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Long-Term Impact of Liming on Soil C and N in a Fertile Spruce Forest Ecosystem

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ABSTRACT

Liming can counteract acidification in forest soils, but the effects on soil C and N pools and fluxes over long periods are less well understood. Replicated plots in an acidic and N-rich 40-year-old Norway spruce (*Picea abies*) forest in SW Sweden (Hasslöv) were treated with 0, 3.45 and 8.75 Mg ha^{-1} of dolomitic lime (D0, D2 and D3) in 1984. Between 1984 and 2016, soil organic C to 30 cm depth increased by 28 Mg ha^{-1} (30% increase) in D0 and decreased by 9 Mg ha^{-1} (9.4% decrease) in D3. The change in D2 was not significant (+ 2 Mg ha^{-1}). Soil N pools changed proportionally to those in soil C pools. The C and N changes occurred almost exclusively in the top organic layer. Non-burrowing earthworms responded positively to liming and stimulated heterotrophic respiration in this layer in both D2 and D3. Burrowing earthworms in D3 further accelerated C and N turnover and loss of soil. The high soil C and N loss at our relatively N-rich site differs from studies of N-poor sites showing no C and N loss. Earthworms need both high pH and N-rich food to reach high abundance and biomass. This can explain why liming of N-rich soils often results in decreasing C and N pools, whereas liming of N-poor soils with few earthworms will not show any change in soil C and N. Extractable nitrate N was always higher in D3 than in D2 and D0. After 6 years (1990), potential nitrification was much higher in D3 (197 kg N ha^{-1}) than in D0 (36 kg N ha^{-1}), but this difference decreased during the following years, when also the unlimed organic layers showed high nitrification potential. Our experiment finds that high-dose liming of acidic N-rich forest soils produces an initial pulse of soil heterotrophic respiration and increases in earthworm biomass, which together cause long-term declines in soil C and N pools.

Key words: Decomposition; Heterotrophic respiration; pH; Net N mineralization; Nitrification; Nitrifiers; Earthworms.

Author Contributions TP conceived the funding, designed the study and was responsible for most of the research. SA and AW took part in field, laboratory and calculation work in the first half of the study. TG made laboratory and calculation work during the second part of the study. JB was responsible for deposition and leaching studies. LH organized the sampling in 2007. BV made the statistical analysis. TP wrote the paper and all co-authors commented on the manuscript.

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HIGHLIGHTS

- Forest liming counteracted soil C and N accumulation during 32 years
- Liming and high soil N status favored earthworm activity
- Burrowing earthworms promoted forest floor decomposition
- Net nitrification responded faster in limed than in unlimed plots

INTRODUCTION

Increasing atmospheric deposition of nitrogen (N), sulfur (S) and protons (H^+) affected many areas in Europe and North America during the three decades following World War II and resulted in soil acidification, nutrient imbalances in trees and forest decline (Schulze and others 1989; Ericsson and others 1993; Linder 1995; Prietzel and others 2006). Forest soil acidification is often associated with increased solubilization of inorganic Al and decreased contents of Ca and Mg in the exchangeable soil pool (Lundström and others 2003a). Forest decline symptoms were reported from areas with ''acid rain'' in mountain regions in, for example, the Czech Republic, Poland and Germany (Ulrich and others 1979; Murach and Ulrich 1988; Kandler and Innes 1995). Also Swedish soils turned out to be affected by soil acidification, and reinvestigation of coniferous forest soils in southern Sweden showed decreasing pH values of 0.3–1.0 units between 1927 and 1982–1984 (Hallbäcken and Tamm 1986). Similar values were recorded for beech and other deciduous stands in southern Sweden for the period from 1949 to 1984 (Falkengren-Grerup 1987; Falkengren-Grerup and others 1987).

Addition of lime to reduce the acidity of forests soils was frequently tested in Central and Western Europe, where many trials were set up (Kreutzer 1995; Huber and others 2006a, b; Kunes and others 2007; Court and others 2018). A number of liming experiments were also carried out in Norway, Finland and Sweden, and between 1907 and 1986, about 150 liming trials were initiated in these countries (Staaf and others 1996). Tree growth often remained unchanged in limed spruce, pine and fir stands (Hüttl and Zöttl 1993; Lundström and others 2003a; Børja and Nilsen 2009, Ouimet and Moore 2015). However, there were also examples of increased growth after liming in productive stands of Norway spruce in Sweden (Andersson

and others 1996), Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, in Oregon and Washington (Mainwaring and others 2014) and Masson pine, *Pinus massoniana* Lamb. in China (Li and others 2014).

Forest liming often stimulates the activity of the soil microbial community (Bååth and Arnebrant 1994, Persson and others 1995; Nilsson and others 2001) and population growth of earthworms (Ammer and Makeschin 1994; Persson 2002; Chan and Mead 2003). While C and N pools in the organic layer often decrease (Kreutzer 1995; Persson and others 1995; Huber and others 2006a; Paradelo and others 2015), at least one study shows increased C and N pools after liming (Derome 1990). Thus, the effect of liming may vary depending on tree species, site fertility and geographical position.

Between 1984 and 1986, six new forest liming experiments with four replicate plots per treatment were set up in Sweden. These experiments covered different climate (latitude 56°N–62°N), fertility ranges and tree species (Norway spruce and Scots pine, *Pinus sylvestris* L.) and were designed to answer questions about tree growth, soil chemistry and soil biological processes in relation to lime type and dose (Bertills 1996). Before 1984, knowledge of soil biological responses to forest liming was limited, and soil biology therefore became a new focus area in the experiments. Hasslöv in SW Sweden had the highest S, N and H^+ deposition among these six experimental sites. This site was selected for long-term studies as being a good representative of N-rich sites in southern Sweden and in having space enough (10 ha) for a large experiment with even-aged trees.

The aim of this study was to determine the effects of different doses of dolomitic lime on soil C and N pools and soil C and N mineralization at Hasslöv over 32 years. We hypothesized that the control plots (without lime) would become increasingly acidified through tree growth and acid deposition, whereas small to moderate lime doses would compensate for external acidification, and high doses would increase pH even at deeper soil layers. We further hypothesized that the soil C and N pools in the unlimed plots would increase, because the spruce stand was the first tree generation after a *Calluna* heathland. Afforestation of heathlands in NW Europe has often resulted in increased soil organic matter (SOM) (Bárcena and others 2014). This can depend on a combination of high N availability, high tree production and low SOM decomposition (Hyvönen and others 2008) as well as low pH. We also hypothesized in these afforested systems that liming would cause smaller increases

in C and N pools than in unlimed plots, because of an increased decomposition of SOM. Accelerated decomposition of N-rich substrates by liming at Hasslöv was also expected to increase net N mineralization and subsequent oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) by pH-sensitive ammonium oxidizers, followed by oxidation to nitrate (NO_3^-). Increased pH might also favor earthworms, both epigeic (living in the topsoil) and burrowing species that increase vertical bioturbation (for example, transport surface organic matter to subsurface mineral soil) and stimulate C and N mineralization. At the start of this long-term liming experiment, we had no clear ideas of when different processes would occur, but assumed that SOM decomposition and net N mineralization rates would increase in parallel with increased pH at the different soil depths.

MATERIALS AND METHODS

Site Description

The study was conducted in the Hasslöv liming experiment situated on the horst Hallandsåsen (56° 24′ N, 13° 00′ E, 190 m a.s.l.) about 10 km from the coastline of the Laholm Bay in southwestern Sweden (Figure 1). Annual mean temperature and precipitation were 7.0°C and 1000 mm, respec-

tively, during the reference period of 1961–1990, and 8.0°C and 1200 mm during the period of 2000–2016 (SMHI 2017). Average bulk deposition in 1990–1995 was 51 mmol m^{-2} y^{-1} of SO_4^{-2} and 138 mmol m^{-2} y^{-1} of N (43% as NO_3^-), corresponding to about 16 kg S ha^{-1} y^{-1} and 19 kg N ha^{-1} y^{-1}, respectively (Lundström and others 2003a). Recent estimates for the region (2012–2014) indicate much lower S deposition (4–6 kg S ha^{-1} y^{-1}) and slightly lower deposition of inorganic N (14–16 kg N ha^{-1} y^{-1}) (Karlsson and others 2015).

The Hasslöv site is a former *Calluna vulgaris* (L.) Hull heathland with scattered *Vaccinium myrtillus* L., *Deschampsia flexuosa* (L.) Trin., *Juniperus communis* L., *Betula* spp. and various herbs. *Calluna* heathlands on glacial till in this area are mostly associated with podzolic soils, mor layer horizons of 5 cm depth and pH values of about 4 in the mor layer, but site-specific data were not available when the site was planted with spruce seedlings.

In 1949, the site was planted with 4-year-old seedlings of Norway spruce, and the stand was considered as 40-year old at the start of the experiment in 1984 (Table 1). Site fertility was high for Swedish conditions and had an estimated site index in 1994 of G34 [the mean height (m) of the 100 largest spruce trees (by diameter) per ha at
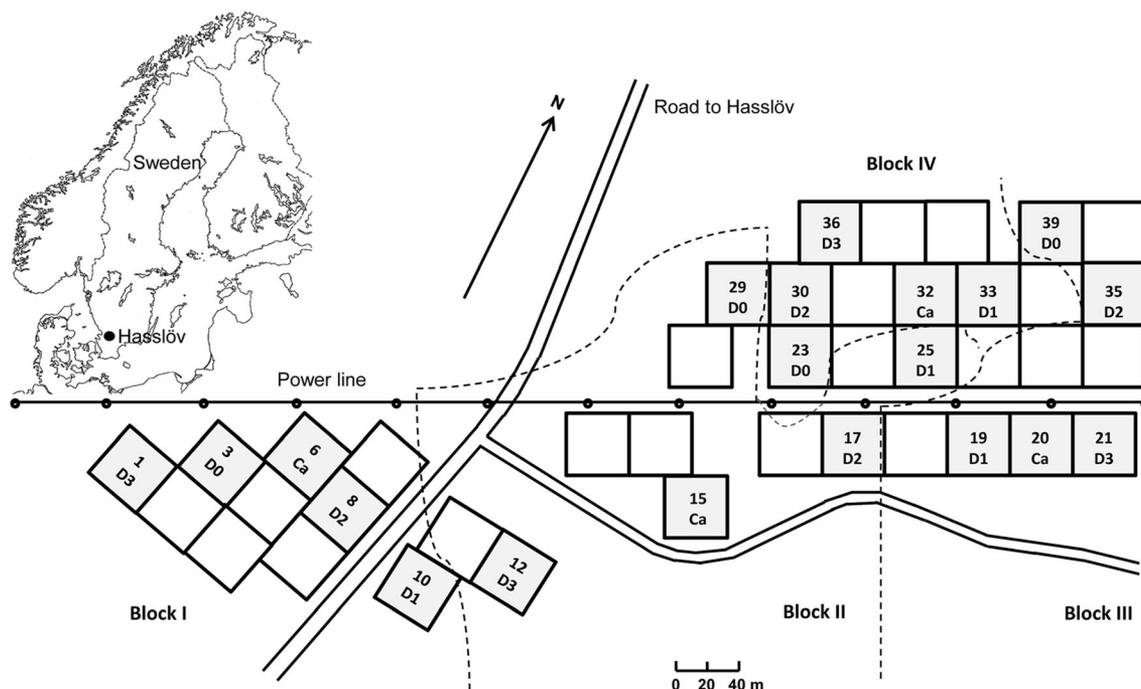
Figure 1. Location and design of the liming experiment at Hasslöv showing all 40 m × 40 m plots. The plot numbers and treatments (see Table 2) used in the present study are shown. The grouping in blocks is based on similarities in tree basal area and plot vicinity.

Table 1. Stand Data Per ha (Mean of Four Plots Per Treatment) at the Start of the Hasslöv Experiment in 1984, Standing Volume 2016 and Total Volume Yield Including Thinning Removals 1984–2016. Data from Ulf Johansson (pers. comm.)

Treatment	Age in 1984 (years)	Stems (no.)	Mean diam. (cm)	Basal area (m^2)	Standing volume in 1984 (m^3)	Standing volume in 2016 (m^3)
D0 (control)	40	1500	15.9	29.4	223	631
D2	40	1572	15.7	30.0	231	665
D3	40	1536	16.0	31.2	242	605

an age of 100 years] (Elfving 2003). The stand was thinned in 1979–1982 (before treatment), 1989 and 1997, when trees representing 20–25%, 15–22% and 20–23% of the current basal area were harvested, respectively. Logging residues (tops and branches) were left on site. The final measurements reported here were conducted immediately prior to harvest and replanting of the site in 2016 and 2017.

The soil profile is classified as a Typic Haplorthod (USDA 1999) and Haplic Podzol (FAO 1988). The O horizon (about 5 cm thickness in 1984) from top to bottom consists of a litter (Oi) layer, a layer with intermediately decomposed organic matter (Oe) and a layer with highly decomposed organic matter (Oa), which we call L, F and H layers, respectively. Because of high organic matter content in the upper 0–5 cm mineral soil, a typical E horizon could not be clearly distinguished. Below the O horizon, the horizon depths were: A/E 9 cm, Bhs 12 cm, Bs 18 cm and BC 15+ cm (Lundström et al. 2003b). The soil was a sandy loamy till, and stoniness varied between 27 and 42% to a depth of 20 cm according to the rod penetration method developed by Viro (1952). At the start of the experiment in 1984, soil pH (H$_2$O) was 4.15 in the FH layer, and 4.08, 4.15 and 4.27 in the 0–5, 5–10 and 10–20 cm mineral soil layers, respectively. Base saturation (BS) calculated as BS/CEC$_{pH7.0}$ *100 was 11.5% in the FH layer, and 4.5%, 3.2% and 2.7% in the mineral soil layers below.

Experimental Design

The field experiment was laid out in 1984 comprising 40 experimental plots divided into four replicate blocks (one replicate per block), each including ten treatments (Figure 1).

The experimental plots each had a size of 40 m × 40 m (25 m × 25 m sampled as net plots, omitting 7.5 m buffers) that were treated once at the start of the study (Bertills 1996). In the present study, five of these treatments were selected, namely control (D0), ground/crushed limestone (Ca) and three doses of ground/crushed dolomite (D1, D2 and D3) (Table 2). Some of the treatments (D0, D2 and D3) have been prioritized in the present study, whereas Ca and D1 have only been included in studies of pH and inorganic N.

Soil Sampling

Samples for determination of soil C and N pools were taken in June 1984 just before treatment. After treatment, samples for C and N pools as well as fluxes (C and N mineralization) were taken in September 1984, October 1986, November 1990, September 2000, March 2007, April 2009 and October 2016. Soil samples for determination of pH and concentrations of NH_4^+ and NO_3^- were taken more frequently, especially during the initial 6 years. Treatments, sample sizes and soil depths studied varied between years. In 1984 and 2007, all five treatments (D0, Ca, D1, D2 and D3) were sampled, whereas only D0, D2 and D3 were sampled in 1990, 2000, 2009 and 2016. In general, 4–9 sample spots per plot were selected inside each 25 m × 25 m net plot. From these spots, litter layer (L) and humus layer (FH) samples were taken with a cylindrical 250-cm^2 metal frame. The mineral soil at 0–5 and 5–10 cm depths was taken with a 10 cm × 10 cm metal frame starting from the bottom of the 250 cm^2 hole. The mineral soil at 10–20 and 20–30 cm depths was sampled with a 15.9 cm^2 cylindrical corer from the bottom of the 10 cm × 10 cm hole. Samples from the same soil layer were pooled for each plot to obtain mean values for each soil layer. In 2016, nine samples per plot were taken using 10 cm × 10 cm frames for the L and FH layers and a 15.9 cm^2 soil corer for the 0–30 cm mineral soil. The estimates of C and N soil pools in the mineral soil were reduced by 30% to compensate for stoniness.

Treatment of Soil Samples

Samples in plastic bags were transported in coolers to the laboratory, where they were stored in field-moist conditions at 4–5°C before analyses. Litter (L

Table 2. Treatments in the Hasslöv Experiment (Bertills 1996)

Addition	Symbol	Dose (kg ha^{-1})	Grain size
Control (no amendment)	D0	0	0
Limestone (CaCO$_3$)	Ca	1750	19% < 0.25 mm; 32% < 0.5 mm; 79% < 1 mm; 100% < 2 mm
Dolomitic lime [CaMg (CO$_3$)$_2$]	D1	1550	50% < 0.15 mm; 70% < 0.3 mm; 98% < 1 mm
	D2	3450	
	D3	8750	

layer) samples were sorted by hand, and living plant parts (mosses, grasses, herbs) and branches greater than 5 mm diameter were removed remaining litter (that is, needles, twigs, cones, moss and bark-flake litter). Soil samples from the FH layer were passed through a 5-mm mesh and mineral soil through a 2-mm mesh. Stones, gravel and coarse roots greater than 2 mm in diameter were discarded, whereas fine roots (0–2 mm diam.) were used for further analysis in 2016.

The litter and sieved soil material from each sample was carefully mixed and divided into sub-samples for analysis of total C and N concentrations, inorganic N concentration, water-holding capacity (WHC), C mineralization and net N mineralization. The analytical procedures for each of the variables are described in Supplementary Information on ''Laboratory treatment''.

Extrapolation to the Field

Extrapolation to the field was made by multiplying estimates of C mineralization, net N mineralization and net nitrification rates obtained in the laboratory at 15°C (expressed per g of C) by (1) the amount of C per soil layer, (2) the number of days per year (365), (3) a temperature-dependent factor (F_{ST}) and (4) a moisture-dependent factor (F_{SM}). F_{ST} (Equation 1) was calculated for each soil layer and month (Persson and others 2000) with input data from measured and modeled soil temperature (ST) and soil moisture (SM) at 10 cm depth at Hasslöv. The response function for soil moisture (F_{SM}) was based on Seyferth (1998), who found a linear relation between relative water content (x) and C mineralization rate:

$$F_{ST} = (ST-ST_{min})^2/(T_{ref}-T_{min})^2 \qquad (1)$$

$$F_{SM} = 0.8x + 0.2 \qquad (2)$$

where ST is the soil temperature in the field (°C), ST_{min} is the minimum temperature for C mineral-

ization ($-6.2°C$), T_{ref} is the laboratory incubation temperature (15°C) and x is the fraction of optimum soil moisture. Our laboratory condition of 60% WHC was considered as x = 1 in Equation 2 (optimum soil moisture) as well as the winter moisture at Hasslöv corresponding to 68% water content in the FH layer and 30% water content in the upper mineral soil.

After integration for the whole year, the correction factor for converting the rates obtained in the laboratory at 15°C to those in the field soil was estimated to be 0.46 (Supplementary information, Table S1). The same correction factor was used for the whole soil profile and for both C and net N mineralization.

Earthworm Effects

In addition to temperature and moisture, C and N fluxes are also affected by earthworm activity. The presence of earthworms stimulates C and N mineralization (Haimi and Huhta 1990; Edwards and Bohlen 1996; Uvarov 2016), and extrapolation to the field thus needs an estimate of the ''earthworm factor'' to be complete. The mean abundance and biomass of earthworms at Hasslöv during the period of 1984–2016 in the D0, D2 and D3 treatments were estimated to be 37, 187 and 247 ind. m^{-2} and 0.24, 1.18 and 1.72 g dry wt m^{-2}, respectively (T. Persson, in prep.). The epigeic earthworms *Dendrobaena octaedra* and *Dendrodrilus rubidus* made up 66 and 21% of the biomass. Burrowing earthworms (*Lumbricus rubellus*, *L. terrestris*, *Aporrectodea caliginosa* and *A. rosea*) became abundant only during the last two samplings. Therefore, *D. octaedra* was selected for studies of the ''earthworm factor'' in microcosm studies.

A microcosm study with FH materials from Hasslöv showed that the presence of a dense population of the earthworm *D. octaedra* (similar to that in the D3 treatment during most of the experimental period) increased respiration rate by 10% and net N mineralization rate by 65% in compar-

ison with earthworm-free microcosms (T. Persson, in prep.). Based on the microcosm study and the biomass estimates, the field estimates of respiration and net N mineralization were increased by 10% (respiration) and 65% (net N mineralization) for D3, 7% and 44% for D2 and 1% and 9% for D0 in comparison with the estimates lacking earthworms.

Data Treatment

Mean (± SE) soil concentrations, soil pools and soil fluxes were estimated for each of the three treatments (D0, D2 and D3), six soil depths (L, FH, 0–5 cm, 5–10 cm, 10–20 cm and 20—30 cm) and five occasions (1984, 1990, 2000, 2009 and 2016) with blocks as replicates ($n = 4$). For some variables (pH and inorganic N), the Ca and D1 treatments were also included. The results of the different treatments were compared for both individual years and cumulative values over 32 years by means of ANOVA with blocks as replicate using the general linear model (GLM) procedure in SAS for Windows (version 9.1).

RESULTS

Soil pH

Addition of lime to the soil surface increased soil pH, and the increase was dependent on the dose of lime. Maximum pH(H_2O) was reached between 4 and 10 years after lime addition in the FH layer after which pH decreased in all treatments (Figure 2). In the 10–20 cm soil layer, pH in the D0 and D2 treatments was 4.2 and 4.4, respectively, in year 5, and the difference of 0.2 pH units between these treatments stayed relatively stable throughout the experimental period. In the D3 treatment, pH increased from 4.2 to 5.0 during the 32 experimental years in the 10–20 cm soil layer. The pH effect in the Ca and D1 treatments was slightly lower than in D2, but lack of observations obscured the comparison with other treatments.

Soil C Pools

In June 1984, before the lime addition, the total C pool was slightly higher in the plots that later received the D2 treatment (Table 3; Figure 3A). No change in C pools could be detected during the first 6 years, but between 1990 and 2000 the amount of C increased in the D0 treatment, and the increase continued until 2016 (after 32 years). At that time, the C pool had significantly increased by 2.8 (SE = 0.37) kg C m^{-2} since the start of the experiment (Figure 3A; Table 3). The corresponding
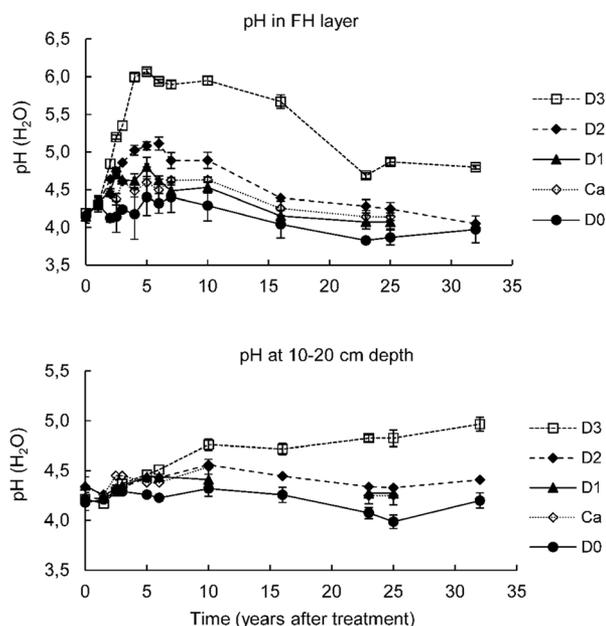

Figure 2. Mean soil pH (± SE) in the FH and 10–20 cm soil layers and different treatments (D0 = control, Ca = 1750 kg $CaCO_3$ ha^{-1}, D1 = 1550 kg dolomite ha^{-1}, D2 = 3450 kg dolomite ha^{-1}, D3 = 8750 kg dolomite ha^{-1}).

95% confidence interval (c.i.) of the increase was 1.6–4.0 kg C m^{-2} or 16–40 Mg C ha^{-1}. No change over time in C pools could be detected in the D2 treatment, whereas a tendency of decline in C pools was found in the D3 treatment.

The decline in D3 depended on a strong reduction in C in the LFH layer (Figure 3B). This decline was partly counteracted by an increase of 0.6 kg C m^{-2} ($P < 0.05$) in the 10–30 cm soil layers (Table 3). The decline in D3 differed between plots where both burrowing and non-burrowing earthworms were present (D3+, $n = 3$) and where only non-burrowing earthworms were present (D3−, $n = 1$) (Figure 3B).

The 32-year increase in total C to 30 cm depth was significantly higher ($P < 0.01$) in D0 than in both D2 and D3. No statistical difference in total C was found between D2 and D3 ($P = 0.08$). In the organic LFH layer, the C pool increased significantly more ($P < 0.001$) in the D0 treatment than in D2 and D3, and the increase in D2 was significantly higher ($P < 0.01$) than in D3, where the C pool decreased over the 32-year period. When the plots containing burrowing + non-burrowing ($n = 3$) and non-burrowing ($n = 1$) earthworms were compared, the D3 plots with burrowing earthworms showed a marked loss of C, whereas the D3 plot with only non-burrowing earthworms had similar C as D2 (Figure 3B).

Table 3. Mean Soil C Pools (kg m^{-2}, $n = 4$) and Mean Increase ($\pm$ SE) During the Experimental Period of 32 years in Different Soil Layers and Treatments

| Years from start	1984 0	1990 6	2000 16	2007 23	2009 25	2016 32	Mean Increase	SE Increase
D0 (control)								
L	0.75	0.93	0.35	1.07	0.73	1.01	0.26	0.13
FH	2.84	2.58	4.22	3.15	4.51	4.91	2.07	0.21
0–5 cm	1.47	1.76	1.75	1.64	1.73	1.63	0.16	0.15
5–10 cm	1.17	1.08	1.31	1.22	1.27	1.31	0.14	0.19
10–20 cm	1.84	1.70	1.86	2.01	1.99	1.84	0.00	0.04
20–30 cm	1.36	1.36	1.26	1.46	1.52	1.53	0.17	0.14
LFH + 0–30 cm	9.43	9.42	10.75	10.55	11.76	12.23	2.80	0.37
D2								
L	0.75	0.66	0.51	0.80	0.58	0.82	0.07	0.05
FH	2.92	2.88	2.82	2.49	3.18	3.07	0.15	0.29
0–5 cm	1.63	1.70	1.82	1.59	1.74	1.69	0.07	0.28
5–10 cm	1.27	1.29	1.33	1.16	1.21	1.22	− 0.06	0.10
10–20 cm	2.03	2.08	2.16	2.01	2.08	2.07	0.04	0.29
20–30 cm	1.46	1.40	1.28	1.38	1.40	1.41	− 0.05	0.12
LFH + 0–30 cm	10.06	10.01	9.92	9.43	10.19	10.28	0.23	0.36
D3								
L	0.75	0.80	0.29	0.74	0.59	0.69	− 0.06	0.18
FH	2.90	2.46	2.49	2.04	1.57	1.56	− 1.35	0.56
0–5 cm	1.62	1.89	1.70	1.37	1.53	1.53	− 0.10	0.11
5–10 cm	1.31	1.18	1.36	1.10	1.35	1.32	0.01	0.16
10–20 cm	1.83	1.70	1.94	1.96	2.17	2.11	0.29	0.15
20–30 cm	1.12	1.17	1.38	1.53	1.57	1.43	0.31	0.09
LFH + 0–30 cm	9.54	9.19	9.16	8.73	8.76	8.64	− 0.90	0.48

Soil N Pools

Mean soil N pool to a depth of 30 cm increased from 0.46 to 0.57 kg m^{-2} in the D0 treatment during the experimental period, whereas changes in D2 (0.48–0.50 kg m^{-2}) and D3 (0.47–0.44 kg m^{-2}) were less pronounced (Table 4; Figure 3C). The increase in total soil N was, on average, 35 ± 5 (mean $\pm$ SE) or 18–51 (95% c.i.) kg ha^{-1} y^{-1} in D0 during the experimental period. The corresponding figures were 7 ± 8 (mean $\pm$ SE) for D2 and $- 10 \pm 6$ kg N ha^{-1} y^{-1} for D3. Only D0 showed a significant increase, and this increase was higher than that in D2 ($P < 0.05$) and D3 ($P < 0.01$). No significant difference in total soil N was found between D2 and D3 ($P = 0.09$).

In the organic LFH layer, the soil N pool in D0 showed a significantly higher increase than in D2 ($P < 0.01$) and the three plots in D3 with burrowing earthworms (D3+) ($P < 0.001$) (Figure 3D). The increase in D2 was significantly higher ($P < 0.01$) than in D3+.

C/N Ratios

C/N ratios differed between soil layers and treatments. In the D0 treatment, the mean C/N ratio decreased from the litter layer (28) and humus layer (24–25) to the upper 0–5 cm of the mineral soil layer (19–20), but below this layer, the C/N ratio did not change with increasing depths (18–19). The mean C/N ratio in the organic LFH layer was significantly higher in D0 than in D2 ($P < 0.01$), which in turn was higher than in D3 ($P < 0.05$) over the entire experimental period (Figure 4). The C/N ratio showed a significant decrease over time with the exception for D3. The tendency of increasing C/N ratio in the D3 treatment at the end of the study period can be explained by the change in the proportions between L layer (high C/N) and FH layer (low C/N), because the amount of L remained relatively unchanged, whereas that of the FH layer decreased. Averaged over the whole soil profile down to a depth of 30 cm, the C/N ratio was 20.4 for the D0 and D2 treatments and 19.4 for the D3 treatment with no change over time.
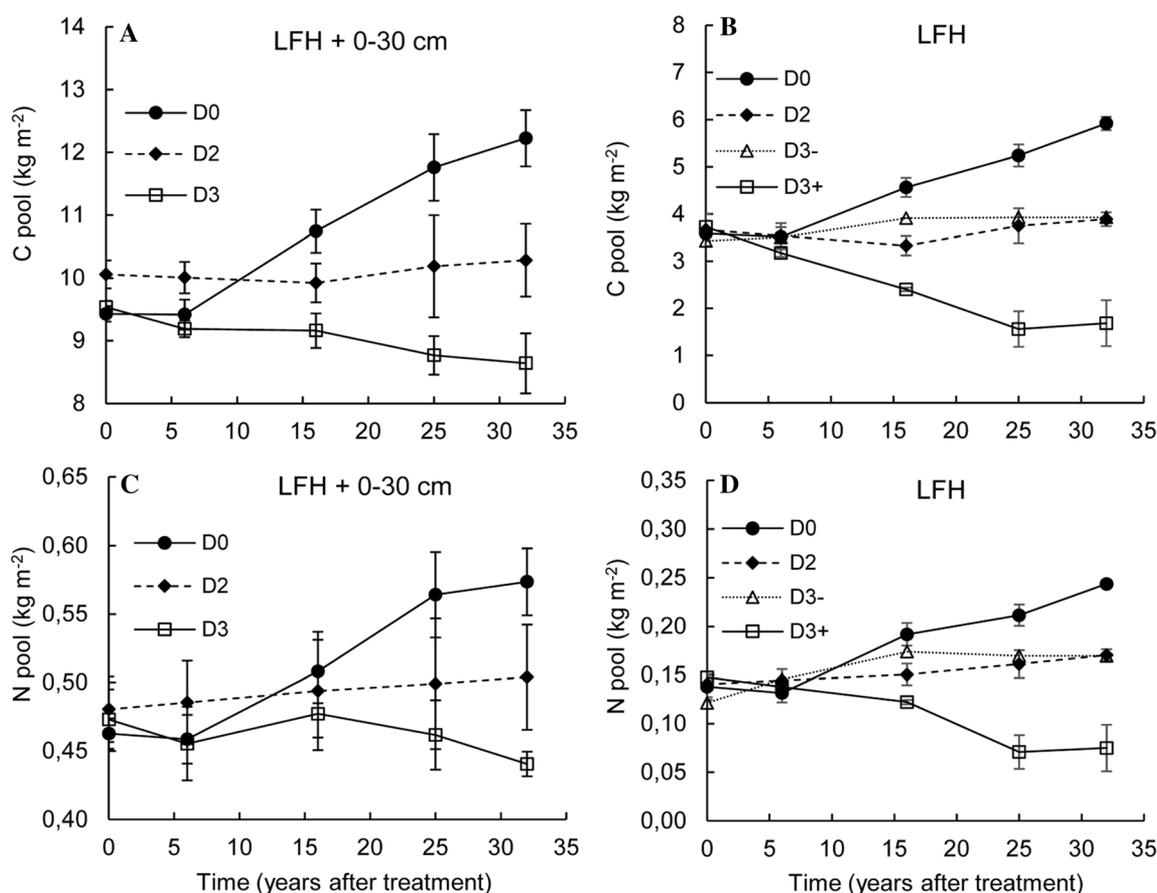
Figure 3. Mean C and N pools ($\pm$ SE) in the soil profile to a depth of 30 cm in the mineral soil (**A** and **C**) and in the organic LFH layer (**B** and **D**) in the D0, D2 and D3 treatments. D3$-$ and D3$+$ indicate plots where only non-burrowing earthworms were found (D3$-$, one plot) and where also burrowing earthworms were found (D3$+$, three plots).

Heterotrophic Soil Respiration

C mineralization rates in the laboratory (at 15°C and 60% WHC) over 20–30 days showed that C mineralization rates (per g of C) decreased with increasing soil depth (Figure S1, Supplementary information). Mean C mineralization rate in the FH layer differed significantly between D0, D2 and D3 and was 39% and 101% higher in D2 and D3, respectively, than in D0 when averaged over the experimental period (Figure 5A). The difference between treatments was smaller in the mineral soil.

When the laboratory C mineralization rates were combined with the soil C pools (Table 3) taking soil temperature (equation 1), soil moisture (equation 2) and the "earthworm factor" into consideration, the heterotrophic respiration in the field (R_H field) could be calculated. The estimate of annual R_H showed a sharp increase for D3 in the first 6 years, followed by a marked decline toward the end of the study, a less sharp increase for D2 fol-

lowed by a smaller decline and a continuous increase for D0 (Figure 5B). The decline in D3 between year 6 and 25 was correlated with decreasing C in LFH plots where burrowing earthworms were found (Figure 3B). Mean annual R_H field was estimated to be 4.63 ± 0.10, 5.34 ± 0.03 and 5.60 ± 0.30 (mean $\pm$ SE) Mg CO_2–C ha^{-1} y^{-1} over the whole study period, and cumulative R_H was 148 ± 3, 171 ± 1 and 179 ± 10 Mg CO_2–C ha^{-1} in D0, D2 and D3, respectively (not shown).

The mean difference ($\pm$ SE) in cumulative R_H field after 32 years was 23 ± 2 Mg CO_2–C ha^{-1} between D2 and D0 and 31 ± 12 Mg CO_2–C ha^{-1} between D3 and D0. These estimates were slightly lower than the differences found in C-pool accumulation of 26 Mg C ha^{-1} between D0 and D2 and 37 Mg C ha^{-1} between D0 and D3 of (Figure 3, Table 3). The higher estimates of cumulative R_H in D2 and D3 compared to D0 could explain 88% and 84% of the difference in soil C pools, respectively.

Table 4. Mean Soil N Pools (kg m^{-2}, $n = 4$) and Mean Increase ($\pm$ SE) During the Experimental Period of 32 years in Different Soil Layers and Treatments

Years from start	1984 0	1990 6	2000 16	2007 23	2009 25	2016 32	Mean Increase	SE Increase
D0 (control)								
L	0.026	0.034	0.011	0.040	0.026	0.041	0.016	0.005
FH	0.113	0.097	0.181	0.129	0.185	0.202	0.090	0.012
0–5 cm	0.078	0.090	0.087	0.085	0.090	0.082	0.004	0.008
5–10 cm	0.066	0.063	0.070	0.068	0.071	0.071	0.005	0.011
10–20 cm	0.104	0.097	0.095	0.108	0.110	0.096	− 0.008	0.003
20–30 cm	0.077	0.077	0.063	0.078	0.082	0.081	0.004	0.007
LFH + 0–30 cm	0.463	0.459	0.508	0.508	0.564	0.574	0.111	0.016
D2								
L	0.026	0.022	0.017	0.028	0.020	0.033	0.007	0.002
FH	0.115	0.122	0.134	0.108	0.141	0.138	0.023	0.012
0–5 cm	0.086	0.088	0.091	0.081	0.090	0.087	0.001	0.013
5–10 cm	0.070	0.070	0.071	0.062	0.066	0.065	− 0.004	0.008
10–20 cm	0.111	0.112	0.113	0.107	0.112	0.111	0.000	0.018
20–30 cm	0.074	0.071	0.067	0.070	0.071	0.070	− 0.004	0.005
LFH + 0–30 cm	0.480	0.485	0.494	0.456	0.499	0.504	0.023	0.025
D3								
L	0.026	0.033	0.009	0.027	0.022	0.026	0.001	0.007
FH	0.116	0.107	0.126	0.097	0.074	0.072	− 0.043	0.029
0–5 cm	0.087	0.095	0.087	0.073	0.084	0.084	− 0.003	0.005
5–10 cm	0.077	0.069	0.076	0.063	0.078	0.073	− 0.003	0.007
10–20 cm	0.106	0.091	0.103	0.106	0.118	0.112	0.006	0.009
20–30 cm	0.062	0.060	0.076	0.084	0.086	0.073	0.011	0.003
LFH + 0–30 cm	0.473	0.455	0.477	0.451	0.462	0.441	− 0.032	0.021
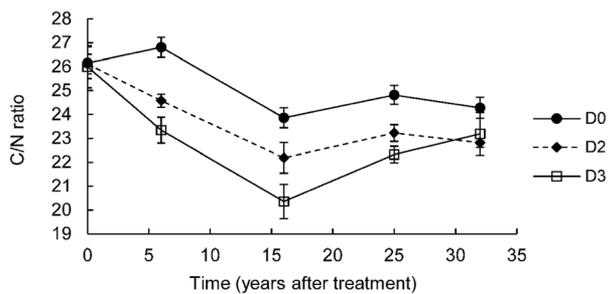
**Figure 4.** C/N ratio in the organic LFH layer in the D0, D2 and D3 treatments.

Mean Residence Time of Soil C

Mean residence time (MRT) of soil C was calculated as the quotient between the soil C pool in each soil layer and the corresponding estimate of R_H field. MRT increased with increasing soil depth from about 4 years in the L layer to 75–100 years in the 20–30 cm mineral soil layer (Supplementary information, Table S2). MRT had a tendency to decrease with increasing dose of lime, and in the FH layer, this decrease was significant ($P < 0.05$) between the D0 and D3 treatments.
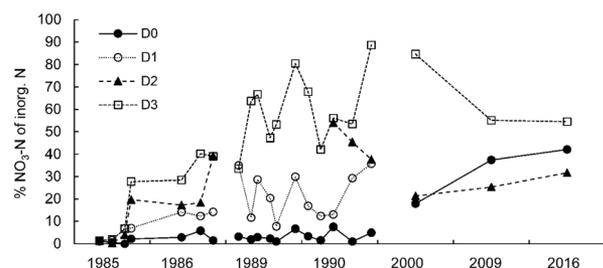
Inorganic N

KCl-extractable inorganic N (NH$_4$–N + NO$_3$–N) concentration in soil varied between soil layers, treatments and seasons. During the initial years, the proportion between NH$_4$–N and NO$_3$–N was markedly affected by liming. The share of NO$_3^-$ was highest in D3 followed by D2, D1 and D0 (Figure 6). The D0 treatment had initially (1984–1990) low NO$_3^-$ concentration in the organic LFH soil layer, but after 1990 the NO$_3^-$ levels increased in D0 and became similar to or even higher than in the D2 treatment.

The amount of KCl-extractable inorganic N down to a depth of 30 cm in the mineral soil was initially 7–9 kg ha^{-1} but increased over the course of the study period to around 30 kg ha^{-1} in the D0 treatment and to around 20–25 kg ha^{-1} in the D2 and D3 treatments (Figure 7A). The amount of extractable NO$_3$–N in D3 increased rapidly between 1986 and 1990 (years 2 and 6) and stayed at about the same level between year 6 and 32. The amount of NO$_3$–N increased at a slower rate in the D2 and D0 treatments, but in years 25 and 32 there was no
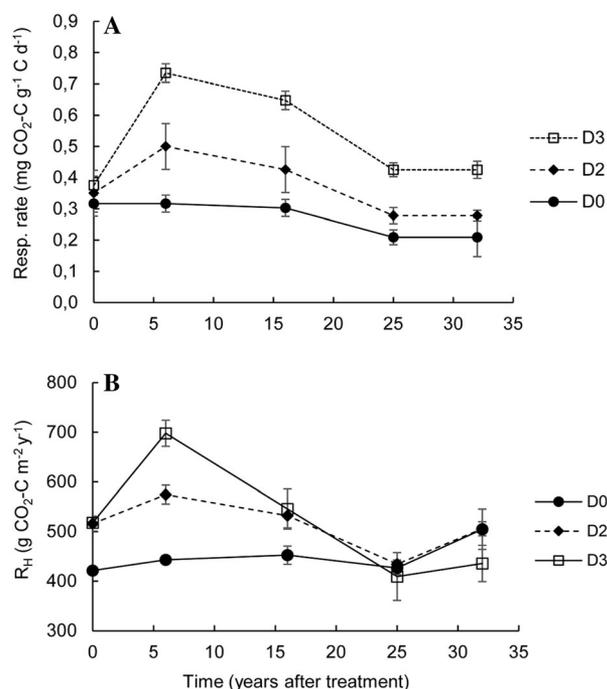
Figure 5. Mean respiration rate (± SE) in the FH (humus) layer measured in the laboratory at 15°C and 60% water-holding capacity (WHC) in the D0, D2 and D3 treatments (**A**), and annual mean (± SE) heterotrophic respiration (R_H) extrapolated to the field for the LFH + 0–30 cm mineral soil layers (**B**). The samples in year 0 were taken 4 months after treatment.

Figure 6. Per cent NO_3–N of total KCl-extractable inorganic N (NH_4^+–N + NO_3^-–N) concentrations in the FH layer in different treatments (see Table 2).

significant difference in extractable NO_3–N between the D0, D2 and D3 treatments (Figure 7B).

Net N Mineralization and Nitrification

Net N mineralization was studied in the laboratory in 1984 (4 months after treatment, not replicated), 1990, 2000, 2009 and 2016 at 15°C. Net N mineralization rate (expressed per kg of C) was highest in the L layer followed by the FH layer and the min-

eral soil layers (Figure S2, Supplementary information).

Extrapolation to the field (taking soil temperature, soil moisture and earthworm stimulation into consideration) showed that net N mineralization ranged from 120 to 278 kg N ha^{-1} y^{-1} (Figure 7C). On average, 66% of net N mineralization down to 30 cm depth occurred in the LFH layer and 88% in the topsoil (LFH + 0–10 cm mineral soil).

The estimates of net nitrification (potential nitrification) showed a clear difference between the D0 and D2 treatments on the one hand and the D3 treatment on the other during the initial 16 years after treatment, but the difference decreased at the end of the experimental period (Figure 7D). The main change seemed to occur in the FH layer, in which net nitrification in D0 was only 0.1 and 3 kg NO_3–N ha^{-1} y^{-1} in 1984 and 1990 (year 0 and 6), respectively, but fairly high (63 ± 4 kg NO_3–N ha^{-1} y^{-1}) in 2016 (year 32) despite low pH (4.0). By 1990, the D3 treatment had resulted in substantial NO_3^- formation. The mineral soil at 10–30 cm depth had a nitrification potential that seemed independent of treatment and length of the study period (not shown).

DISCUSSION

Soil C in Control Plots

The mean increase in soil organic C (SOC) in the D0 treatment (0.87 Mg C ha^{-1} y^{-1}) during the 32-year experimental period was unexpectedly high but can partly be explained by the site history. In a meta-analysis on SOC change following afforestation in Northern Europe, Bárcena and others (2014) found that afforestation of heathlands (as at Hasslöv) with conifer trees resulted in a non-significant decline of SOC pool during the first 30 years after afforestation followed by a significant increase in stands older than 30 years. The results of our study that followed a 40-year-old spruce stand over 32 years to an age of 72 years are in line with the meta-analysis. A similar result was found for an 87-year chronosequence of Norway spruce planted on arable land at Tönnersjöheden in the same region (30 km from Hasslöv), where the soil C pool increased by 0.65 Mg C ha^{-1} y^{-1} (Vesterdal and others 2007) viewed over the entire chronosequence. The conifer plantations with the highest increase in SOC pools were situated in areas with relatively high N deposition and high precipitation (Vesterdal and others 2007; Bárcena and others 2014). Common to all these studies, including ours, was that significant SOC increases
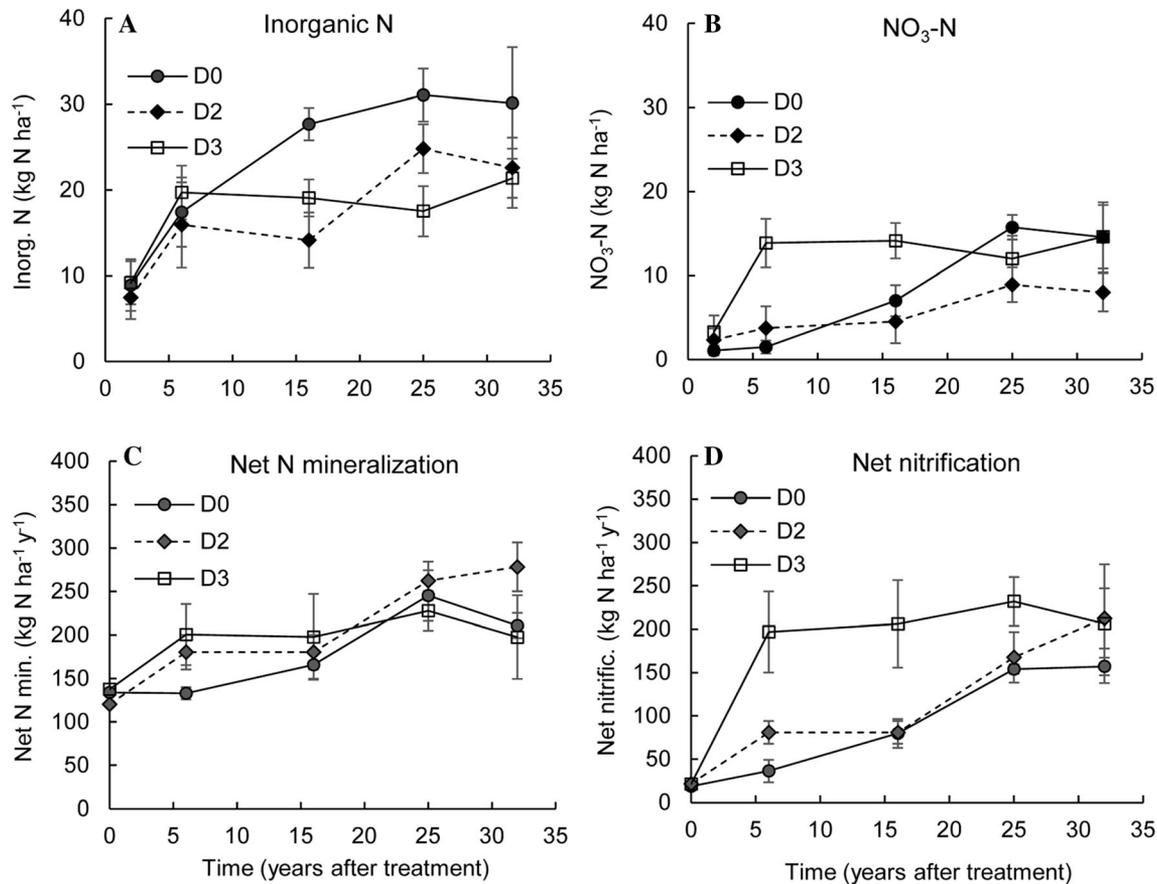
Figure 7. Mean amount of KCl-extractable inorganic N (NH_4–N + NO_3–N) and NO_3–N in the LFH + 0–30 cm mineral soil layers (**A** and **B**, upper panel), and mean annual net N mineralization and potential nitrification extrapolated to the field in the D0, D2 and D3 treatments (**C** and **D**, lower panel). The data from 1984 (time 0) represent just one plot.

were restricted to the LFH layer ("forest floor") and did not occur in the 10–30 cm mineral soil.

The increase in the SOC pool shows that the above- and belowground input of litter C to the soil system was higher than the output through heterotrophic respiration (R_H) and dissolved organic C (DOC). DOC leaching at 50 cm depth at Hasslöv was estimated to be 1.2% of R_H (Nilsson and others 2001); thus, R_H was the main output of soil C. The increase in R_H from 1984 to 2016 in D0 was correlated with increased SOC pools (Figs. 3A, B and 8). The relatively low estimate of R_H in D0 (4.6 Mg CO_2–C ha^{-1} y^{-1}) over the entire experimental period can be explained by the fact that the D0 soil had both high N availability and low pH. High N availability slows down SOM decomposition (Nohrstedt and others 1989; Berg and Matzner 1997; Berg 2000; Hobbie 2008; Hyvönen and others 2008). Several mechanisms have been suggested to explain why high N availability results in low respiration, for example, increase in microbial carbon-use efficiency (CUE), decrease in lignolytic

enzyme activity and decrease in microbial biomass (Riggs and Hobbie 2016). Experimental acidification of forest soils shows that reduction in pH reduces C mineralization (Persson and Wirén 1993).

Soil C After Liming

In contrast to the D0 treatment, the SOC pool in D2 did not show any significant change, and the SOC pool in D3 had a tendency to decline over the experimental period. However, the decline in D3 was restricted to the LFH and 0–5 cm mineral soil layers, whereas the SOC pool increased in the 10–30 cm layer (Table 3). The SOC decline in the upper soil layers could be explained by a higher mean rate of R_H in D3 than in D0. Six years after treatment (1990), the R_H rate in the FH layer was more than twice as high in D3 as in D0 (Figures 5A and 8). In 1990, the FH layer in D3 consisted of a loose layer of earthworm excrements, whereas it consisted of organic matter interwoven with fine roots and fungal hyphae in the D0 treatment. As the
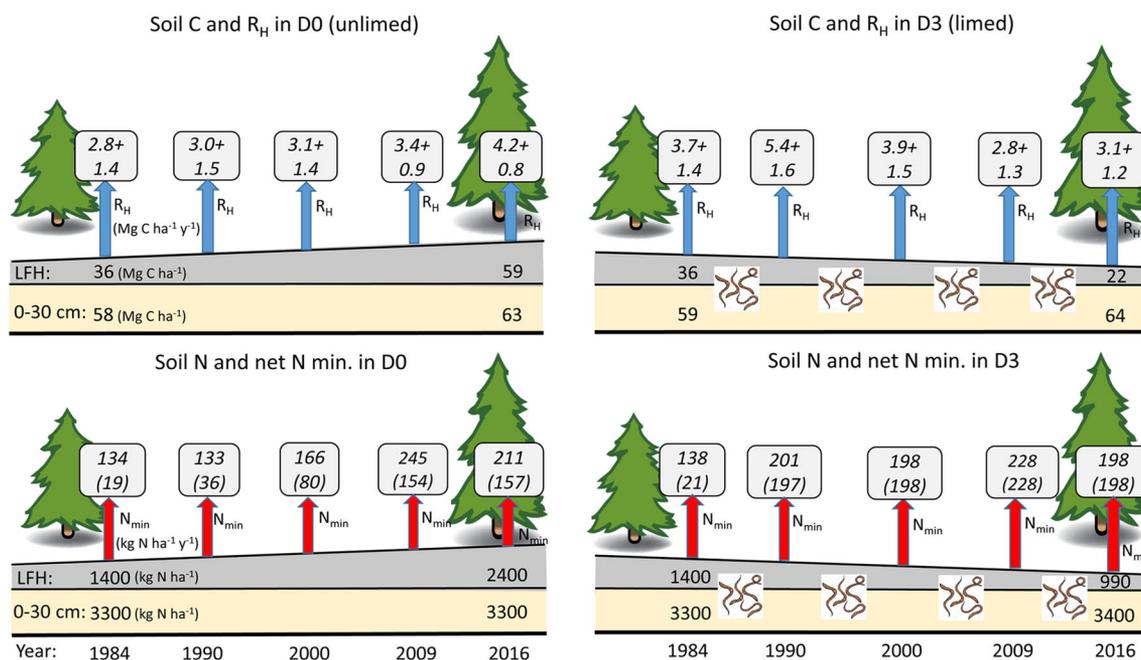
Figure 8. Overview of major changes in soil C and N pools and fluxes during 1984–2016. Soil C and N pools increased in the D0 treatment and decreased in the D3 treatment. The changes mainly occurred in the LFH layer (grey color). Heterotrophic respiration (R_H, estimates for LFH + 0–30 cm mineral soil layers presented separately) was initially higher in D3 than in D0 because of higher pH and earthworm stimulation of litter and SOM decomposition. At the end of the study, annual R_H in LFH became lower in D3 than in D0 because of substrate reduction in the FH sublayer. Both net N mineralization (here called N_{min}) and potential nitrification (figures in parentheses) increased faster in D3 than in D0. High earthworm numbers are indicated in the D3 soil. Burrowing earthworms accelerated C and N pool decline in D3 between 2000 and 2016.

respiration measurements were conducted with sieved soil lacking earthworms, the 11% higher R_H rate in D3 than in D0 was probably due to an effect of pH. Increased pH increases SOM solubility (Andersson and others 2000; Curtin and others 2016) and reduces Al concentration, which often results in higher abundance and biomass of bacteria (Bååth and Arnebrandt 1994; Illmer and others 1995; Joner and others 2005). Earthworm feeding, defecation and mixing of the organic material including disruption of fungal hyphae seem to increase the bacterial/fungal ratio (Rousk and others 2009, 2010; Dempsey and others 2011).

The SOC increase in the 10–30 cm mineral soil in D3 (0.188 kg ha^{-1} y^{-1}) could be explained by DOC leaching from topsoil to subsoil layers but also by earthworm bioturbation (Taylor and others 2019) and by root litter accumulation. In a field study at Hasslöv between 1993 and 1998, Nilsson and others (2001) showed that DOC leaching from the LFH layer and the A horizon was higher in D3 than in D0. About 80–90% of the DOC that leached from the LFH layer was adsorbed in the B horizon (10–30 cm in the mineral soil) regardless of treatment. They estimated the DOC adsorption to be

202 kg ha^{-1} y^{-1} in D3, which was almost identical with the D3 estimate in Table 3 (see above). However, earthworm bioturbation can also explain SOC transfer from the LFH layer to the mineral soil (see below), but in that case incorporation of organic matter should probably be most pronounced in the upper mineral soil. A third explanation can be root litter accumulation in the mineral soil. Therefore, although the data on DOC adsorption by Nilsson and others (2001) seem to fit the data in Table 3, earthworm and fine-root effects can probably also contribute to some SOC increase in the mineral soil.

Earthworm Role in SOM Decomposition and Mineralization

The interaction of earthworms with soil microorganisms plays a key role in litter and SOM decomposition (Hoeffner and others 2019). Earthworms can increase stabilization of SOC by forming soil aggregates (for example, Zhang and others 2013), but most long-term studies on earthworm impact indicate that the balance of mineralization and stabilization of C is in favor of mineralization

(Fahey and others 2013a, b; Moore and others 2013; Yavitt and others 2015; Lubbers and others 2017). Different earthworm species affect soil respiration differently (Haimi and Huhta 1990; Wessels and others 1997; Lubbers and others 2013, 2017; Uvarov 2016). In temperate forests in North America, the invasion of non-native European earthworms (for example, *L. rubellus*, *L. terrestris* and *A. caliginosa*) has often resulted in forest floor reduction (Scheu and Parkinson 1994; Bohlen and others 2004; Frelich and others 2006; Yavitt and others 2015; Jennings and Watmough 2016).

The epigeic earthworms *D. octaedra* and *D. rubidus* were the only earthworm species found at Hasslöv during the first 15 years of the experiment. Both species can tolerate pH(H_2O) values around 4, but they prefer higher pH. They responded with a sharp peak in abundance (from 30 to 700 ind. m^{-2}) and biomass (from 0.2 to 5 g dry wt m^{-2}) 3–6 years after the D3 treatment followed by a decline to about 200 ind. m^{-2} (T. Persson, in prep.). In a microcosm study with the presence or absence of *D. octaedra* in limed FH layer materials from Hasslöv, the presence of this species in abundances similar to those found in the D2 and D3 treatments increased CO_2 evolution rate by about 7% and 10%, respectively, in comparison with worm-free soil (T. Persson, in prep.).

The burrowing species *L. rubellus*, *A. caliginosa*, *A. rosea* and *L. terrestris* appeared later, but only in some of the limed plots at Hasslöv. The earthworm biomass in D3 viewed over the entire experimental period was on average 1.7 g dry wt m^{-2}, of which the epigeic *D. octaedra* and *D. rubidus* contributed 88% and the burrowing earthworms 12% (T. Persson, in prep.). The delayed arrival of the burrowing species can be explained by the fact that most limed plots were surrounded by acidic soil. Only three D3 plots with short distances to suitable habitats (a forested pasture, an electric power line and a grassy glade) had moderately high abundance (40–170 ind. m^{-2}) and biomass (0.8–2 g dry wt m^{-2}) of burrowing earthworms at the end of the study (2009 and 2016).

When the pH and earthworm effects were added, total R_H over the whole experimental period (to a depth of 30 cm in the mineral soil) was estimated to be 15% and 21% higher in D2 and D3, respectively, than in D0.

SOM Decomposition at Hasslöv

Heterotrophic respiration (R_H) was initially higher in D3 than in D0 because of higher pH and stimulation of litter and SOM decomposition by earthworms. At the end of the study, R_H (per year) became lower in D3 than in D0 because of substrate reduction in the FH layer (Table 3, Figs. 5B and 8). The mean cumulative R_H of 148, 171 and 179 Mg CO_2–C ha^{-1} in D0, D2 and D3, respectively, calculated for the entire experimental period, indicated that the SOC pool would be 23 and 31 Mg CO_2–C ha^{-1} lower in D2 and D3 than in D0. These estimates, based on laboratory measurements extrapolated to the field, were slightly lower than the differences based on field sampling of 26 and 37 Mg SOC ha^{-1}. The relatively small differences between the laboratory-estimated R_H and the field-measured SOC pools indicated that SOM decomposition in the field at Hasslöv was predominantly due to saprotrophic organisms and not to ectomycorrhizal (EM) fungi.

In N-poor forest soils, some EM fungi are able to mobilize organic N in N-containing phenolic substances in boreal forest litter and soil by means of oxidative extracellular enzymes (Bödeker and others 2014; Lindahl and Tunlid 2015; Shah and others 2016). This can result in SOM decomposition and release of CO_2 (Lindahl and Tunlid 2015). However, EM peroxidase is less active when concentrations of inorganic N are high (Bödeker and others 2014; Nicolás and others 2019) as was the case in the organic layer at Hasslöv (0.1–0.7 mg N g^{-1} C), indicating low contribution of EM fungi to SOM decomposition.

Field data on EM fungi at Hasslöv point in the same direction. Jacobsson (1993) conducted a fruit-body inventory of fungi at Hasslöv during 1985 to 1992. He found that liming resulted in a decrease in EM fruit bodies and an increase in saprotrophic fungi. In addition, the number of mycorrhizal species was very low at Hasslöv in comparison with other fruit-body inventories in Sweden. The study thus indirectly supports the results on low impact of EM fungi on CO_2 release.

Comparison with other C studies

The mean SOC pool to a depth of 5 cm (in the mineral soil) at Hasslöv increased by, on average, 780 and 90 kg ha^{-1} y^{-1} in the D0 and D2 treatments and decreased by 470 kg ha^{-1} y^{-1} in the D3 treatment. The SOC pool change in relation to D0 was thus − 690 kg C ha^{-1} y^{-1} for D2 and − 1250 kg C ha^{-1} y^{-1} for D3. These estimates resemble the results from the 80-year-old Norway spruce forest at Höglwald in southern Germany (C/N ratio 21–24 in the FH layer). For the latter site and the same soil depth, Kreutzer (1995) reported a reduction of 1000 kg C_{org} ha^{-1} y^{-1} during 7 years

in the limed plot that had received 4 Mg dolomite ha^{-1}. From the same experiment, Anderson (1998) reported almost three times higher microbial biomass (SIR) and twice as high CO_2 evolution rate after 6–8 years in the limed compared to the unlimed plot. The Höglwald results on annual soil C decline (with 4 Mg dolomite ha^{-1}) are similar to those found at Hasslöv (with 8.75 Mg dolomite ha^{-1} and longer experimental period).

In a study of four 37–42-year-old liming experiments in one beech and three spruce stands in SW Sweden (C/N ratio 20–26 in the FH layer), Persson and others (1995) found that high doses of lime (9–10 Mg ha^{-1}) significantly reduced the SOC pools to a soil depth of 50 cm compared to the unlimed plots. The reduction varied between 8.5 and 24.3 Mg C ha^{-1}, and the lime treatment resulted in a mean decline of 437 kg C ha^{-1} y^{-1}. Almost all changes occurred in the top organic layer.

In contrast to the six liming experiments with high N status mentioned above, Bergholm and others (2015) found no significant difference in soil C pools between unlimed and limed plots ($n = 3$) in a Norway spruce forest at Farabol, 16 years after the first lime application (0.5 Mg CaCO$_3$ ha^{-1} added during 12 years). The Farabol site was located about 100 km east of Hasslöv but differed in having only about 50% of the N deposition at Hasslöv, a needle litter concentration of 0.75% N in contrast to 1.8% at Hasslöv and a C/N ratio in the FH layer of 28 in contrast to 24 at Hasslöv, indicating N-poorer conditions at Farabol. Even poorer N conditions (C/N 30–58 in the FH layer) were reported from 14 Swedish liming experiments, which were reinvestigated in 1980–1982 by Hallbäcken and Popović (1985). These experiments were started between 1913 and 1971 and had lime doses of 5 and 10 Mg ha^{-1}. In these experiments, SOC pools in limed and unlimed treatments were similar. A series of 40 liming experiments in Norway spruce and Scots pine in Finland with a lime dose of 2 Mg ha^{-1} were reinvestigated after 20 years (Derome 1990). The soils had low N content (C/N 26–48 in the FH layer). Here, liming resulted in considerable accumulation (10–50% increase) of organic matter in the humus layer but in no change in the mineral soil. Increased cover of ground vegetation, particularly grasses, seemed to be the most plausible explanation of the increase in organic matter in the humus layer, as liming had a slightly negative or no effect on stand growth (Derome 1990).

In a 145-year-old, N-rich (forest floor C/N = 21–25) European beech (*Fagus sylvatica*) forest in Germany, Bauhus and others (2004) compared C and N soil pool changes after an eight-year period following the creation of canopy gaps and lime application. Although liming with 3 Mg dolomite ha^{-1} did not change the C and N pools in the intact forest stand, it reduced the pools in the F and H layers and increased them in the 0–10 cm mineral soil when added to the forest gaps. The authors suggested that earthworms and other soil fauna were probably responsible for this change, using increased inputs of herbaceous litter in the limed gaps as an energy source. In the limed stand, the input of such litter was relatively low, which might have been unfavorable for earthworm activity.

Based on these studies, it can be concluded that effects of liming on the SOC pool are dependent on both soil N status and dose of lime. Although liming of N-rich soils seems to decrease the SOC pool, liming of N-poor soils does not change or increase the SOC pool.

In contrast to most other studies, Melvin and others (2013) reported significantly higher C and N stocks in the forest floor (C/N = 20–23) in a mixed hardwood forest (mainly *Fagus grandiflora, Acer rubrum* and *Betula alleghaniensis*) 19 years after lime addition (6.9 Mg of CaCO$_3$ + MgCO$_3$ ha^{-1}) in comparison with unlimed areas. The areas consisted of two limed and two unlimed catchments in Woods Lake Watershed, New York, USA. Baseline data on C and N stocks at the start of the trial are, however, lacking in the paper. It is therefore not possible to distinguish between ''lime'' and ''site'' effects in this publication.

Soil N Pool Change at Hasslöv

Total soil N pool in the D0 treatment increased by 35 ± 16 (95% c.i.), that is between 18 and 51 kg ha^{-1} y^{-1}, during the experimental period. The increase (also in the lower range of the confidence interval) was higher than expected, because bulk deposition and throughfall deposition in the area were estimated to be only 15–20 kg N ha^{-1} y^{-1} (Lundström and others 2003b; Karlsson and others 2015; J Bergholm, pers. comm.). However, these estimates did not cover the entire experimental period and do not account for all possible N inputs to the forest ecosystem.

The Hasslöv forest is situated on top of a ridge and is surrounded by agricultural areas, so in addition to the regional bulk deposition of N, the forest may act as a collector of inorganic N because of the forest edge effect (Remy and others 2016). Foliar uptake of N may have contributed to plant N (Harrison and others 2000; Sparks 2009) and thereby soil N through litterfall N.

Biological N fixation can also be a source of N, but N-fixing plants (legumes, alder etc.) were practically absent in the dense spruce forest at Hasslöv. Associative N-fixing cyanobacteria in feather moss can add up to 1–2 kg N ha^{-1} y^{-1} under low rates of N deposition (DeLuca and others 2002) but inputs were likely much lower at Hasslöv due to down-regulation by high rates of N deposition (DeLuca and others 2008).

In the lime treatments, the N pool in D2 had a tendency to increase (mean +7 kg N ha^{-1} y^{-1}) and in D3 to decrease (mean − 10 kg N ha^{-1} y^{-1}). The total difference in soil N pools between D0 and D3 cannot entirely be explained by leaching losses. A leaching study at Hasslöv during 1988–1993 estimated NO_3–N leaching at 15 cm depth to be 0.9, 11 and 22 kg N ha^{-1} y^{-1} in D0, D2 and D3, respectively (J Bergholm, pers. comm.), and even the latter figure is too low to explain the total difference in soil N pools between D0 and D3. We therefore assume that the loss of N in especially D3 was also caused by gaseous N losses (NO, N_2O and N_2) through denitrification (cf. Lubbers and others 2013). Denitrification can sometimes be the dominant pathway of NO_3-N removal from forest ecosystems (Fang and others 2015; Morse and others 2015).

Comparison with Other N Studies

The mean soil organic N pool (± 95% confidence limits) to a depth of 5 cm in the mineral soil at Hasslöv increased by 32 ± 7 and 8 ± 12 kg N ha^{-1} y^{-1} in the D0 and D2 treatments. The soil N pool decreased by 23 (range 14–33) kg N ha^{-1} y^{-1} in the three D3+ plots with burrowing earthworms. This meant a mean difference of 24 and 55 kg N ha^{-1} y^{-1} in D2 and D3+ in relation to D0. The decline in D2 at Hasslöv was very similar to that reported from the Höglwald study in Germany (see above) that showed a decline between 1984 and 1991 of 20 kg N ha^{-1} y^{-1} after liming in comparison with the control plot (Kreutzer 1995). At 40 cm depth, the mean annual efflux of inorganic + organic N was 23 kg ha^{-1} y^{-1} higher than in the control plot. A follow-up study by Huber and others (2006a, b) showed mean leaching of NO_3–N of 20 kg ha^{-1} y^{-1}.

The study of four 37–42-year-old liming experiments in SW Sweden in N-rich ecosystems (C/N ratios 20–26 in the humus layer, see above) showed great variation in N-pool decline after liming (2–34 kg N ha^{-1} y^{-1}) and a mean loss of 15 kg N ha^{-1} y^{-1} (Persson and others 1995).

At Farabol with N-poorer conditions than at Hasslöv, there were no significant differences between control and limed treatments in soil N pools (Bergholm and others 2015).

Inorganic N Pool

At Hasslöv, the amount of KCl-extractable inorganic N (NH_4–N + NO_3–N) in D0 plots showed an increase from about 8 to 30 kg N ha^{-1} during the experimental period. The N demand of young trees is believed to be greatest during canopy buildup and decreases once canopy closure is reached (Miller and Miller 1988; Wilson and Emmett 1999; Hansen and others 2007). In 1984, at the start of the experiment, the trees at Hasslöv were 40 years old and had passed the most intense period of canopy closure. The N demand of the maturing trees probably decreased after this period resulting in higher availability of N to the soil organisms and more mineralized N to be found in the soil. The D2 and D3 treatments also resulted in an increase (from 8 to 20–25 kg N ha^{-1}). The smaller (but statistically insignificant) increase in inorganic N in D3 in relation to the D0 treatment can probably be explained by (1) reduced cation exchange sites as a consequence of less organic matter and (2) more NO_3^- production followed by NO_3^- leaching and denitrification. NO_3^- is vulnerable to leaching, and at Hasslöv the content of NO_3^- was always higher in the D3 than in the other treatments.

The marked increase in extractable NO_3-N in the D0 treatment between 1990 and 2009, which is when the trees were between 46 and 65 years old, indicates increased probability for NO_3^- leaching, though leaching was not determined after 1993.

Net N Mineralization and Nitrification

The mineralization studies indicated high N turnover. Net N mineralization rate (per g of C and day) was higher in the L layer than in any of the other soil layers. This can be explained by unusually high litter N concentration (18 mg g^{-1} dry wt), which indicates that the shed needles probably contained high amounts of arginine (Näsholm 1994) as an important source of N during decomposition and mineralization. Viewed over the entire experimental period and including all soil layers to a depth of 30 cm, net N mineralization doubled over time from 120–140 kg N ha^{-1} y^{-1} in 1984 to 200–280 kg N ha^{-1} in 2016 (Figs. 7C and 8). The net N mineralization estimates seemed to be balanced by (1) root uptake (allocated to biomass accumulation, needle/twig litterfall, fine-root turnover and mycorrhizal turnover) and (2) leaching/denitrification (data not shown here).

Enumeration of ammonia-oxidizing bacteria (AOB) with the MPN method in the FH layer at Hasslöv showed that the number of AOB (genus *Nitrosospira*) was below the detection level in D0 plots (pH 4.2) in 1990. In the limed D2 and D3 plots, increasing numbers of AOB (range from 10^3 to 10^5 bacteria g^{-1} dry wt) correlated well with increasing pH (Klemedtsson and others 1999).

The results of the mineralization and nitrification studies in the laboratory supported the results of the field soil samples. After 1990, also the D0 plots showed increased nitrification potential despite low pH(H_2O) in the FH and 0–5 cm mineral soil layers (pH 3.9 and 3.7, respectively) in 2009 and 2016. These results indicate the presence of acid-tolerant nitrifiers, which may have benefitted from the higher abundance of NH_4–N during the aging of the forest stand. Such nitrifiers can be ammonia-oxidizing bacteria (AOB) adapted to acidic conditions (De Boer and Kowalchuk 2001) or ammonia-oxidizing archaea (AOA) adapted to low substrate (ammonia) concentrations (Leininger and others 2006; Prosser and Nicol 2012; Hu and others 2014; Li and others 2018).

An increase in net N mineralization and net nitrification with stand age has also been observed in other forest systems, as, for example, in soils from 31–33- to 80–100-year-old hardwood forests in Indiana, USA (Idol and others 2003). In an oak–hornbeam chronosequence in central France, Trap and others (2009) also found high net N mineralization in the oldest stand (99 years old), but here net nitrification decreased with age.

Studies by Persson and Wirén (1995) at ten Norway spruce forest sites (age 28–71 years) in southern Sweden and eastern Denmark showed that net N mineralization to 50 cm soil depth ranged from 35 to 105 kg N ha^{-1} y^{-1}. Throughfall N deposition ranged from 10 to 31 kg N ha^{-1} y^{-1}. Almost no net nitrification could be detected in the FH layer where pH (H_2O) was lower than 4.0. NO_3^- was formed in FH layers with pH values of 4.0–4.5, but the nitrification was never complete. In contrast, net nitrification was almost complete (no NH_4^+ remaining after incubation) at a depth of 10–50 cm, where pH was 4.1–4.5. Almost no net nitrification was detected in the FH layer in the young stands (28–40 years) and stands with the lowest N deposition. High net nitrification in the FH layer occurred in some of the 45–70-year-old stands, but not all. Other factors than age, for example, N deposition, tree vitality and type of nitrifiers, probably played a role. In conclusion, net nitrification in the acidic FH layer at Hasslöv (pH 3.8–4.0) during 2007–2016 was higher than at the other sites in the same region.

Earthworms and Soil N Status

Acid forest soils usually have low populations of earthworms, and liming can increase their populations by increasing soil pH and calcium concentration. Earthworms also need nutritious food for population growth. Steinwandter and others (2019) tested whether litter of different qualities would affect the life history traits of the detritivorous earthworm *L. rubellus*. Low-quality (dwarf-shrub) litter resulted in delayed development, low biomass and low cocoon production in contrast to high-quality (grass/herb) litter. Hendriksen (1990) found that the abundance of *Lumbricus* earthworms was negatively correlated with the C/N ratio of the food ingested. Earthworms and enchytraeids contain about 10% N in their body mass (Persson 1983; Schröter and others 2003; T. Persson, pers. comm.) and need to consume large amounts of litter and soil to fulfill their nutritional requirements to grow and maintain the body-mass N concentration. Consequently, litter/soil with high N concentration (for example, C/N 20–25) should be more favorable to body and population growth than litter/soil with low N (for example, C/N 30–40) concentration. A positive earthworm response (high reproduction and greater growth rate) that increases microbial SOM decomposition thus seems to be dependent on both (high) pH and (high) soil N status.

CONCLUSIONS

The result at Hasslöv supports findings of other studies showing that afforestation with Norway spruce on former *Calluna* heathlands in areas with high N deposition will increase soil C and N pools. Liming reduced the expected increase in soil C and N pools in the 3.45 Mg ha^{-1} treatment and decreased C and N pools in the 8.75 Mg ha^{-1} treatment, particularly in the organic FH layer. When comparing among liming studies in general, experiments at high N sites (as at Hasslöv) show substantial C and N losses compared to liming of low N sites. The decline in the SOC pool after liming in our study coincided with increases in heterotrophic soil respiration (R_H) rate. Increased R_H rate after liming could be explained by both a direct pH effect (increased SOM solubility and reduced Al levels) and an indirect (positive) effect of pH on earthworms, which stimulate litter frag-

mentation and microbial activity. In addition to high pH, earthworms also need N-rich food to reach high abundance and biomass. This can explain why liming of N-rich soils decreases C and N pools fueled by high earthworm activity in the organic LFH layer, whereas liming of N-poor soils with few earthworms shows no change in soil C and N. The increase in extractable inorganic N and net N mineralization over time was interpreted as an effect of decreasing tree demand for N after canopy closure. In the first 6 years of the study, extractable NO_3^- and net nitrification increased much faster in the highest lime treatment than in the other treatments, indicating that increased pH promoted growth of acid-sensitive ammonia-oxidizing bacteria. During the following period, NO_3^- levels became high also in the control treatment indicating activity of acid-tolerant nitrifiers. To fulfill the aim to reduce CO_2 export to the atmosphere and NO_3^- leaching to the groundwater, we conclude that liming of N-rich soils (especially in high doses) should be avoided.

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