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Long-term effects of liming on soil physico-chemical properties and micro-arthropod communities in Scotch pine forest

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Abstract

We tested the long-term effects of liming on soil micro-arthropods in a stand of Scotch pine on former drift sand in the Netherlands. To counteract the effects of acidification, liming was applied in increasing quantities from 0 (control), 3, 6, 9 and 18 ton ha⁻¹ on experimental plots over the course of 1985 and 1986. Soil samples for chemical analyses and those for extraction of soil micro-arthropods were taken in October 2017, 32 years after application. Liming did restore the buffer capacity of the soil and did increase pH and increased plant biomass of the understory. Liming, however, also created P limitation, due to Al precipitation and an excess of free Ca to bind on. The consequence of the observed P limitation was a significant decrease in herbivorous and herbofungivorous micro-arthropods, whereas fungivorous and opportunistic herbofungivores were unaffected. P availability in acidified soils had become limited, due to higher N input that also caused acidification and due to remediation with added buffer material. Decomposition of organic matter was accelerated and increased N release to the system. The forest ecosystem had become P limited, where it originally was N limited or N and P co-limited.

Keywords Phosphate availability · Aluminium · Calcium · Soil fauna · Decomposition

Introduction

To counteract the effects of acidification due to atmospheric deposition of nitrogen and sulphur compounds from traffic, industrial and agricultural emissions, which cause severe

ecological problems on a global scale (Vitousek et al. 1997; Galloway et al. 2008; Bobbink et al. 2010; Sutton et al. 2014), liming of forest soils has often been applied both in Europe (e.g. Huettl 1989; Boxman et al. 1994; Boxman and Roelofs 2006; Court et al. 2018) and in North America (e.g. Moore et al. 2012). Various effects of forest soil liming experiments on soil biota have been investigated ever since: the changed role of mycorrhizae, where liming in general decreased the relative proportion of smooth mycorrhizae in favour of hairy types (Bakker et al. 2000) and acidophilic species are replaced by more generalist ones (Rineau and Garbaye 2009). Changes in faunal groups have been evaluated by Deleporte and Tillier (1999), who report an increase in Lumbricid worm numbers in response to liming (1.5 ton ha⁻¹) after 22 years, but did not find any difference in numbers of oribatid mites and springtails. Chagnon et al. (2001) showed a decrease of numbers of epigeic collembolan species in the short term (2 years) after liming with 2 and 20 ton ha⁻¹ carbonated lime and an increase in numbers of some endogaeic species. Geisen and Kampichler (2004) did not find any clear signal in collembolan communities after forest liming in their evaluation of the effects after 5 subsequent years of 3, 6 and 9 ton ha⁻¹ applied lime. Hågvar and Amundsen (1981) report on the short-term effect of liming with 3 ton ha⁻¹ of burnt lime

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(CaO) and show oribatids to decrease in general and a number of species specifically, while numbers of a uropodid mite species increase with liming.

Among these varying results on soil fauna, only Chagnon et al. (2001) and Geisen and Kampichler (2004) provided extensive soil chemical data to possibly come to an explanation. Kreuzer (1995) reported in detail on the soil chemical processes following liming: increase of buffer capacity, which is one of the intended goals of liming, but also an increase of available N, because of stimulated decomposition. The latter could be seen as a setback, while increased N load through deposition is one of the problems to solve. Haynes and Swift (1988) came to the same conclusion and, moreover, paid also attention to P availability in acid soils which have a high phosphate-fixing capacity. The low phosphate availability may have affected soil fauna composition and activity. The P concentration in the soil explained a 72% positive relation of shifts in mass-abundance of soil animals (Mulder 2010). Vogels et al. (2017) reported a P limitation in detritivorous insects on heathlands after continuous N deposition, resulting in acidification and grass encroachment, causing serious nutrient imbalances. Attempts to restore the heathland by sod-cutting even amplified P limitation as with the organic matter almost all P was removed, while N deposition continued, resulting in increasing N:P ratios on the longer term. In a factorial experiment with addition of lime, phosphate or a combination of both on sod-cut, formerly grass-encroached, dry heathland, Siepel et al. (2018) showed a vast increase of herbivore numbers after P addition on a spodic dystrudept (see Soil Survey Staff 1999), suggesting that these animals indeed are P limited on heathlands on these mineral-poor sandy soils. Especially numbers of herbivorous grazers were stimulated (10 times more in P added plots compared with control plots), but less in combination with lime (only 4 times more in P and lime added plots than in control plots). Effects on fungivore numbers were mixed: browsers (feeding on fungal content) decreased, while the small number of fungivorous grazers increased. The effects of liming in this experiment have been discussed. Added P may be bound to the added lime and becomes less available (Haynes 1982). As this P addition experiment showed only the reaction 3 years after application, added phosphate may still be stored in plant material and may become available after a longer timespan again. So, we were looking for a situation where P availability may be influenced by management actions on mineral-poor sandy soils on a longer time-scale. These mineral-poor sandy soils in the Netherlands have low amounts of plant available P (e.g. Boxman et al. 1994 measured 0.035–0.141 mmol P kg⁻¹ in the topsoil of a humic haplorthod), where P may become limited rather quickly. In comparison with the data on P of Chagnon et al. (2001) and Geisen and Kampichler (2004), P availability in these Dutch soils was 10–30 times lower.

In the current paper, we report for the first time the combination of a complete soil biochemical analysis with microarthropods identified to the species level, which makes it possible to group the species in feeding guilds and analyse the reaction patterns of these feeding guilds to changed soil chemical properties on the long term after various levels of liming. We have revisited an old but very well-documented experiment with liming of forest soils (Dilz et al. 1988), where carbonated lime has been added in quantities of 3, 6, 9 and 18 ton ha⁻¹. Assumed that these amounts of carbonated lime indeed decreased P availability, despite an increased decomposition of organic matter (Haynes 1982; Kreuzer 1995), we expect to find decreasing numbers of herbivorous microarthropods with increasing lime quantities. We expect no change in numbers of fungivorous micro-arthropods, as fungi have a much lower N:P ratio compared with plants (15 for fungi and resp. 23 and 53 for herbaceous and woody plants; Reiners 1986). Siepel and De Ruiter-Dijkman (1993) distinguished also two mixed feeding strategies on plant material and fungi: herbofungivorous grazers and opportunistic herbofungivores. As the latter were defined by trehalase activity (trehalose is a storage sugar found in temperate regions predominantly in fungi), this group also feeds on active fungi and is expected to show no relation to liming either. Decomposition is not thought to be influenced negatively, as fungivores that stimulate fungal growth (see Hanlon and Anderson 1979; Siepel and Maaskamp 1994) will not be influenced by the supposed decrease in P availability. On the contrary, we expect a better decomposition with liming in line with the results of Haynes and Swift (1988), Kreuzer (1995) and Court et al. (2018). So, in summary, we expect a decreasing role of micro-arthropod herbivores, but not fungivores, with the increased lime application. As fungivores have a much larger influence on decomposition than herbivores (Siepel and Maaskamp 1994), we expect an increase in the decomposition rate because of the elevated pH.

Methods and material

Study site

The study site is a 55 years old forest stand of Scotch pine on a typic quartzipsamment (Soil Survey Staff 1999), afforested former drift sand, near the city of Harderwijk, the Netherlands (52.315° N, 5.664° E). The forest stand is on a flat plane at 18 m a.s.l. In 1985, a series of experiments were set up, varying from application of several types of manure (of calves and ducks), a factorial experiment with additions of P, Ca, Mg and K, and a range of added carbonated lime in quantities of 3, 6, 9 and 18 ton ha⁻¹ and an untreated control (see Fig. A1). All experimental plots are triplicated and 22 × 25 = 550 m² in

size. At the start of the experiment, $\text{pH}_{[\text{CaCl}]}$ was around 4.1–4.2 in the mineral soil (mixed top 25 cm) (Dilz et al. 1988). Carbonated lime for agricultural use, powdered, having around 2% Mg, was added in quantities of 3 ton (autumn 1985), 6, 9 ton (in equal amounts in autumn 1985 and 1986) and 18 ton (in equal amounts in autumn 1985, 1986 and spring 1987), to prevent vegetation damage. Thinning of the canopy was done in 2001 as regular forest management measure; no other management activities have been conducted ever since. Understory vegetation differed highly among plots as a result of liming: in the control plots wavy-hair grass (*Deschampsia flexuosa*), and alder buckthorn (*Rhamnus frangula*) are frequent and even some heather (*Calluna vulgaris*) can be found, while with increasing amounts of lime, species like enchanter's-nightshade (*Circaea lutetiana*), wood avens (*Geum urbanum*), bittersweet (*Solanum dulcamare*) and bramble (*Rubus fruticosus* agg.) show up or become more frequent. Plant biomass of especially bramble is increased ever since the start of the liming.

Soil chemical analysis

On 16 October 2017, samples were taken for soil chemical analyses. Each sample (4 subsamples bulked and mixed, core 5 cm) was taken from the top 25 cm of the mineral soil. We determined organic matter content by weight loss-on-ignition. Soil pH, NO_3^- , NH_4^+ and exchangeable ion concentration were determined by mixing fresh soil (17.5 g) with 50 mL 0.2 M NaCl solution. The pH of the solution was measured immediately using a combined pH electrode (radiometer and a TIM840 pH meter). Plant available P was determined by bicarbonate extraction (3 g of dry soil shaken with 100 mL of 0.5 M NaHCO_3) (Olsen et al. 1954), and cation exchange capacity and base saturation by mixing an amount of dry soil equivalent of 5 g fresh soil in 200 mL 0.2 M SrCl (Liu et al. 2001). Soil extracts were collected under vacuum conditions with Teflon pore water samplers (rhizon, Eijkelkamp Agrisearch Equipment, The Netherlands). Total soil elemental concentrations were determined. Two hundred milligrams of soil, homogenised and dried, was digested for 17 min with 4 mL 65% HNO_3 and 1 mL 30% H_2O_2 (Milestone Ethos D – Microwave Labstone) (Jin et al. 1999). Samples for Autoanalyzer Analyses were collected and stored at -18°C until further analysis; ICP-samples were collected and stored at 4°C . The total concentration of Ca, Mg, Al, Fe, Mn, P, S, Si and Zn were measured with an Inductively coupled plasma spectrophotometer (ICP-OES, ICAP 6300 ARCOS MV, Spectro). NO_3^- , NH_4^+ and PO_4^{3-} concentrations were determined colorimetrically with a Seal auto-analyser III with using salicylate, hydrazin sulphate and ammoniummolybdate/

ascorbic acid reagent, respectively. Cl^- was determined colorimetrically with a Bran+Luebbe auto-analyser III using mercuritiocyanide. Na^+ and K^+ were determined with a Technicon Flame Photometer IV Control (Technicon Cooperation).

Sampling of micro-arthropods

On 17 October 2017, in each of the limed plots and the control plots four soil cores (\varnothing 5 cm) of 5 cm depth: 100 cm^3 content plus litter were sampled. The cores were taken in the middle of the plots, 1 m apart of each other. The cores were extracted on a Tullgren funnel for 7 days. During that period, the temperature was increased from 35 to 45 $^\circ\text{C}$. Ethanol 70% was used as conservation fluid and micro-arthropods obtained were put into lactic acid 40% for clarification and identification (Siepel and van de Bund 1988). Nomenclature and identification for the main groups is according to Weigmann (2006) for Oribatida, Karg (1993) for Gamasina, Karg (1989) for Uropodina and Bretfeld (1999), Potapow (2001), Dunger and Schlitt (2011) and Jordana (2012) for Collembola. Species were grouped to feeding guilds after Siepel and De Ruiter-Dijkman (1993) in herbivorous grazers, herbivorous browsers, fungivorous grazers, fungivorous browsers (grazers feed on the cell walls as well and have respectively cellulase and chitinase activities, browsers on the contents only), opportunistic herbofungivores (including plant cell walls and fungal cell contents, i.e., cellulase and trehalase activity), herbofungivorous grazer (plant and fungal cell walls) and predators (general or specialised on nematodes or arthropods).

Statistical analysis

Soil properties (like percentage soil organic matter, moisture content, and concentration of various elements) were regressed (using *lm* in R) against the tons of added lime (i.e. treatment levels 0, 3, 6, 9 and 18) and separately against the log of treatment levels (after adding 1). For each soil property or feeding guild-specific micro-arthropod abundance variable, we compared models with logged or untransformed liming levels and selected the better fitting model based on the sum of squared residuals on the untransformed scales. The regression models of micro-arthropod abundances included plot as a random variable because multiple samples were taken per plot (i.e. mixed-effect models, *lmer* in R). We also constructed models with liming as a factor to see which treatments differed from the control treatment. The canonical correspondence analysis (using *cca* of the *vegan* package in R) was performed with soil chemical properties and micro-arthropod abundances per feeding guild. Additional multivariate analyses can be found in the online appendix.

Results

Soil chemistry and morphology

Soil and especially litter layer morphology changed in the sequence of increased liming after 32 years. With an increasing amount of lime used, the litter F-horizon thickness decreased until virtually absent at 18 ton ha⁻¹ (Figs. 1 and A2). Soil organic matter content in the top 25 cm mineral soil was around 3% in all treatments, while pH(NaCl) increased with liming from 3.5 (control) to 4.9 (18 ton ha⁻¹) (Fig. 2). Base saturation increased with the amount of liming from < 10% (control) to around 90% (18 ton ha⁻¹), which is predominantly exchangeable Ca²⁺ and to a lesser extent Mg²⁺ (Fig. 3). Free Al³⁺ decreased with increasing liming and the Al³⁺/Ca²⁺ ratio even more so (Fig. 4). Nitrogen, both as NO₃⁻ as well as NH₄⁺ forms, increased with liming, but plant available P (P_{Olsen}) decreased (Fig. 5). Detailed soil chemistry data can be found in Siepel et al. (2019).

Soil micro-arthropods

In total, 129 micro-arthropod species have been identified (in 23,487 individuals). Mites formed the majority (87.5%), followed by springtails (11.7%); of the smaller groups of Protura, Diplura, Symphya and Pseudoscorpionida, one species per group was found (0.8% of total micro-arthropods). The abundance of micro-arthropods was highest in the control

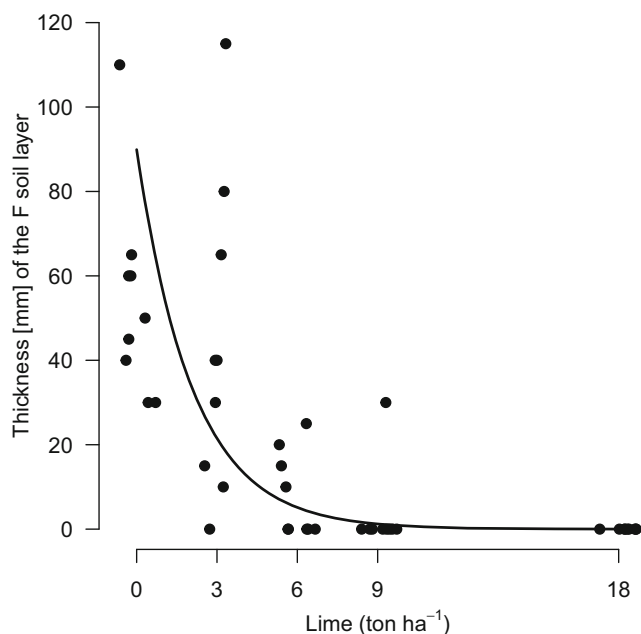


Fig. 1 Thickness (mm) of the F soil layer. Three samples were taken in each of the 15 plots. The effect of liming is highly significant ($z = -5.37$, $p < 0.001$) in the fitted generalized linear mixed-effect model (log link and plot as a random variable). Points are spread out horizontally for visual clarity

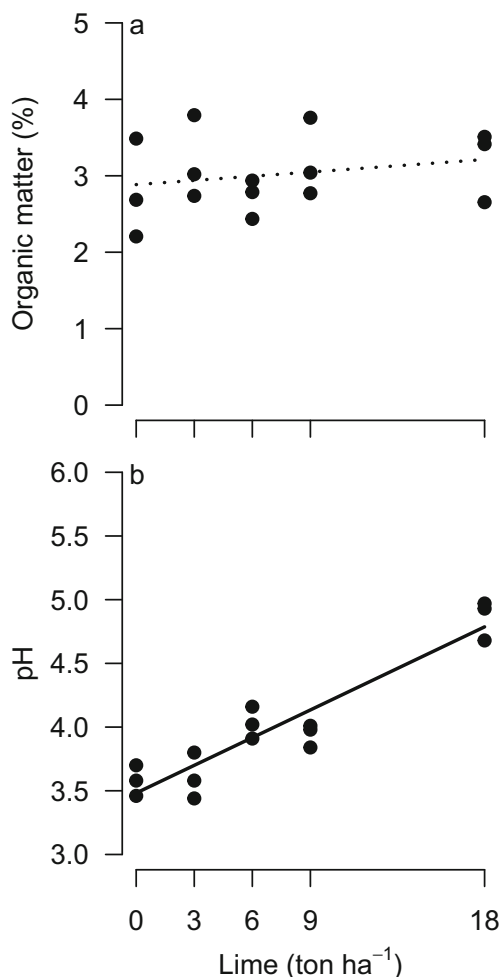


Fig. 2 **a** Average soil organic matter (%) and **b** pH in each of the 15 plots. The pH significantly ($t = 9.76$, $p < 0.001$) increased with liming. Organic matter showed no relationship ($t = 0.906$, $p = 0.381$) with the amount of applied lime. Also, none of the treatment levels differed significantly from the controls for organic matter. For pH, the 6, 9 and 18 ton ha⁻¹ treatments differed significantly from the controls

plots ($255,250 \pm 37,811$ ind m⁻²) and lowest in the plots with 18-ton lime ($161,334 \pm 5779$ ind m⁻²). Species richness, diversity and evenness did not differ significantly along the liming gradient (Figs. A3–A5). The PCA (Fig. A6) showed the soil chemical variables of the sampled plots. The first principal axis explained 63.0% of the total variance and covers primarily the effects of added lime (increased pH, Ca²⁺, Mg²⁺ and NO₃⁻ and decreased Al³⁺ and P). The second principal axis explained another 19.8% and covers soil organic matter and NH₄⁺). In the CCA performed at the level of feeding guilds (Fig. 6), the first axis represented 49.5% of the total variance, the second axis 24.6%. Soil chemistry explained 55.9% of the variance, with P and Al being strongly associated with the first axis, N to a lesser extent with the second axis, and Ca, pH and Mg with both axes. Numbers of herbivorous micro-arthropods are presented in Fig. 7a. Decrease of numbers with increasing quantities of lime was highly significant

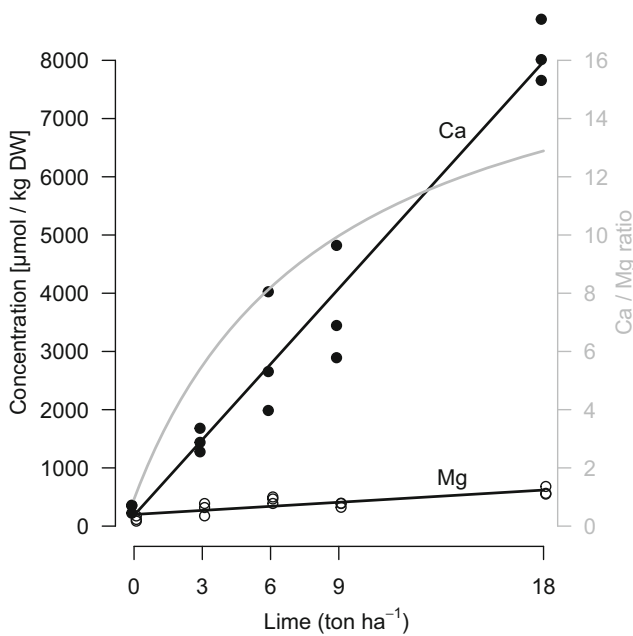


Fig. 3 Calcium and Mg concentrations in each of the 15 plots. Both significantly ($t > 3.85$, $p < 0.001$) increased with liming (black lines), while the Ca/Mg ratio (grey line) of the modeled concentrations went up from 0.93 in the control plots to 12.89 at 18 ton lime per hectare. When liming was treated as a factor, all liming treatments differed significantly from the controls, except for the 3 ton ha^{-1} addition in the Ca model

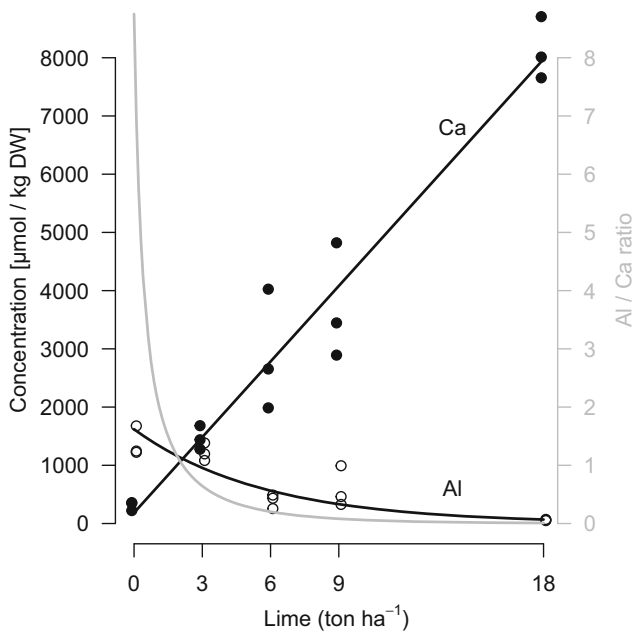


Fig. 4 Calcium and Al concentrations in each of the 15 plots. While Ca significantly increased with liming (black line, $t = 16.16$, $t < 0.001$), Al decreased (black line, $t = -9.63$, $p < 0.001$). The Al/Ca ratio (grey line) of the modeled concentrations decreased from 8.75 in the control plots to 0.0086 at 18 ton lime ha^{-1} . When liming was included as a factor, the 6, 9 and 18 ton lime ha^{-1} treatments differed significantly from the controls, in both the Ca and the Al model

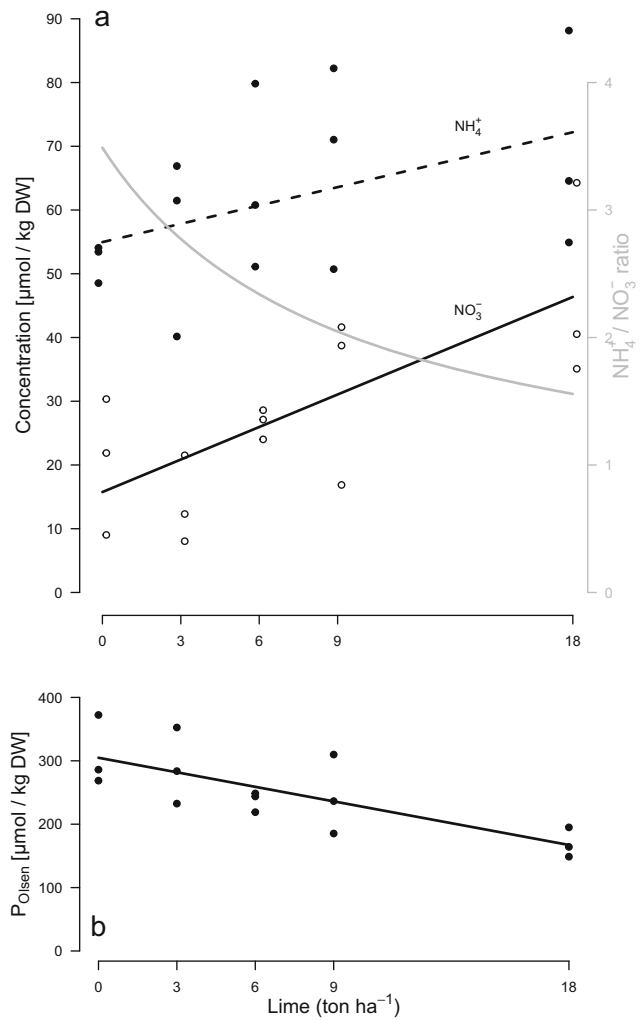


Fig. 5 **a** Ammonium and NO_3^- and **b** P_{Olsen} concentrations in each of the 15 plots. Nitrate increased significantly with liming (black, uninterrupted line, $t = 3.92$, $p = 0.0018$, while $t = 1.81$ and $p = 0.094$ for NH_4^+). The $\text{NH}_4^+/\text{NO}_3^-$ ratio (grey line) of the modeled concentrations decreased from 3.49 in the control plots to 1.56 at 18 ton lime ha^{-1} . P_{Olsen} decreased significantly ($t = -4.19$, $p = 0.0011$) with added lime. When liming was included as a factor in the models, only the 18 ton lime ha^{-1} treatment (for NO_3^- and P_{Olsen}) differed significantly from the controls in the case of NO_3^- and P_{Olsen} , while none of the treatment effects were significant for NH_4^+

($t = -4.5$). Dominant species here are the oribatid mites *Atopacarus striculus* (C.L. Koch, 1836), *Steganacarus magnus* (Nicolet, 1855) and *Platynothus peltifer* (C.L. Koch, 1839), the collembolan *Lepidocyrtus lignorum* (Fabricius, 1793) and the symphylian *Symphylellopsis subnuda* (Hansen, 1903). Fungivores, however, did not decrease with increasing lime quantities (Fig. 7b); $t = 0.7$ (no relation with lime quantities). All dominant species here were oribatid mites: the fungivorous grazers *Eniochthonius minutissimus* (Berlese, 1904), *Oppiella nova* (Oudemans, 1902) and *Puncctoribates punctum* (C.L. Koch, 1839) and the fungivorous browsers *Suctobelbella acutidens* (Forsslund, 1941) and *S. subcornigera* (Forsslund, 1941). In

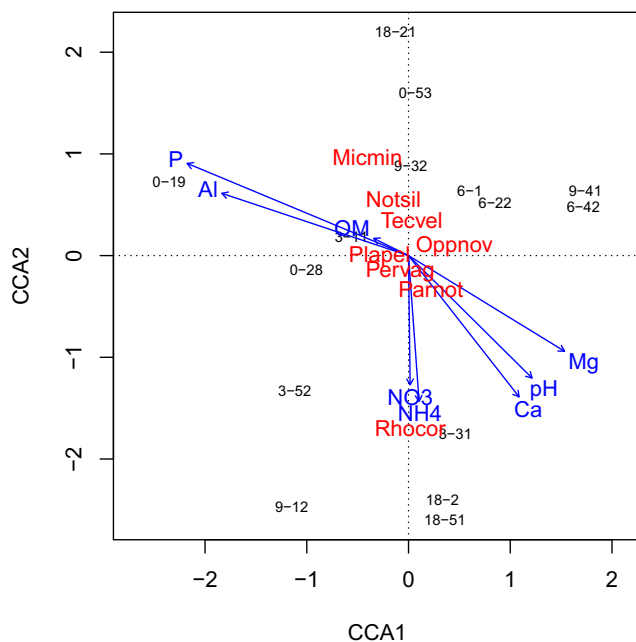


Fig. 6 Canonical correspondence analysis of the micro-arthropod communities (organised by feeding guilds) and soil chemical properties. Species abundances were averaged per plot and summed per feeding guild prior to analysis. Numbers indicate liming treatment and plot number (see Fig. A1), respectively. The first CCA axis represents 49.5% of the variation, the second axis 24.6%. The soil chemical properties explained 55.9% of the variance. Afterwards, eight abundant species were added to the plot: the herbivorous grazer *Platynothrus peltifer* (Plapel), the fungivorous grazers *Oppiella nova* (Oppnov), the fungivorous browser *Parisotoma notabilis* (Parnot), the herbofungivorous grazers *Nothrus silvestris* (Notsil) and *Microtritia minima* (Micmin), the opportunistic herbivorous grazer *Tectocephus velatus* (Tecvel) and the general predators *Rhodacarus coronatus* (Rhocor) and *Pergamasus vagabundus* (Pervag)

Fig. 7c and d, results of the mixed feeding guilds are presented, where the herbofungivorous grazers show a significant negative relationship with liming (Fig. 7c, $t = -4.2$, dominant species: the oribatids *Nothrus silvestris* (Nicolet, 1855) and *Rhyssotritia duplicata* (Grandjean, 1953)), whereas the opportunistic herbofungivores show no relationship with liming (Fig. 7d, $t = -1.4$, dominant species: the oribatids *Adoristes ovatus* (C.L. Koch, 1839) and *Tectocephus velatus* (Michael, 1880)). Data on the abundance of all micro-arthropod species in this project can be found in Siepel et al. (2019).

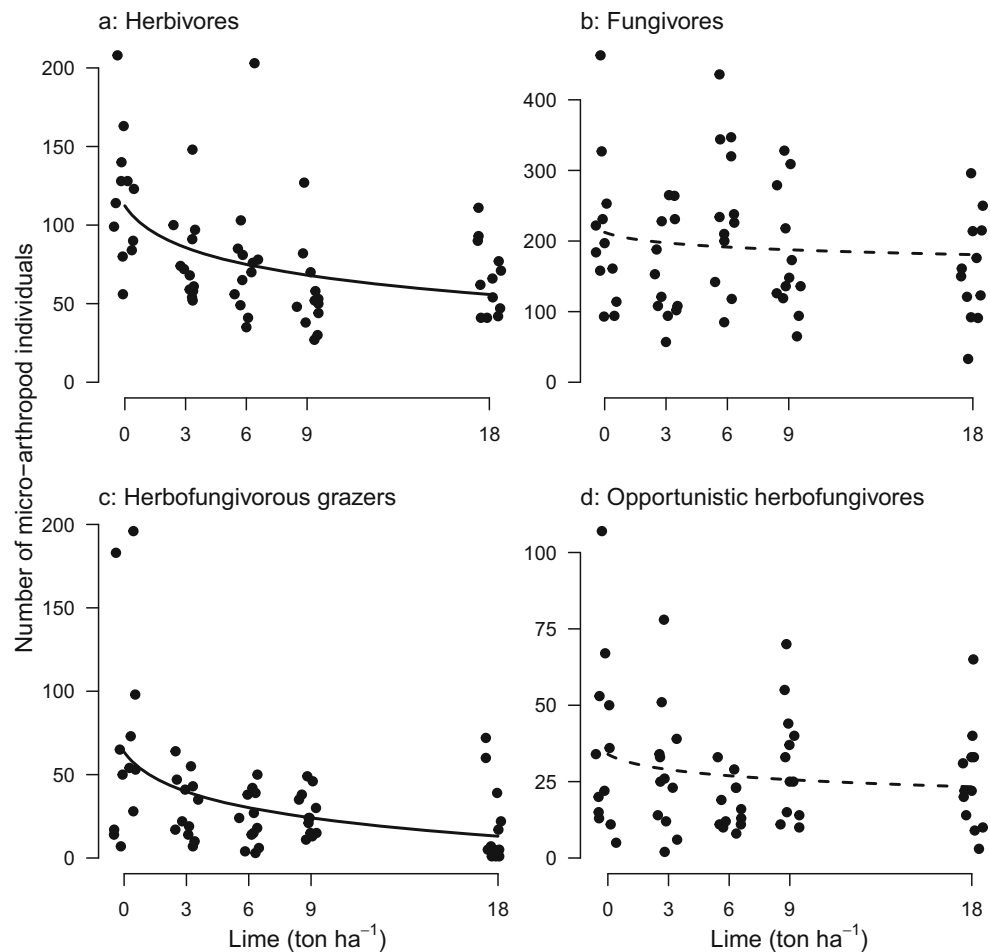
Discussion

Soil chemistry and morphology

Liming seems to meet its initial goal of increasing the pH and increasing the base saturation. With increasing pH Al precipitates, and thus the concentration of free Al^{3+} is decreasing. Along with an increase of free Ca^{2+} , the $\text{Al}^{3+}/\text{Ca}^{2+}$ ratio drops significantly and one may assume that the negative effects of

acidification have been largely alleviated. However, there is also a downside of this ecological restoration technique: the amount of free Ca^{2+} is inconceivably high for a Scotch pine stand on former drift sand (Boxman and Roelofs 2006 give values of around $100 \mu\text{M kg}^{-1}$, whereas our highest values are above $8000 \mu\text{M kg}^{-1}$, Fig. 3), which may lead to a competition in uptake with Mg^{2+} for plants and possible chlorosis (at 18 ton ha^{-1} Ca/Mg ratio > 14 in plot 21, compared with 1.9 in control plot 19). Another side effect is the increase of NO_3^- and NH_4^+ . As the recent acidification is caused by increased N deposition, an increase of freely available N is quite undesirable, as this enhances the eutrophication by N compounds. This increase has been reported before (Haynes and Swift 1988; Kreuzer 1995; Court et al. 2018) and is the result of increased decomposition of plant material: the inhibiting effect of acid remnants of incomplete decomposition is buffered by the dissolution of lime. The ratio $\text{NH}_4^+/\text{NO}_3^-$ is decreasing, which can be seen as positive, as more plants are able to take up NO_3^- than NH_4^+ (Van den Berg et al. 2005, De Graaf et al. 2009), which may increase understory plant diversity. Another negative effect of liming is apparently the decrease of plant available PO_4^{3-} . Soil phosphate chemistry is quite complicated and many results seem contradictory if pH, P concentration itself and free Ca^{2+} concentrations are not taken into account. The process, as we consider here, has been started by the acidification process, setting free Al^{3+} , (see control plots for concentration without buffering). Raising pH from this point may result in precipitation of Al^{3+} with PO_4^{3-} , the new formation of amorphous Al-P bindings ($\text{Al}(\text{OH})_2\text{H}_2\text{PO}_4$; stable at $2.5 < \text{pH} < 8.5$), and the high concentration of free Ca^{2+} may lead to the formation of new hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH} \cdot 2\text{H}_2\text{O}$) (Haynes 1982). Also Aarnio et al. (2003) found a binding effect of liming as they state that the amount of soluble P was less in limed than in non-limed apatite-treated soils; these soils had comparable levels of PO_4^{3-} in an acid soil (mineral coarse podzolic sand, $\text{pH} = 3.6$, measured in water, $[\text{PO}_4^{3-}] = 0.16 \mu\text{mol kg}^{-1}$). At higher pH levels, no effect on phosphate availability is seen (Chagnon et al. 2001; $\text{pH} = 4.2$, measured in water; Geisen and Kampichler 2004, $\text{pH} = 4.2\text{--}6.2$, measured in 0.01 M CaCl_2 ; Court et al. 2018; $\text{pH} = 4.5$, measured in water). The pH measured in 0.01 M CaCl_2 is about 0.6 lower than measured in water (Blume et al. 2010). As McDowell et al. (2002) state: Al solubility plays a major role in determining P solubility and consequently P release to the soil solution. Moreover, the decrease of free Al^{3+} relieves its toxic effect on PO_4^{3-} uptake by plant roots (De Graaf et al. 1997). Also, the enhanced mineralisation of the organic layer may have released more P (Haynes 1982). However, as we see the strong development of understory vegetation (especially bramble) with increasing lime addition, the plant may have acquired and stored more P from the soil at higher levels of liming.

Fig. 7 Number of micro-arthropods in each of the samples. **a** Herbivores (both browsers and grazers). **b** Fungivorous (both browsers and grazers). **c** Herbofungivorous grazers. **d** Opportunistic herbofungivores. Solid lines indicate a significantly negative effect of liming in the fitted mixed-effect model (samples nested within plots, *t*-statistics of the four analysis are -4.461 , -0.728 , -4.188 and -1.402 , respectively). Dashed lines indicate non-significant effects of liming. Please note the differently scaled *y*-axes. When liming was included as a factor in the analyse, all treatments differed significantly from the controls in **a** and **c**, none in **b** and only 6 ton ha^{-1} in **d**



Increased decomposition, seen from increased NO_3^- and NH_4^+ concentrations in soil with increasing amounts of lime supplied, can also be seen morphologically: the litter layer is almost completely vanished in the 18 ton ha^{-1} (Fig. A2) and gradually decreases with increasing amounts of lime (Fig. 1). Where in the control site a tiny spodic horizon develops in the top soil (just below the litter layer, see Fig. A2), in the 18 ton ha^{-1} organic matter is completely mixed with the top soil, creating a small A1 horizon. Especially macrofauna is active in the limed sites, feeding on the combination of fungi and organic matter (e.g. larvae of Mycetophilidae, Bibionidae and Tipulidae). Abundances of these larvae can temporarily be very high. In comparable situations 74 specimens of *Dilophus febrilis* (Linnaeus, 1758) were found per 10 g organic matter (Siepel 1990). Earthworms are not present in these young afforested drift sand soils.

Consequences for soil micro-arthropods

Differences in sampling depth between the standard protocol for micro-arthropods (Siepel and Van de Bund 1988) and that for chemical analyses (Dilz et al. 1988), respectively top 5 and 25 cm, are inconsequential. However, the majority of soil

micro-arthropods is found in the top 5 cm (around 95% in Ducarme et al. 2004) and probably even more in our soil type. Sampling the top 25 cm for soil chemistry may have diluted the data somewhat compared with when we would have measured just the top 5 cm. The presented patterns in soil chemistry can therefore be considered conservative. The decline of the F-layer could be seen as the cause of the lower number of micro-arthropods one could argue. However, all feeding guilds then should decrease in about the same way. We see a clear pattern of decrease in the herbivores but not in the fungivores; the latter is more or less constant throughout the range of liming. Moreover, two mixed feeding guilds in which both plant and the fungal matter is digested show comparable patterns. Opportunistic herbofungivores that digest plant cell walls and fungal cell contents (these have both a cellulase and trehalase activities; Siepel and De Ruiter-Dijkman 1993) act like fungivores and do not show a significant decrease with liming. These mites gather P from active living fungal material by definition. On the other hand, the herbofungivorous grazers that digest both plant cell walls and fungal cell walls appear to feed on dead fungal material next to plant organic matter (Siepel and De Ruiter-Dijkman 1993). Where living fungal hyphae contain a N:P ratio of 15 (Reiners 1986), dead

hyphae, or fungal cell walls alone have hardly any P left. P amounts of fungal cell walls are between 0.3 (*Aspergillus* sp.; Ruiz-Herrera 1967) and 0.7% (*Penicillium notatum*; Applegarth 1967). So feeding on dead fungal material in combination with plant organic matter yield as much phosphate as just herbivory and herbofungivorous grazers decline significantly with liming comparable with herbivores. It appears from the chemical data that the probable causal factor of the decline of the numbers of herbivorous (and herbofungivorous) micro-arthropods is in the decreasing availability of phosphate. Hågvar and Amundsen (1981) found a decrease of *Nothrus silvestris*, *Nanhermannia* sp. and *Sellnickochthonius zelawaiensis* all herbofungivorous grazers, and also a decrease of the opportunistic herbofungivore *Tectocepheus velatus* in a study on liming of heathland 3 years after application, although these numbers were low. Siepel et al. (2018) found a slight increase of opportunistic herbofungivores after the same short term after liming of a sod-cut heathland. Chagnon et al. (2001) found a decrease of both epigeic and endogaeic Collembola 2 years after liming, but they did not differentiate in herbivores or fungivores, just as Geisen and Kampichler (2004), who did not find any pattern in species composition 5–9 years after repeated liming. Collembola in our study follows the general pattern of the micro-arthropod total, but less strong compared with the mites.

In conclusion, long-term effects of liming confirm earlier results of increased pH and buffer capacity as aimed for, and also increased decomposition with higher amounts of available N. Availability of phosphate appears to decrease with liming in nutrient-poor sandy soils, which is enhanced by previous aluminium release through acidification. When this limitation cascades in the food web, one might expect effects on predatory species having less prey to feed on (Michalko et al. 2018). So, liming does not restore the original type of forest as it was before N deposition and acidification, but it pushes the system in a completely new direction, with a change in plant species composition in the understory and limitation of phosphate which might have consequences for animal life when applied on a larger scale. Alternatives for liming, such as finely ground igneous rocks (rock powder), might overcome a potential shock-effect, but probably has the same long-term effect on phosphate availability, as this is caused by the earlier release (acid deposition) and precipitation of aluminium (after buffering), and the excess of buffer material for P to bind on. To lift the P limitation with P addition, either as part of some rock powders, either as extra mineral addition, may give rise to unwanted situations as well, as long as the ongoing N deposition is much too high. In the near future, we must elucidate the complex interaction of rock powder addition (potentially in combination with mineral-bound P addition), on soil biochemical and biological processes. Especially the role of a diversity of ecto- and ericoid mycorrhizas must be explained as a supplier of organic bound P

in interaction with arbuscular mycorrhizas, when mineral-bound P is added as well. Also, a detailed P fractionation is necessary to explain the total contents and availability of P in different soil compartments.

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