilegaard, K., Lindroth, A., Grelle, A., Bernhofer, C., Grunwald, T., Aubinet, M., Ceulemans, R., Kowalski, A.S., Vesala, T., Rannik, U., Berbigier, P., Loustau, D., Guomundsson, J., Thorgeirsson, H., Ibrom, A., Morgenstern, K., Clement, R., Moncrieff, J., Montagnani, L., Minerbi, S., Jarvis, P.G., 2000. Respiration as the main determinant of carbon balance in European forests. Nature 404, 861-865.
- Verhagen, F.J.M., Laanbroek, H.J., Woldendorp, J.W., 1995. Competition for ammonium between plant roots and nitrifying and heterotrophic bacteria and the effects of protozoan grazing. Plant and Soil 170, 241-250.
- Zak, D.R., Groffman, P.M., Pregitzer, K.S., Christensen, S., Tiedje, J.M., 1990. The vernal dam - plant microbe competition for nitrogen in northern hardwood forests. Ecology 71, 651-656.

8 Appendix

1. Map of the Hässlöv site set-up.