Status and restoration of soil microarthropod communities in forests impacted by nitrogen deposition.



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Status and restoration of soil microarthropod communities in forests impacted by nitrogen deposition

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Chapter 1

Introduction

Nitrogen deposition

Elevated nitrogen (N) deposition is causing large problems for nature in most parts of Western Europe and specifically in the Netherlands (van Breemen and van Dijk 1988). Through the burning of fossil fuels and the use of fertilization there is high emission of ammonia (NH_3) and nitrate (NO_3 -) that is also deposited into the nature areas (Pearson and Steward 1993; Bobbink et al. 2010). During pre-industrial times, biologically reactive nitrogen was often the limiting factor in many forest soils, since N enters the soil ecosystem through atmospheric deposition, which contrasts with other elements, for example phosphorus (P), that is already in the soil and becomes available through the weathering of parent rock material. (Vitousek and Howarth 1993; Vitousek et al. 1997; Galloway et al. 2004). As a result, life has been selected to deal with low nitrogen availability. The greatly elevated levels of atmospheric nitrogen deposition has therefore caused large changes in the environment and has disrupted ecological systems through many pathways (Bobbink et al. 2010; Nijssen et al. 2017):

1) Excess nitrogen deposition causes high level of soil acidification due to increased rate of nitrification (van Breemen and van Dijk 1988). During nitrification, i.e. the conversion of ammonia to nitrate, H^+ molecules are released, which promotes soil acidification (Gundersen and Rasmussen 1990; Chen et al. 2023). This process has resulted in large reductions in soil pH in many Dutch forests and the subsequent leeching of important base ions (calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^+)) (Aber et al. 1995; Stevens et al. 2009). Soil acidification, additionally, leads to the release of aluminium (Al^{3+}) from the soil buffering system, which is toxic for plant roots (Rout et al. 2001; Rahman et al. 2018).
2) A higher nitrogen input additionally causes stoichiometric imbalances of C:N and N:P ratios in the soil (Cleland and Harpole 2010; Sardans et al. 2012). These soil imbalances cascade up to plants as they take up more N in comparison to P, leading to higher plant N:P ratios (Güsewell 2004).
3) Excess nitrogen deposition furthermore causes a reduction in plant species richness. Nitrogen deposition increases the productivity and supports the growth of fast-growing plants, causing asymmetrical competition and eventually a loss in plant species richness (Bobbink et al. 1998; Roem and Berendse 2000; Farrer and Sudding 2016).
4) Excess nitrogen deposition negatively affects invertebrates as well (Nijssen et al. 2017). Loss of plant species can lead to a loss of invertebrates dependent on that plant. But even if plants persist, their imbalanced stoichiometry can result in P-limitation for consumers. Because P is an element crucial for the growth and reproduction of organisms (Elser et al. 2000), impairment of growth and reproduction follows (Vogels et al. 2024). P-limitation has an allometric effect as well, since larger species will require more P to complete their development, and therefore leads to the disappearance of larger species (Elser et al. 1996; Mulder 2010).

1.2 Restoration of ecosystems affected by nitrogen deposition

Because of the long history of excess nitrogen deposition in the Netherlands, many restoration methods to mitigate the nitrogen deposition have been tried, but none have proved to be sufficient so far. Sod cutting (i.e. removing the topsoil layer) was often used in heathlands, and occasionally in forests as well (Boxman and Roeloefs 2006), to remove excess nitrogen from the soil, however it also removed phosphorus and other important elements (Vogels et al. 2020). This in combination of continued nitrogen deposition has further increased the stoichiometric imbalance (Siepel et al. 2018). Liming is used to mitigate the soil acidification, but while it is effective in increasing the pH level (Boxman and Roeloefs 2006; Court et al. 2018; Jansone et al. 2020), its fast dissolution accelerates the mineralisation of nitrogen and negatively impacted soil N:P ratio (Siepel et al. 2019; Vogels et al. 2021).

Silicate rock powders (SRP's) in recent times have emerged as an alternative for these previous restoration methods. SRP's are finely grounded rock materials that are usually by-products of mining. They contain carbonates and can therefore buffer the pH in the soil and SRP's additionally contain many minerals, such as Ca, K and Mg, that can replenish the soil with base cations that have been lost due to the soil acidification (Lewis et al. 2021; Taylor et al. 2021; van der Bauwhede et al. 2024a). The additional benefits of SRP's are that they weather more slowly than lime, which should prevent high mineralisation rates that elevate nitrogen levels and make it more beneficial for the long-term (Taylor et al. 2021; van der Bauwhede et al. 2024a; van der Bauwhede et al. 2025). The early results look promising in regard to mitigating some of the negative nitrogen deposition impacts (van der Bauwhede et al. 2024b; Findeisen et al. 2025), however its effect on the soil fauna community has not yet been tested thoroughly (Vicca et al. 2021).

1.3 Soil microarthropods

My thesis will focus on the status of an important soil fauna group: the soil microarthropod community. The microarthropod community consists of a variety of animal groups that range typically from 0.2 mm to 2 mm. Mites (Acari) and springtails (Collembola) are the most common groups, but Diplura, Pauropoda, Protura, Pseudoscorpionida and Symphyla are also part of the microarthropod group.

Soil microarthropods are a very abundant and species-rich part of the ecosystem (Zaitsev and Berg 2001). They furthermore have crucial roles in the nutrient cycling and litter decomposition processes in forest ecosystems (Seastedt 1984; Filser 2001). Fungivorous grazing species for example are important because they can breakdown the cell walls of fungi, which means that they can feed on dead fungal biomass and throughout this process important nutrients in the cell walls are released into the system that promote microbial activity (Hanlon and Anderson 1979; Siepel and Maaskamp 1994). Without animals feeding on fungi and recycling nutrients back into the food web, there is a risk that fungal biomass is not decomposed well and those nutrients are locked into the ecosystem.

How soil microarthropods affect litter decomposition is dependent on their diet and mode of feeding. There are a few methods to categorize feeding behaviour in soil microarthropods including carbohydrase activity (Siepel and de Ruiter-Dijkman 1993) or stable isotope analysis (Schneider et al. 2004). The advantage of using carbohydrase activity is that it not only separates herbivores from fungivores, but it can also distinguish browsers (species that feed only on cell content) and grazers (species that feed on both the cell wall and interior cell content) feeding strategies. Fungivorous browsers, in contrast to fungivorous grazers, negatively impact litter decomposition since they only feed on living cells, which are then left senescent (Siepel and Maaskamp 1994).

Similar to feeding strategy, dispersal ability differs between species as well. Some mites can disperse through phoresy or wind and therefore can disperse quickly (Siepel 1994). In contrast, most oribatid species, the mite group most abundant in stable soil ecosystems, rarely have phoresy or wind dispersal and therefore typically do not have a great dispersal ability (Ojala and Huhta 2001). Springtail species are generally expected to be less limited by dispersal than non-phoretic mite species since many springtail species have a furcula, which allows them to disperse passively through wind (Santorufo et al. 2021).

Many soil microarthropod species are generally vulnerable to disturbance in the soil and will prefer stable soil conditions with no environmental extremes (Siepel 1996a). The large soil disruption in their ecosystem caused by elevated nitrogen deposition could therefore be problematic. Abiotic stress from nitrogen deposition and a reduction in food availability and quality are all factors that could reduce soil microarthropod richness and, as I have established in the previous paragraph, many species are poor colonisers, which means that if species get lost in a location they might not be able to restore their population on their own (Siepel 1996b).

1.5 Old oak forests

Most of my research is located in the old oak forest habitat in the Veluwe. The old oak forest habitat (H9190 in the EU habitats directive) is dominated by the pedunculate oak (*Quercus robur*) and naturally occurs in sandy, acidic and nutrient poor areas (Evans 2006). For these reasons it is also particularly vulnerable to the effects of excess nitrogen deposition and this habitat has been observed to be in a degraded state: organic matter has accumulated and oak tree vitality has declined (Hommel et al. 2020).

To investigate the restoration of this habitat type, a large research project funded by Provincie Gelderland was established in 2020 to test the effectiveness of two silicate rock powder types (Eifelgold and Soilfeed) in 10 different old oak forest locations scattered around the Veluwe. The research presented in my thesis is part of this project and aimed to understand the role of the soil microarthropod community, which are expected to be important in combating the observed accumulation of soil organic matter.

1.6 Knowledge gaps and research questions

The main goal of my thesis is to investigate the role of soil microarthropods in the restoration of nitrogen-impacted forest ecosystems. I will do so with answering three key knowledge gaps: 1) the current status of the soil microarthropod community in Dutch forests on acidic, nutrient poor soils, 2) how the current soil microarthropod community in these forests function in their role in the nutrient cycling process and 3) to study the effectiveness of two restoration methods (silicate rock powder application and soil inoculation).

The first research aim will be discussed in Chapters 2 and 3. Chapter 2 is large dataset study comparing old forest soils and young forest soils to assess how dispersal limitation affects the species richness of the soil microarthropod community. Among the old forest soils are both old forests and old hedgerows in the original camp landscape. These old hedgerows are remnants of old forests, but adjacent to agricultural land and therefore have been subjected to constant P-influx, which allows for a study on how P availability affects the soil microarthropod community. Chapter 3 will evaluate the status of the aforementioned old oak forest habitat to explore how accumulative excess nitrogen deposition and the observed degraded abiotic state has affected the richness of the soil microarthropod community using the old forest soils from Chapter 2 as a baseline.

In Chapter 4 I use a large-scale litterbag experiment to explore the relationship of soil microarthropods and litter decomposition of *Quercus robur*. In 9 forests I have tracked the litter decomposition and soil microarthropods for 21 months and studied how the richness of fungivorous grazers affect the litter decomposition.

Chapter 5 evaluates the effectiveness of two restoration methods: silicate rock powder application and soil inoculation using a full factorial experimental set up in five old oak forest locations. Silicate rock powder is used to improve abiotic soil conditions necessary to support a diverse soil microarthropod community and soil inoculation has the aim to increase the species richness since many species will not be able to arrive in the restored sites.

Chapter 2

Soil age and soil phosphate content shape microarthropod communities of Dutch forest ecosystems

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Abstract

Soil microarthropods, particularly mites, are key contributors to the decomposition of plant litter and nutrient cycling in forest ecosystems, where they can reach high levels of biodiversity. However, their diversity can be impacted by soil disturbances such as soil compaction or organic matter removal and by phosphorus (P) limitation, driven by nitrogen (N) deposition.

This study compares microarthropod communities across forest locations differing in soil age and soil phosphate levels, using a trait-based approach focused on the species' feeding guild and body size. We compared 3 old forest soils, 19 young forest soils, and 10 old hedgerow soils. The old hedgerow soils resembled old forest soils in age, but have higher P-availability, allowing us to disentangle these effects. We hypothesized that older soils, with minimal disturbance, will support higher species richness due to their poor colonization abilities.

Our results show that older soils have indeed a higher microarthropods species richness than young soils. Greatest differences in the species richness and abundance were observed in (herbo)fungivorous grazers, a group of mites essential for decomposition. Consequently, young forest soils are expected to exhibit a less efficient decomposition process. The higher P-availability in old hedgerow soils likely explains their greater richness of herbivorous grazers and the higher abundance of larger mite species, by creating a more efficient trophic transfer that supports larger bodied consumers. Our findings indicate that differences in body size and feeding guild correspond to differences across forest soils in terms of age and P-availability of forest soils.

Introduction

Decomposition of plant litter is an important component in the nutrient cycling process in forests, which returns organic matter and nutrients to the soil and makes nutrients available again for plants roots and soil fungi (Attiwill and Adams 1993; Foster and Bhatti 2006; Krishna and Mohan 2017). Soil microarthropods play an important role in this nutrient turnover in forest soils (Verhoef and Brussaard 1990; Carrillo et al. 2011). Many species in this group are detritivores and their ability to fragment litter material stimulates decomposition processes (Seastedt 1984; McCary and Schmitz 2021) while their grazing on fungi stimulates microbial activity (Hanlon 1981; Siepel and Maaskamp 1994; Janoušková et al. 2018).

The community of soil microarthropods can reach high levels of species richness on a small local scale (Zaitsev and Berg 2001; Siepel et al. 2009). However, the mechanisms that give rise to such high levels of biodiversity are not fully understood. Large spatial heterogeneity in the soil habitat and niche differentiation among species, particularly with respect to food sources, are forwarded to explain such high levels of biodiversity (Anderson 1975a; Giller 1996; Nielsen et al. 2010).

One of the largest threats to the biodiversity of soil microarthropods is disturbance of the soil, which can consist of soil compaction or organic matter removal (Maraun et al. 2003; Battigelli et al. 2004; van Eekeren et al. 2022). Major consequences of such disturbance include lack of food availability due to reduction of fungal biomass (Battigelli et al. 2004), lack of soil pores which impedes microarthropod movement (Nielsen et al. 2008), increased rates of desiccation, and stronger thermal fluctuations, all of which can lead to a loss of species in the local soil habitat (Siepel 1996a). Forests are a habitat where soil conditions are stable and predictable for long time periods, representing key prerequisites for a diverse microarthropod soil community. Clearcutting of forests causes a clear disturbance leading to a shift in abundance of species (Moritz 1965; Siepel 1996a; Lóšková et al. 2013) and often a reduction in species richness (Marra and Edwards 1998). Many microarthropod species are additionally poor colonizers (Ojala and Huhta 2004; Lehmitz et al. 2011; Cordes et al. 2022), which means that if species are lost locally, it might take a very long time for them to recolonize areas, if at all. This is consistent with the positive relationship between Oribatida species richness and soil age reported by Zaitsev et al. (2012) for Dutch forests. The poor ability to colonise new sites in non-phoretic Acari differs from that of Collembola which can recolonize new systems more quickly due to the presence of furcula and their ability to passively disperse with wind (Santorufo et al. 2021). Following this, it can be expected that the microarthropod species richness, especially Acari, is higher in forests with the longest time period since the last disturbance event.

Next to their limitation in colonization, phosphate limitation forms another potential major threat for the microarthropod community. P-limitation became common in Dutch nature areas on poor sandy soils due to high nitrogen deposition. Nitrogen deposition causes initially more plant growth, thus organic matter, whereas the acidification effect of this deposition hampers the decomposition. This leads to more uptake of N in plants compared

to P, resulting in elevated N:P ratios (Bobbink et al. 2010; Sardans et al. 2012, Siepel et al. 2019). Soil acidification additionally creates the formation of insoluble aluminum phosphates (Stoddart 1908; Penn and Camberato 2019). Moreover, aluminum toxicity affecting the roots leads also to less P content (Rout et al. 2001). P-limitation as a consequence of nitrogen deposition has been linked to the decline in the invertebrate fauna species richness in Dutch nature areas (Vogels et al. 2013; Vogels et al. 2017). The effect of P-limitation on the microarthropod community is little studied, although Siepel et al. (2018) found that P-limitation is related to an impoverished microarthropod community with, in particular, lower densities of herbivorous mites and mites with larger body sizes. Herbivorous species are more strongly affected by P-limitation than fungivorous species due to plants being less stable in their N:P ratio than fungi (Elser et al. 2000) and, additionally, P-limitation has an allometric effect causing larger species to be more limited by it (Mulder 2010).

Species traits are a valuable tool to find generality in differences in diversity and can help predict which group of species is vulnerable to the disturbance factor (McGill et al. 2006; Verberk et al. 2013). Siepel (1996b) showed that the species loss in microarthropods as a result of disturbance is not random, but rather that interspecific differences in body size, feeding guild and reproduction mode were associated with differences between locations. A few methods for classifying microarthropod feeding behaviour have been created with for example the use of carbohydrase activity (Siepel and de Ruiter-Dijkman 1993) and additionally stable isotope ratios (Schneider et al., 2004). The method of Siepel and de Ruiter-Dijkman (1993) distinguishes the group of (herbo)fungivorous grazers (combination of the feeding guilds fungivorous grazer and herbo-fungivorous grazer) which has an important role in decomposition process of the soil because their chitinase activity gives them the ability to breakdown fungal cell walls. By grazing on fungal cell walls, N stored in the fungal cell walls is released back into the system, increasing N-cycling and the biological activity in the soils (Seastedt 1984; Siepel and Maaskamp 1994).

Our goal in this study is to examine the differences in species richness between old (undisturbed) forest soils and young forest soils (later reforested locations) in the Netherlands and additionally how P-limitation in forests affect this pattern. We use mite feeding guilds and mite body size to link functional groups to the differences in species richness. We analysed previously collected data from 32 locations across the Netherlands. This dataset includes 3 locations with old forest soils, 10 old hedgerow soils that are adjacent to agricultural lands and 19 locations with young forest soils. Locations with old soils have been forest (and forest remnants in the case of the hedgerows) since at least the Middle Ages (Ten Houte de Lange 1977), while young forests were afforested 100-140 years ago.

We hypothesize that old soils (old forest soils and old hedgerows) generally will have a higher species richness of Acari, but no significant difference in the species richness of Collembola due to their ability to disperse faster (Santorufo et al. 2021). Additionally, we predict that the lower mite species richness in young forests will be most pronounced in the (herbo)fungivorous grazer feeding guilds since many species in that group are very poor colonizers (Siepel 1996b). We further hypothesize that old hedgerow soils have a higher

amount of Olsen-P content due to fertilization of nearby agricultural land, which may result in a different species composition compared to old forest soils and young forest soils. Specifically, we expect old hedgerow soils to exhibit a higher species richness and abundance of herbivorous grazers compared to old and young forest soils (Elser et al. 2000) and a higher abundance of large mites in old hedgerow soils compared to old and young forest soils (Mulder 2010).

Methods

Site description

Data are used from 3 old forest soil stands sampled in 1994, 10 old hedgerow soils sampled in 1997 and 19 young forest soil locations sampled in 2000 (Fig. 2.1). A list of all locations is shown in Supplementary Table S2.1. Tthese datasets are, so far, unpublished and have been selected for this study because the study sites are the same habitat type, namely nutrient poor sandy soil.

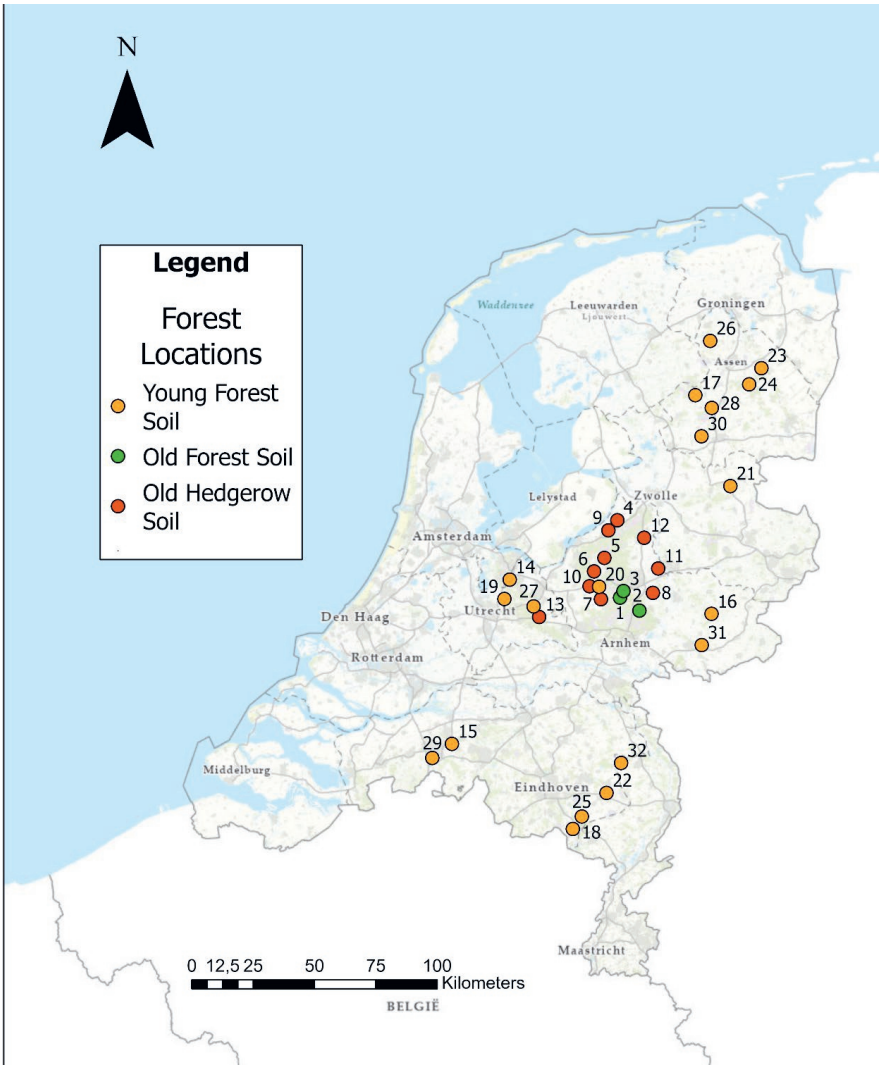


Fig.2.1: *The forest sites included in the project with a label for the site ID (see Table S2.1 for more info). The colours indicate different categories with young forests in yellow, old hedgerows in red and old forest soils in green*

The old forest soil stands are the same as described in Siepel (1996b) and have been forested since at least the Middle Ages and possibly longer. The 10 old hedgerow soils likewise function as old forest soils as they are small strips of old forest remnants that have always remained a forest soil, with which we can expand the number of old-forest soil locations. Moreover, these old hedgerow soils are adjacent to agricultural land and have a constant influx of phosphate due to fertilization. In all stands and hedgerows incidental cutting of trees has been common practice in winter, however the stand has not been removed entirely so the soil has constantly been a forest soil. The 19 young forest soils were afforested from heathland between 1880 and 1930. In those earlier heathland systems sod cutting (i.e., removal of the relatively nutrient rich topsoil layer) was a common agricultural management practise. Sod cutting is a large disturbance factor and has been found to reduce the diversity of microarthropods (Guo 2021), meaning, that these locations were disturbed prior to being reforested.

Sampling procedure and identification

The old forest samples were taken on 03-05-1994, the old hedgerow samples on 22-09-1997 and the young forest samples from April to June in 2000. The sampling procedure was similar in all locations; four samples were taken with an auger and soil microarthropods were then extracted from a Tullgren funnel for one week and collected in 70% alcohol (Siepel and van der Bund 1988). However, there was a small difference in the size of the auger: in old forests and hedgerows the samples were 98.15 cm^3 (5 cm depth and 5 circumference), whereas the younger forests had samples of 132.10 cm^3 (5 cm depth and 5.8 cm circumference). Densities are likewise corrected. The specimens were transferred to 30% lactic acid on a microscope slide for identification. The identification was done to the lowest feasible level, which was species-level in most cases. Due to the fact that different data sources had different people doing the identification, some species in the young forest locations were only identified to the genus or family level, where they were identified to the species level in the old forests and old hedgerows datasets. This includes species in the collembolan genera *Folsomia*, *Hypogastura, Isotoma, Mesaphorura,* and *Onychiurus,* the mesostigmatid genera *Holoparasitus, Hypoaspis, Macrocheles, Parasitus*, and *Zercon,* the oribatid family Brachythoniidae, and genera *Camisia, Suctobelbella,* and *Quadroppia*, the Astigmatina cohort*,* the endeostigmatic genus *Nanorchestes* and family Alycidae and the prostigmatid genera *Scutacarus* and *Siteroptes* as well the orders Diplura and Protura. In order to avoid that the uneven identification standards would affect the analysis, for analyses concerning the species richness and the unique species analysis, all morphotypes within these groups were aggregated into the same genus, family or order name in all datasets.

Trait assignment

All mite species were assigned a feeding guild and size. The feeding guilds were taken from Siepel and de Ruiter-Dijkman (1993) with additions from a personal dataset and are based on the activity of 3 enzymes (trehalase, for fungal cell content, chitinase, for fungal cell walls and cellulase for plant cell walls). The feeding guilds consist of bacterivore, fungivorous browser (only trehalase activity; feeding on fungal cell content only), fungivorous grazer

(trehalase and chitinase activity; feeding on both fungal cell content and cell walls), herbivorous browser (feeding on plant cell content only), herbivorous grazers (cellulase activity; feeding on both plant cell content and cell walls), general predators (predatory mites, not otherwise specified), herbo-fungivorous grazers (activity of all three enzymes mentioned; feeding on both fungal and plant cell walls and contents), omnivores (cellulose and chitinase activity, but without trehalase, feeding on both plant cell walls and arthropod exuviae) and opportunistic herbo-fungivores (cellulase and trehalase activity, are probably mainly herbivorous and lichenivorous and can take advantage of fungal food sources as well). The sizes given are the average body length in µm of each mite species and were taken from identification literature using Karg (1989) for Uropodina, Karg (1993) for Gamasina and Weigmann (2006) for Oribatida.

Soil phosphorus analysis

Soil cores were taken in April 2023 for 18 locations for an Olsen-P analysis, including all 3 old forest soil locations, all 10 old hedgerow soil locations and 5 randomly selected young forest soil locations (Boswachterij de Vuursche, Boswachterij Ruurlo, de Schotkamp, Leusderheide and Vredepeel). As of interest of time, budget and feasibility, only 5 young forest locations were used, as the other young forests in the dataset are scattered around the country. Four soil cores were taken randomly with the standard procedure for microarthropod sampling (5 cm mineral soil depth plus organic matter and litter layer with 5 cm circumference). These four samples were mixed together afterwards in order to create a single soil sample for each location.

Before measuring the Olsen-P level, moisture, organic matter content and soil density was measured in each soil sample. For moisture, fresh soil material was weighted in aluminium trays (40.5 ml), filled to determine soil density and reweighted after oven-drying (48h, 60°C). The fraction of organic matter was calculated by loss on ignition (LOI). Dried soil material was incinerated for 4 hours in an oven of 550°C and reweighted to determine the LOI.

Biologically available P was determined as Olsen-P level (Olsen et al. 1954), by adding 60 ml 0.5 mol/L sodium bicarbonate ($NaHCO_3$) to 3 gram finely grounded soil. The pH of the extraction medium was set on 8.5 with the help of NaOH. The medium was then shaken for 30 minutes in a shaking machine (105 rpm), after which the supernatant was collected under vacuum with a Teflon pore water sampler. This extract was kept in 4°C until further analysis on an Inductively Coupled Plasma Spectrofotometer (ICP; Thermo Electron Corporation, ICP-OES iCAP 6000).

Data analysis

Taxa richness of mites and springtails was estimated for each location and compared between the forest categories using a one-way type II ANOVA with the taxa richness as response variable and the forest category as predictor value. Taxa richness was calculated separately for each feeding guild and differences in richness across locations where then compared among the three forest categories, focussing on the most numerous feeding guilds: (herbo)fungivorous grazers (combination of the two groups most important for decomposition, fungivorous grazers and herbo-fungivorous grazers), herbivorous grazers

and general predators. A Tukey HSD test was used in case of significant results to test for differences between pair of categories.

Additionally, the number of unique taxa was analysed for each forest category (unique is defined as a species that only occurs in one forest category). Since we have unequal number of samples across the forest categories, we also calculated the expected number of unique taxa for each forest category if taxa were randomly distributed across sites. This expected number can be seen as a null model. Numbers of observed unique taxa were compared to numbers of expected unique species and analysed using a chi-square test. The expected sum of the number of unique taxa was calculated by dividing the total possible permutations each taxa that is found in N number of locations can occur in a given forest category by the total possible permutations that taxa can occur in each location (formula 1). The total possible permutations a taxa can occur across each location is calculated using formula (2). Fcat depends on the forest category using the following formula (3) for old forests, formula (4) for old hedgerows and formula (5) for young forests.

$$expected\ number\ of\ unique\ taxa = \Sigma(\frac{\mathrm{Fcat(N)}}{\mathrm{T(N)}}) \quad (1)$$

$$T(N) = \frac{32!}{(32-N)!N!} \quad (2)$$

$$Old\ Forest\ (N) = \frac{3!}{(3-N)!N!} \quad , \quad (3)$$

$$Old\ Hedgerow\ (N) = \frac{10!}{(10-N)!N!} \quad (4)$$

$$Young\ forest\ (N) = \frac{19!}{(19-N)!N!}. \quad (5)$$

The abundance of mites was calculated separately for each feeding guild. First, for each of the four samples of a given location, abundances were summed to a total of 100%, allowing us to express the abundance of each feeding guild as a proportion. The proportions of all samples of a given location were then averaged and arcsine transformed to improve normality. A one-way type II ANOVA was then used on each feeding guild with the proportion of the feeding guild as predictor variable and the forest category as a response variable.

Lastly, the relationship between a mite taxa' body length and its density was analysed per forest category. Mites were divided into two size classes, either small or large, with a cut-off point of 900 µm, which was the threshold size beyond which both species richness and total abundance strongly decreased in our dataset. A two-way type III ANOVA was performed on density as the response variable and with size class (i.e. small or large), forest category and an interaction between size and category were included as predictor variables. The Olsen-P content of our locations was compared using a one-way type II ANOVA with Olsen-P as the response variable and the forest category as predictor variable. Olsen-P was $\log_{10}$-transformed to improve normality.

All statistical analyses were performed on R version 4.2.0 (R Core Team 2022)

Results

Species richness

Old forest and old hedgerow soils harboured on average more taxa of mites compared to young forest sites (ANOVA, $F_{2,29}$=21.06, P<0.001; Fig. 2.2a, Supplementary Table S2.2.1 and Supplementary Table S2.3.1). In contrast, there was no significant difference in springtail taxa richness between forest categories (ANOVA, $F_{2,29}$=1.31, P=0.284; Fig. 2.2b, Supplementary Table S2.2.1 and Supplementary Table S2.3.1). Differences in mite richness between soil categories varied with feeding guild. Higher numbers for taxa that are (herbo)fungivorous grazers in old forest and old hedgerow locations contributed to the difference in taxa mite richness (ANOVA, $F_{2,29}$ =32.41, P<0.001; Fig. 2.2c, Supplementary Table S2.2.1 and Supplementary Table S2.3.1). For herbivorous grazers and for predatory mites, taxa richness also differed between forest categories (Herbivorous grazers, ANOVA, $F_{2,29}$=6.99, P=0.003; Fig. 2.2d, Supplementary Table S2.2.1 and Supplementary Table S2.3.1; General predators, ANOVA, $F_{2,29}$=9.77, P<0.001; Fig. 2.2e, Supplementary Table S2.2.1 and Supplementary Table S2.3.1). For both feeding guilds, the taxa richness was significantly higher in old hedgerow soils than in young forest soils (Herbivorous grazers, TukeyHSD, P=0.002; General predators, TukeyHSD, P<0.001, Supplementary Table S2.3.1).

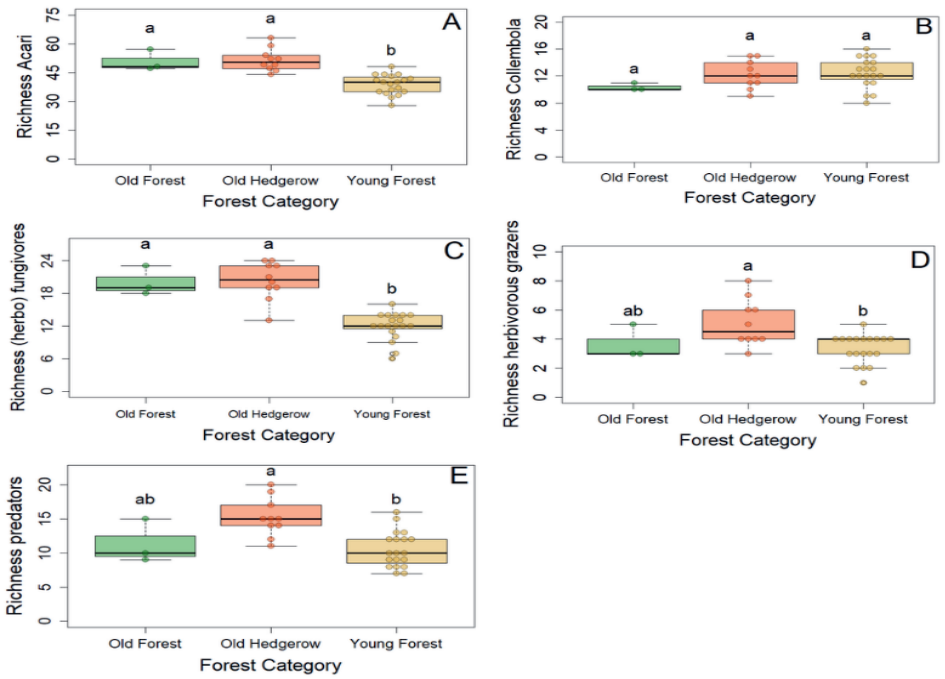


***Fig. 2.2**: Mean Acari taxa richness (a), mean Collembola taxa richness (b), mean (herbo) fungivorous mite taxa richness (c), mean herbivorous grazer mite taxa richness (d) and mean general predator mite taxa richness (e) in each forest category. Significance levels are tested with a Tukey HSD test and illustrated with different letters.*

In addition to old soils having a higher taxa richness for mites compared to young forest soils, these old soil locations also harboured many species that were only observed in one of the three forest categories Since the number of these unique species depends on sampling effort (more unique species will be observed in a given forest category when more locations are sampled), we need to compare observed numbers with the numbers that would be expected by chance (i.e. if all taxa were distributed randomly, see Supplementary Table S2.4). Old soil locations harboured approximately 2-fold to 3-fold more unique taxa than expected by chance (old forests had 12 unique taxa while 5.62 taxa would be expected if randomly distributed, and old hedgerows had 65 unique taxa, while 21.28 taxa would be expected). In contrast, young forests had fewer unique taxa than expected (41 observed vs 50.61 expected). The difference between expected and observed unique taxa was highly significant (χ^2 test, df=2, χ^2=98.28, P < 0.001). When focussing on separate feeding guilds within the mites (Fig. 2.3; Supplementary Table S2.4), it becomes clear that this pattern of higher numbers of unique taxa in old soils applies to most mite feeding guild groups, but is especially pronounced for the fungivorous grazers. Compared to the expected numbers, the old forest soils and the old hedgerow soils had almost twice the number of unique fungivorous grazer taxa higher, while the numbers were about two-fold lower in young forest soils. In contrast, unique herbivorous grazer species were only overrepresented in old hedgerows soils with old forests and young forests having no unique herbivorous grazer taxa.

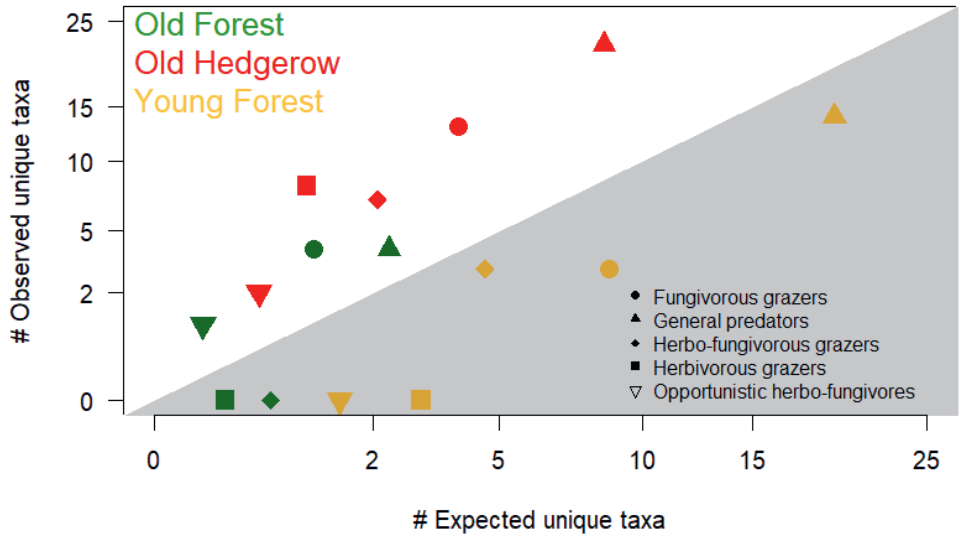


Fig. 2.3: *The number of unique and expected taxa for all three forest categories with several of the more common feeding guild groups of mites. The axis is square root transformed. The line y=x shows the expected values if the number of observed taxa is exactly as would be expected if taxa were randomly dispersed. Colours are used to indicate the forest type and shapes to indicate mite feeding guilds.*

All species that were unique for old forest soils are shown in Supplementary Table S2.5. The most distinctive unique old forest taxa (all occuring in 2 out of 3 locations) were: *Berniniella sigma* (oribatid mite, fungivorous grazer), *Carabodes femoralis* (oribatid mite, opportunistic herbo-fungivore), *Trachytes pauperior* (mesostigmatid mite, general predator) and *Tomocerus minor* (Collembola). The most distinctive unique old hedgerow taxa were: *Oppiella falcata* (oribatid mite, fungivorous grazer, 9 locations), *Xenillus clypeator* (oribatid mite, herbivorous grazer, 7 locations) and *Trichouropoda ovalis* (mesostigmatid mite, general predator, 7 locations) (full list in Supplementary Table S2.6). The most distinctive unique taxa for young forest soils were: *Sphaeridia pumilis* (Collembola, 16 locations), *Ceratozetes mediocris* (oribatid mite, fungivorous grazer, 14 locations), *Pachylaelaps multidentatus* (mesostigmatid mite, general predator, 12 locations) and Pauropoda (10 locations) (full table shown in Supplementary Table S2.7).

Feeding guild distribution

Large difference in the numerical proportions of different feeding guilds across soil categories were observed. Both old forests soils and old hedgerow soils hosted higher proportions for the (herbo)fungivorous grazers compared to young forest soils (ANOVA, $F=7.57$, $P=0.002$; Fig. 2.4, Supplementary Table S2.2 and Supplementary Table S2.3.2). In old forest soils, the (herbo)fungivores were mainly fungivorous grazers with 37.41% (± 7.18 %), while old hedgerow soils had a more even distribution of fungivorous grazers of 27.51% (±2.68 %) and herbo-fungivorous grazers 15.20 % (± 2.62 %). Young forest soils had a higher proportion of herbivorous browsers (21.52 % (± 3.24 %); ANOVA, $F=14.43$, $P<0.001$, Fig. 2.4, Supplementary Table S2.2.2 and Supplementary Table S2.3.2), when compared to old forest soils having 2.96 % (± 2.24) and old hedgerow soils having 1.72 % (± 1.13 %). Differences in these proportions were caused by high densities of small prostigmatid mites like *Microtydeus* sp., which had a mean density of 105,045.27 (±27,224.46) ind./m^2 in young forests compared to 3,225.57 (± 1,032.65) ind./m^2 in old forests and 14,769.70 (± 9,165.44) ind./m^2 in old hedgerows. Despite old hedgerow soils hosting the most species of herbivorous grazers (Fig. 2.2d), the proportions for this feeding guild were comparable across forest categories. This could be attributed to the high abundance of the most common herbivorous grazer, *Platynothrus peltifer*, in every forest category

Body size

The abundance of mites differed by body size, forest type and their interaction (interaction between forest type and body size class, $F_{2,58} = 9.50$, $P<0.001$, Supplementary Table S2.2.3 and Supplementary Table S2.3.3). Mites smaller than 900 µm were more numerous than large mites, but their densities did not differ across soil categories (Fig. 2.5a). In contrast, the density of large mites (>900 µm) was higher in old hedgerow soils than in either old forest soils or young forest soils (Fig. 2.5b). The highest density of large mites in old hedgerows coincides with the highest Olsen-P-content when compared to either old forest soils or young forest soils (ANOVA, $F=8.81$, $P<0.01$; Supplementary Fig. S2.1, Supplementary Table S2.2.4 and Supplementary Table S2.3.4).

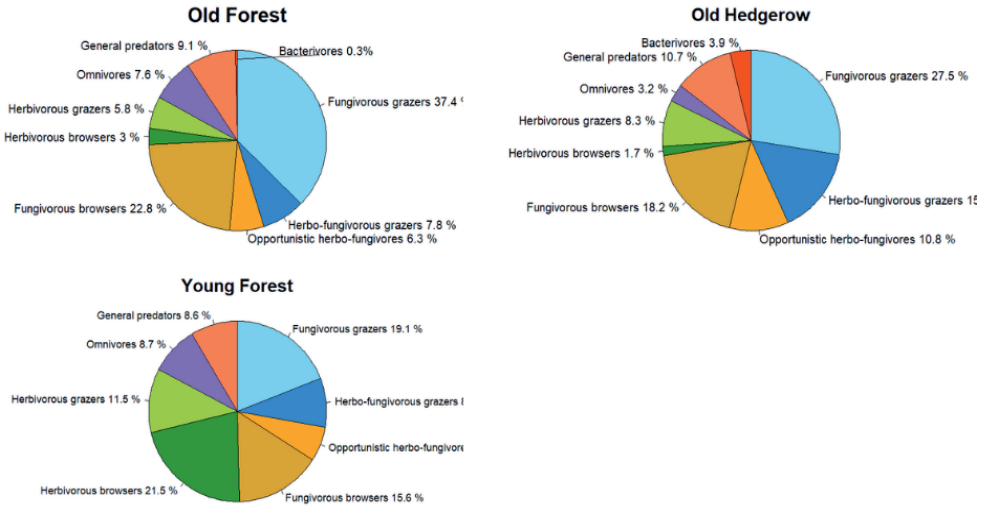


Fig. 2.4: *The average Acari feeding guild distribution of forest locations. Colours are used to indicate the feeding guilds. The blue signifies (herbo) fungivorous grazers, orange and brown the fungi feeders that only feed on cell content, green for herbivorous mites and purple and red for omnivorous and predatory feeding guilds.*

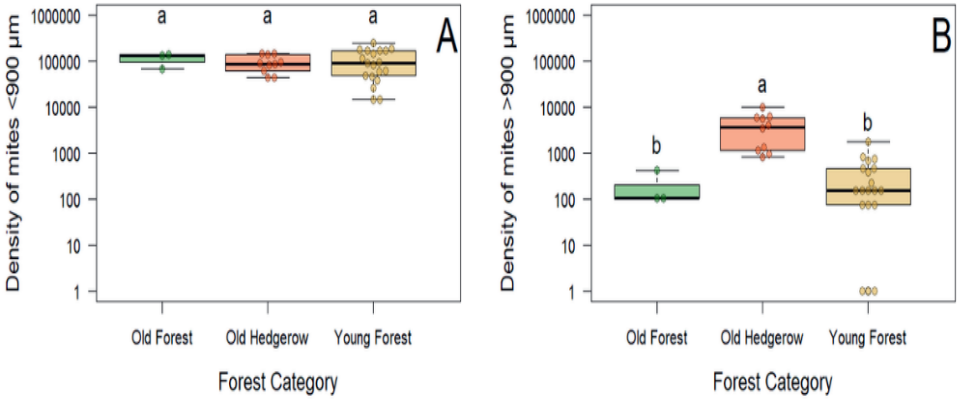


Fig. 2.5: *The density (ind./m^2) of mite taxa smaller than 900 µm (a) and mite taxa larger than 900 µm (b) grouped by forest category. The axis is log_{10} transformed. Significance levels are illustrated with letters (a is significantly higher than b, ab is no significance).*

Discussion

As hypothesized, we find higher taxa richness of mites in old soils than in young forest soils. Both old forests and old hedgerow soils have a higher taxa richness and unique taxa are overrepresented in these two soil types. Note these differences cannot result from slight differences in the auger size used to sample since a (slightly) larger auger was used in young forest soils, we would expect to retrieve more taxa there by chance. Thus, our finding requires a biological explanation. Since Oribatida in particular are known to be poor colonizers (Ojala and Huhta 2004; Lehmitz et al. 2012), the lower richness of mites in young forest soils likely results from their low ability to (re)colonize habitats. Hågvar et al. (2009) studied Oribatida species richness in receding glaciers in Norway and found higher species richness in older soils. Huhta and Niemi (2003) also reported lower Oribatida species richness in birch forests (*Betula* sp.) that were afforested from cultivated fields 26-43 years ago when compared to soils that were forested continuously (Ashwood et al. 2021). In contrast, no difference in diversity was found for Collembola. Many Collembola species are better colonizers than most mite species (Santorufo et al. 2021) and therefore have had a better chance to recolonize these areas. Since many Collembola species are also vulnerable to soil disturbance (Maraun et al. 2003), we expect that the taxa richness of Collembola was also reduced during the sod cutting when the young forests were heathlands, but Collembola communities have since recovered.

Difference in mite richness between forest categories was largest for (herbo)fungivorous grazers. The richness of mites of this feeding guild is about two-fold higher in old forest soils and old hedgerow soils compared to young forest soils. Furthermore, young forest soils harbour not only fewer taxa, but also lower densities of (herbo)fungivorous grazers. This group has an important role in the decomposition process of organic matter because of their chitinase activity, which gives them the ability to breakdown fungal cell walls (Siepel and de Ruiter-Dijkman 1993). In doing so, they release minerals from the (senescent) cell walls back into the system and the recycling process stimulates the growth of fungi (Siepel and Maaskamp 1994; McGonigle 1995). The study of van Eekeren et al. (2022) associated a higher density of (herbo)fungivorous grazer mites with a higher decomposition rate. Therefore, we predict that the young forest locations as a result of the low density and richness of (herbo)fungivorous mites are less efficient in their ability to breakdown organic matter.

While the diversity of fungivorous mites was similar between old hedgerow soils and old forest soils, there was a higher species richness of both herbivorous grazing mite groups (herbo-fungivorous grazers and herbivorous grazers) in the old hedgerow locations compared to young forest soils. When compared to old forest soils this difference was not quite significant, potentially due to the small number of old forest locations in our study. Additionally, old hedgerow soils hosted most unique species of herbivorous grazing mites (16 species when combining herbivorous grazers and herbo-fungivorous grazer mites). In contrast, young forest soils hosted only 3 unique herbo-fungivorous grazers and there were no herbivorous grazers that were unique to old forest soils at all. Potentially, this difference in species richness could be due to P-limitation induced by continuous N-deposition.

Previous studies have found that for nutrient poor ecosystems, such as these forests, N-deposition increases N:P ratio in the plants, and as a result herbivorous species are experiencing P-limitation (Elser et al. 2000; Siepel et al. 2018; Vogels et al. 2023). Camenzind et al. (2021) found that soil fungi mycelia, in contrast to plants, have a relatively homeostatic N:P ratio more similar to animals. This is because fungi have the ability to immobilize and store excess P (Beever and Burns 1981; Gulis et al. 2017). Thus, whereas fungivorous mite species still get sufficient P in a P-limited system, herbivorous species are more likely to struggle in doing so since they can only feed on plants that are more stoichiometrically imbalanced. The old hedgerow soils are in direct contact with agricultural land and have therefore been subjected to constant P-influx due to fertilization. This likely explains the greatly elevated level of Olsen-P in old hedgerow soils compared to young and old forests soils (Supplementary Fig. S2.1).

We found significantly more large mite species (>900 µm) in old hedgerow locations compared to the young forest soils. Old hedgerows also had more large mite species than old forest locations, but this was not significant, probably due to the lower number of old forest locations. We expect that this is an effect of the fertilization in hedgerows because previous studies also found that an increase in P is associated with an increase of species in large size classes (Mulder 2010). Reuman et al. (2009) likewise found that fertilization increases the abundance of larger species in soil faunal communities. P-limitation has been found to be one of the most limiting factors for larger bodied animals (Mulder and Elser 2009; Mulder 2010). P-limitation reduces growth rate and reproduction (Vogels et al. 2024) due it its important role in high-energy adenylates (ATP) and nucleic acids (RNA) that are essential for both growth and reproduction (Elser et al. 1996). Smaller organisms with a rapid development, often in lower trophic levels, have a higher P-content and have a high demand for P in their environment in comparison to slow reproducing species on higher trophic levels. In P-deficient systems, the P-content in organisms will decrease, leading to an inefficient transfer of energy to higher trophic levels and hence a loss of large-bodied fauna (Mulder and Elser 2009).

In conclusion, our study shows the importance of soil age for the microarthropod community; old soils have only experienced minimal disturbance for hundreds of years leading to the accumulation of high numbers of species over time. Given the key role of these animals for the decomposition process, we must avoid disturbing the soil of these old forests to preserve a rich (herbo)fungivorous grazer community. For young forests, it may take a very long time for the soil community to recover naturally. Therefore, assisted colonisation by soil inoculation is an option to be considered for young forests locations. While soil inoculation might help species richness, it cannot solve the problem of P-limitation that are evident in Dutch forests due to continuous N-deposition. Therefore, there is an urgent need to greatly reduce the deposition of nitrogen and develop methods to mitigate P-limitation.

Acknowledgements

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Author contributions

Joren Bruggink: Conceptualization, Methodology, Software, Formal analysis, Data Curation, Visualization, Writing - Original Draft. **Marie-Charlott Petersdorf:** Conceptualization, Writing - Review
and Editing. **Wilco Verberk:** Conceptualization, Software, Visualization, Writing - Review and Editing. **Henk Siepel:** Conceptualization, Resources, Writing - Review and Editing, Supervision.

Chapter 3

The diversity of fungivorous mites in Dutch forests affected by nitrogen deposition and soil acidification

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Submitted

Abstract

The old acidophilous oak forest Natura 2000 habitat (H9190) in the Netherlands is rapidly declining due to excess nitrogen deposition. This leads to soil acidification, leaching of base cations, and an accumulation of soil organic matter. Decomposition of this organic matter is driven by fungivorous microarthropods, yet their response to nitrogen deposition remains poorly understood. We analyzed microarthropod communities in old oak forests and compared them to previous data from other forest sites from the same geographical area and a similar habitat. Analysis of abiotic conditions have shown that old oak forests are characterized by low soil pH, imbalanced foliar nutrient ratios, and our study of the microarthropod community found that this coincided with a reduced richness and abundance of especially fungivorous mites. We propose that nitrogen-driven declines in ectomycorrhizal fungal diversity, that are known from literature, limit fungivorous mite communities, contributing to slower decomposition and causing organic matter to accumulate. Effective restoration of soil conditions and fungal diversity and abundance will also require enhancing fungivore populations, potentially through soil inoculation.

Introduction

Excess nitrogen deposition causes problems in the Netherlands for many ecosystems. The high increase in nitrogen availability stimulates productivity in plants and causes asymmetrical competition (Farrer and Suding 2016) in which fast-growing species are facilitated and subsequently outcompete slow-growing plants. Additionally, accumulation of nitrogen deposition causes stoichiometric imbalance in foliar nutrient levels, for example in the nitrogen: phosphorus ratio (N:P), leading to a limitation in crucial nutrients (Sardans et al. 2012; Siepel et al. 2019; Vogels et al. 2020). Furthermore, excess nitrogen deposition leads to soil acidification, which causes leaching of important cations such as calcium (Ca^{2+}), potassium (K^{+}) and magnesium (Mg^{2+}) from the soil (Schulze 1989; Aber et al. 1995; De Vries et al. 2014) and leads to the release of aluminum (Al^{3+}), which is toxic for plant roots (Shortle and Smith 1988; De Graaf et al. 1997; Rout et al. 2001; Rahman and Upadhyaya 2020). As a result, plant species richness declines (Bobbink et al. 2010; Cleland and Harpole 2010; Nijssen et al. 2017).

Furthermore, excess nitrogen deposition slows down the decomposition process in forest soils (Frey et al. 2014; Zhou et al. 2017). Nitrogen deposition increases decomposition rates on a short term, but significantly decreases the decomposition after two or more years (Janssen et al. 2010). Initial high N levels were therefore associated with a slower net decomposition (Berg and Matzner 1997). Therefore, the higher the N input, the higher the amount of residual N in the soil organic matter.

The decomposition in the soil is affected by fungivorous soil microarthropods, which include mites that graze on fungal cell walls. These species recycle important nutrients which become available in the soil and stimulate microbial activity (van der Drift and Jansen 1977; Siepel and Maaskamp 1994; Filser 2002; Janoušková et al. 2018). The study of Siepel and de Ruiter-Dijkman (1993) delineated mite feeding guild groups based on enzyme activity with two feeding guilds (fungivorous grazers and herbo-fungivorous grazers) having chitinase activity that enables them to break down soil fungal cell walls. However, it is not yet clear how these fungivorous grazers are affected by high nitrogen deposition and whether they are more sensitive compared to other soil microarthropods, which includes herbivores and predatory mites, but also fungivore browsers, which can digest only the cell contents of fungi and thus do not recycle nutrients that are locked in the fungal cell walls such as P (Siepel and de Ruiter-Dijkman 1993). Studies investigating the effects of nitrogen deposition on soil microarthropods found mixed results on the abundance of mites (Ochoa-Heuso et al. 2014; Pequero et al. 2021; Ge et al. 2023), but only few studies have identified to species level (Gan et al. 2013; Mitchell et al. 2016), which is necessary to distinguish feeding guilds in mites (Siepel and de Ruiter-Dijkman 1993) and thereby understanding the status of the fungivorous grazers.

In this study we investigate the status of fungivorous mites in the Natura 2000 old acidophilous oak forest habitat (H9190 in the EU habitats directive), in the Veluwe area in central Netherlands. The old acidophilous oak forest habitat type constitutes forest stands dominated by pedunculate oak (*Quercus robur*) on poor sandy soils (Evans 2006); it is

naturally acidic and nutrient poor, which makes it particularly vulnerable to negative nitrogen deposition effects such as soil acidification (Simkin et al. 2016). In these forests, we observe a strong decline in tree vitality, an increase in tree mortality and an accumulation of soil organic matter (Hommel et al. 2020).

Considering the observed degraded state of the old acidophilous oak forest habitat, we expect to find a low density and diversity of soil microarthropods. We will focus on the fungivorous mite community because of the role they play in the decomposition of soil organic matter that is stagnating in this habitat. To put the fungivorous mite community in context, we will compare the species richness and abundance of fungivorous mites in the old acidophilous oak forest habitat with previous records from 1994 taken in locations of the closely related Natura 2000 habitat H9120. These 1994 samples were found to be examples of high microarthropod richness relative to other forest soils in the Netherlands (Siepel 1996b; Bruggink et al. 2025) and therefore will serve as a reference for a good microarthropod status within a similar habitat and the same geographical area.

Methods

Study location information

The old oak forests are classified as habitat type H9190 in the EU habitats directive and are characterized as forest stands with dominance of *Quercus robur* and *Betula pendula* on acidic, nutrient poor sandy soils (Evans 2006). Pedunculate oak (*Quercus robur*) is the dominant tree species in all locations and in some locations there is a dominance of European blueberry (*Vaccinium myrtillus*) in the undergrowth. For this study we will use data from 6 different old oak forest sites spread across the Veluwe (Fig. 3.1). Measurements in the top 10 cm of mineral soil indicated that these old oak forest sites in general suffer from soil acidification, as they exhibited low base saturation levels (average of 12.89 ± 1.23 %), high soil exchangeable Al/Ca-ratios (average of 2.93 ± 0.36 mol/mol; and low soil pH_{NaCl} levels (average of 2.99 ± 0.04) (Sitters et al. 2026). Additionally, chemical analyses of leaves from the pedunculate oak trees in the study sites clearly showed the trees were limited by Ca (mean N:Ca ratio of 8.54 ± 0.25), K (mean N:K ratio of 5.43 ± 0.18) and P (mean N:P ratio of 21.84 ± 0.57) (Sitters et al. 2026). The acidic soil conditions are therefore a major health risk for the oak trees (Cronan and Grigal 1995; Mellert and Göttlein 2012; Wellbrock and Bolte 2019).

Sites we use as reference are in the closely related Natura 2000 habitat type H9120. The variety of this habitat type is characterized as beech-oak forests. (Evans 2006). This habitat is similar to the H9190 old oak forest in that it is also acidic and present in nutrient poor sandy soil areas. We use data for three different locations sampled in 1994 (Siepel 1996b) having very old (undisturbed) forest soil, being continuously forested since at least the Middle Ages and all present in the same geographical area as the Veluwe.

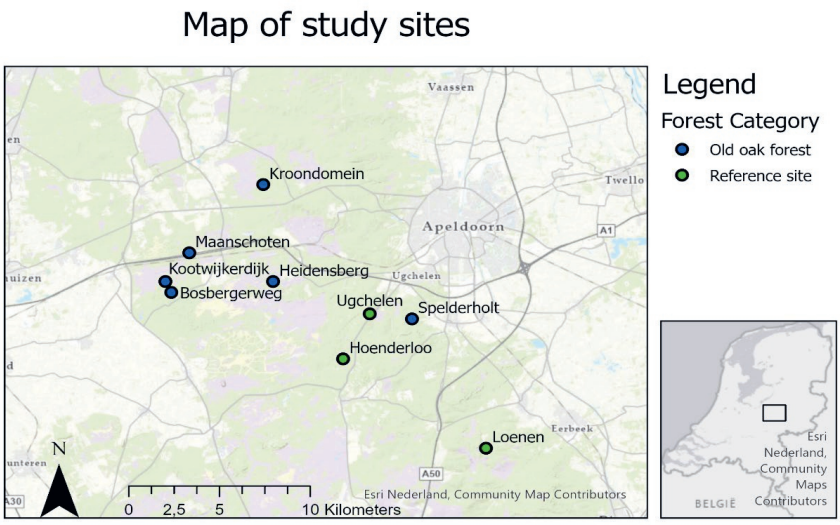


Fig. 3.1*: Map of the 9 study locations. 6 locations in blue are in the H9190 old oak forest habitat and three locations in green are in the H9120 beech-oak forest habitat.*

Microarthropod sampling procedure

Thirty-nine samples were taken from 6 old oak forest sites between February and April 2020. Due to logistic constraints from Covid19 regulations, we did not have an equal number of samples for all locations, but each site was represented with at least 4 samples. For the reference sites in the H9120 habitat we used 12 samples from 3 locations that were taken on May 3, 1994 (Siepel 1996b). All samples were taken with a soil auger to 5 cm mineral soil depth (including litter on top) and 5 cm circumference. A Berlese-Tullgren funnel extraction was used to extract the soil microarthropods out of the soil samples with first 3 days at 35°C and then 4 days at 45°C and conserved in 70% ethanol (Siepel & Van de Bund, 1988). Afterwards microscope slides were prepared by transferring the microarthropods to slides with 30% lactic acid. Identification was done to the lowest possible level (species in most cases, otherwise to the genus level). In the reference samples of 1994, most Prostigmata taxa were only identified to either the family or genus level. We therefore harmonized the identification levels for both datasets by pooling most Prostigmata species into either the genus or family level.

Each mite taxon was assigned to a feeding guild according to Siepel and de Ruiter-Dijkman (1993). Hereby we focused on the fungivorous feeding guilds and made a distinction between species that rely solely on fungi as a food source (fungivorous browsers and fungivorous grazers) and species that can feed both on fungal and plant material (herbo-fungivores group). The herbo-fungivores group contains the herbo-fungivorous grazer and opportunistic herbo-fungivore feeding guilds (Siepel and de Ruiter-Dijkman, 1993). These two groups are further compared to the herbivorous mites (merging of the feeding guilds herbivorous grazer and herbivorous browser) and the predatory mites.

Statistics

Linear mixed-effects models using site as a random effect and forest category (Either old oak forest or reference) as predictor variable were used to analyze the differences in soil microarthropod density, soil microarthropod species richness and proportion of feeding guilds. Linear mixed effect models were chosen since they are robust and appropriate for the unbalanced design (Cnaan et al. 1997). The microarthropod density was $\log_{10}$ transformed to improve normality. In each sample were the total number of mites within each feeding guild group summed to get a proportion of feeding guild group. A logit transformation was then performed on all proportion values to improve normality of proportional data (Warton and Hui 2011). All statistics were performed on R (R Core Team 2025) and the nlme package was used to fit the linear mixed-effect models (Pinheiro et al. 2025).

Results

Microarthropod density and diversity

The old oak forests had a mean density of 64,511 (± 9,943) individuals per m^2, which is more than twofold lower than the reference samples, which had a mean density of 160,175 (± 30,867) individuals per m^2 (lme, $t_{1,7}$=- 2.859, P=0.017; Supplementary Table S3.1). This coincided with a >30% lower species richness of microarthropods in old oak forests (26.62 ± 1.92 species per sample) than the reference samples that had an average of 41.17 (± 2.95) species per sample (lme, $t_{1,7}$ = -2.899, P=0.023; Supplementary Table S3.1).

Density and diversity of fungivorous mites

The total density of mites within each feeding guild group was lower in the old oak forest compared to the reference locations, however the strongest effect was with the fungivorous mite group (both fungivorous browsers and fungivorous grazers) being about four-fold lower in the old oak forest locations (21,377 ± 5,548 individuals per m^2 compared to 81,572 ± 19,470; Supplementary Table S3.1). This is also reflected in the proportion of the fungivorous feeding guild group which was 28.98 % (± 3.36) in the old oak forest, whereas fungivorous mites contributed to 51.80 % (± 5.51) of all mites in the reference locations (lme, fungivorous browsers: $t_{1,7}$ = -2.396, P = 0.048; fungivorous grazers: $t_{1,7}$ = -2.580, P = 0.037; Fig. 3.2; Supplementary Table S3.1). The proportion of herbo-fungivores, in contrast, were not different between the two forest categories (lme, herbo-fungivores, $t_{1,7}$ = 0.035, P = 0.973, Fig. 3.2; Supplementary Table S3.1). Old oak forest had a higher proportion of herbivores than the reference locations, but this was not statistically significant (lme, Herbivores, $t_{1,7}$ = 1.754, P = 0.123; Fig. 3.2, Supplementary Table S3.1)

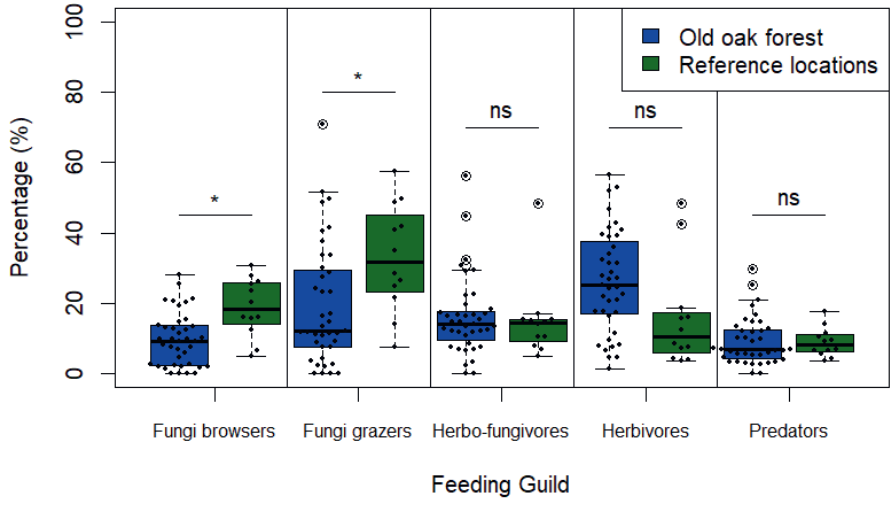


***Fig.3.2**: The proportion of mite feeding guild groups in H9190 old oak forest (nlue) and the reference locations (green). Signficance levels of the linear mixed effects models are displayed with bars and symbols with ns = not signifiant, * = P < 0.05.*

The mean species richness of fungivorous mites differed strongly and significantly (lme, fungivorous browsers, $t_{1,7}$ = -4.925 , P=0.002; fungivorous grazers, $t_{,1,7}$ = -2.629, P = 0.034, Fig. 3.3, Supplementary Table S3.1) and was more than two-fold lower in the old oak forest (7.00 ± 0.89) than the reference locations (17.00 ± 1.49). Within the old oak forest there were a few samples with still high fungivorous mite species richness, which were mostly from the Maanschoten location (highlighted in red in Fig. 3.3). In addition to the difference in fungivore richness, the old oak forests locations had a lower richness of herbo-fungivores (lme, $t_{1,7}$ = - 2.236, P = 0.050; Fig. 3.3, Supplementary Table S3.1), predators (lme, $t_{1,7}$ = -2.846, P = 0.025; Fig. 3.3; Supplementary Table S3.1) and herbivores (lme, $t_{1,7}$ = -1.666 , P = 0.140; Fig. 3.3, Supplementary Table S3.1) compared to the reference locations.

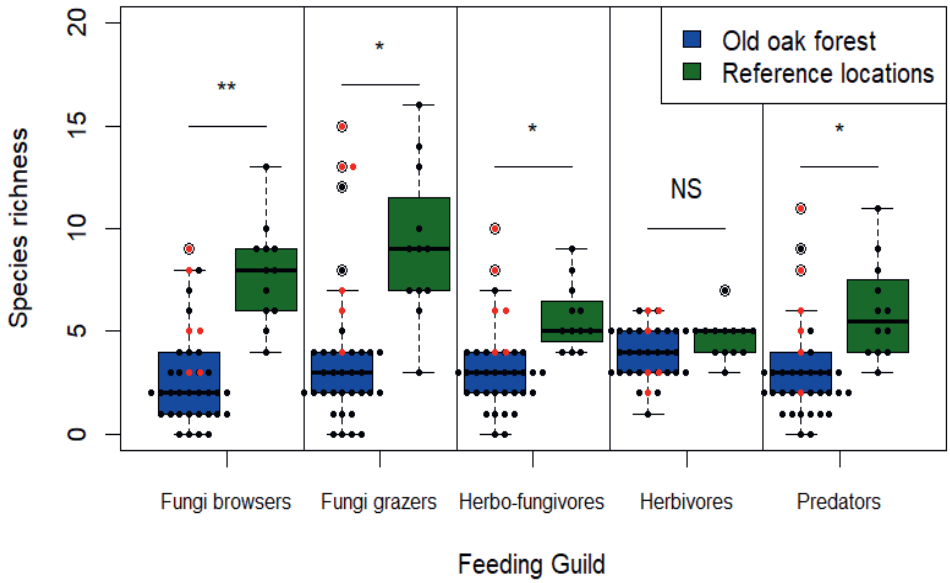


Fig. 3.3*: The species richness of mite feeding guild groups in H9190 old oak forest (blue) and the reference locations (green). The samples in red are fungivorous species richness from the Maanschoten location. Signficance levels of the linear mixed effects models are displayed with bars and symbols with ns = not signifiant, * = P ≤ 0.05, ** = P ≤ 0.01, and *** = P ≤ 0.001*

Discussion

The old oak forests in the Netherlands are strongly impacted by acidification resulting from N deposition. The poor state of this habitat is reflected in the impoverished microarthropod community, with lower numbers of individuals and species when compared to reference locations.

Our results indicate that fungivorous mite species in particular are affected by accumulative nitrogen deposition effects. Similarly, in a 17-year long experiment Gan et al. (2013) noted a reduction in diversity of fungivorous oribatid species with nitrogen addition. In their paper Gan et al. (2013) argue that species that rely more on fungal hyphae are more negatively impacted by nitrogen deposition than species that feed on litter. Our results corroborate that hypothesis. For example, differences between our study location and the reference locations were smaller for the herbo-fungivorous mites that can feed both on fungi and plants, contrasting with the strong differences observed for fungivorous browser and grazer mites that feed exclusively on fungi. Since the feeding of fungivores on fungal hyphae cell wall has a positive effect on the soil respiration of microbes (Siepel and Maaskamp 1994), we hypothesize that the lack of the fungivorous grazer group is at least in part a contribution to the accumulation of soil organic matter and may contribute to essential nutrients being unavailable, locked into recalcitrant biomass such as fungal cell walls. Indeed, analysis of the leaf chemistry of pedunculate oak shows that there is a limitation of P, Ca and K (Sitters et al. 2026), mirroring the acidification effects reported for plants in heathlands on similarly acidified soil (Vogels et al. 2020).

While there is still much unclear about the exact feeding patterns of fungivorous microarthropods, a few studies have indicated that ectomycorrhiza (ECM) could be an important food source for many fungivorous mite species. For instance, Schneider et al. (2005) found that three oribatid species selected for certain ectomycorrhizal fungi, with the fungivorous grazer *Oribatula tibalis* being the most selective. Furthermore, the oribatid species *Oppiella nova* (fungivorous grazer) was positively impacted by the presence of ectomycorrhizal species (Remén et al. 2008), while *Nothrus silvestris* and *Tectocephalus velatus*, which are both species that also feed on plant material (Siepel and de Ruiter-Dijkman 1993), were not impacted as strongly (Remén et al. 2010). These studies indicate that at least certain oribatid species preferably feed on ECM fungi and likely depend on the presence of ECM to thrive in an ecosystem.

The fungal community is affected by elevated nitrogen deposition as well, with studies pointing out that in particular the ECM fungi are strongly reduced in diversity and community composition (Arnolds 1991; Allison et al. 2007; Cox et al. 2010; Morrison et al. 2016). When plants are not limited by nitrogen they allocate less C to the mycorrhiza, which results in a reduced mycorrhizal biomass by 15% as noted by the meta-analysis done by Treseder (2004). Therefore, the diversity of ECM communities in temperate European forests is driven by nitrogen deposition and soil pH (Suz et al. 2014). In the Netherlands, Arnolds (1988) noted a decrease in ECM fungal fruit bodies and concluded that this decline was

strongest in habitats on poor, acid soils that are most vulnerable to accumulative nitrogen deposition effects.

Not all old oak forests locations are in the same state of decline. As briefly highlighted in the results section, the location Maanschoten had a couple of samples with high fungivorous species richness. From a fungal fruitbodies inventory done in four old oak forest locations in 2023 and 2024 we know that this particular location also had a relatively high density and species richness of fungal fruitbodies compared to other old oak forest locations, further indicating that this location was still in a reasonable biotic state compared to the other locations (Supplementary Table S3.2).

In conclusion, soil acidification has led to the accumulation of soil organic matter, while the microarthropods that could play a role in nutrient turnover are in decline due to high nitrogen levels. Fungivorous grazing microarthropods are crucial by stimulating fungal growth and thereby the decomposition process in forest soils. We hypothesize that the decline of fungivorous grazing mite species in forests affected by nitrogen deposition is a large contributor in the observed accumulation of soil organic matter. It is therefore necessary to immediately mitigate the negative effects of nitrogen deposition in the old oak forest habitat and improve the soil pH and buffer capacity. Furthermore, the diversity of fungivorous microarthropods should be stimulated. While it has been shown that the ECM diversity can improve relatively quickly in some areas of the Netherlands after slowing down nitrogen deposition (van Strien et al. 2017), many fungivorous mite species do not have the same level of dispersal capacity (Ojala and Huhta 2001; Bruggink et al. 2025). Therefore, assisted colonization by soil inoculation, in addition to improving the abiotic status of the forests, might be a necessary additional measure to restore fungivorous microarthropod diversity and restore decomposition processes in the soil.

Acknowledgements
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Author contributions
Joren Bruggink: Conceptualization, Methodology, Software, Formal analysis, Data Curation, Visualization, Writing - Original Draft. **Marie-Charlott Petersdorf:** Conceptualization, Writing – Review and Editing. **Wilbert L.P Jansen:** Data Curation, Writing – Review and Editing. **Emiel Brouwers:** Data curation, Writing – Review and Editing. **Judith Sitters:** Data Curation, Writing – Review and Editing. **Maaike J. Weijters:** Data Curation, Project Management, Writing – Review and Editing. **Wilco Verberk:** Conceptualization, Supervision, Writing - Review and Editing. **Henk Siepel:** Conceptualization, Resources, Writing - Review and Editing, Supervision.

Chapter 4

Fungivorous microarthropod richness related to stagnation of litter decay in nitrogen impacted *Quercus rob*ur forests

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Submitted

Abstract

Fungivorous grazing soil microarthropod species (fungivorous grazers and herbo-fungivorous grazers) play an important role in the litter decomposition process of forests by stimulating microbial activity and thereby enhancing the breakdown of recalcitrant lignin-bound compounds during the later phases of decomposition. Excess nitrogen deposition is known to threaten the species richness of fungivorous microarthropods, which potentially impedes decomposition in nitrogen-impacted forests.

We examined the relationship between the microarthropod community and plant litter decomposition in a 21-month litterbag experiment using *Quercus robur* in forests vulnerable to nitrogen deposition. The early phase of decomposition was dominated by herbivorous species that exploit high nutrient availability in freshly decomposing litter, while fungivorous species became dominant after 6 months. At this stage, we observed a stagnation in litter decomposition for nine months, indicating inefficient breakdown of the lignin-bound compounds.

We found that the richness of both fungivorous grazers and herbo-fungivorous grazers richness was positively correlated with leaf mass loss, but in different stages of the experiment. Fungivorous grazers were more important during the stagnating months of 9-12 when there was plenty of litter left, while herbo-fungivorous grazers were more important in the final months of decomposition reflecting their feeding ecology of mostly feeding on dead fungal matter.

Because nitrogen deposition supresses the decomposition of recalcitrant litter, it may partly explain the stagnation observed in our study. We propose that the reduction of fungivorous microarthropod species richness is an important factor in causing this slower litter decomposition in nitrogen-impacted forests and to the accumulation of soil organic matter.

Introduction

The decomposition of leaf litter is an important part in the nutrient cycling process in forest ecosystems. During this process, nutrients, for example nitrogen and phosphorus, are released from organic matter and made available again for uptake by plants (Attiwil and Adams 1993; Foster and Bahtti 2006). The rate of decomposition can be affected by multiple factors among which are various abiotic factors such as climate (Aerts 1997; Liski et al. 2003) and leaf chemistry (Gosz et al. 1973; Bonanomi et al. 2013) or biotic factors such as the quality of leaf litter (Rosenfield et al. 2020).

The decomposition process can be divided into different phases (Berg and Matzner 1997). In the early phase of decomposition, it is dominated by the decomposition of cellulose where non-lignified polysaccharides are rapidly released from the substrate (Whittinghill et al. 2012; García-Palacios et al. 2016; Gill et al. 2021). During this phase the decomposition rate is linearly related to the concentrations of N, P and S until an accumulated mass loss between 26 and 36 percent is reached (Berg and Matzner 1997). After the rapid breakdown of easily degradable material, the latter phases are dominated by the slower breakdown of more recalcitrant lignin-bound polysaccharides and other aromantic compounds (Gill et al. 2021). During this phase there is an important role of fungi that can successfully breakdown lignin (Bani et al. 2018). The final stage of decomposition is the moment when decomposition of leaf litter appears to have been stopped, only leaving certain recalcitrant fragments (Berg and Matzner 1997).

Often it has been assumed that climate and litter quality are the dominant factors regulating litter decomposition (Bradford et al. 2016), however, the faunal contribution to the decomposition process is important as well (Seastedt 1984; Verhoef and Brussaard 1990). Macroarthropods contribute to decomposition by fragmenting or shredding leaf material (McCary and Schmitz 2021). Microarthropods support the decomposition process in various ways too: herbivorous grazing microarthropods, which are species that have ability to breakdown cellulose (Siepel and de Ruiter-Dijkman 1993), contribute in a similar manner as macroarthropods by directly feeding on the leaf litter material. Additionally, fungivorous grazers play an important role in decomposition due to their ability to stimulate microbial activity by breaking down the chitinase in (old) fungal cell walls, which releases nutrients stored herein (Hanlon and Anderson 1979; Siepel and Maaskamp 1994; McGonigle 1995; Filser 2001).

The study of Gan et al. (2013) found a reduction of fungivorous microarthropod species in response to excess nitrogen deposition. Since many of those species play an important role in the decomposition of the recalcitrant material, it is expected that the decomposition in later phases is inhibited when forests are subjected to high nitrogen levels for a long period of time.

In this study we will analyse the litter decomposition of *Quercus robur* leaves in various forest sites on poor, acidic sandy soils and study the decomposition process in relation to the phases of microarthropod communities in litterbags. These forest locations are particularly vulnerable to acidification effects of nitrogen deposition (Simkin et al. 2016) due to low

buffering capacity and this habitat has been observed to have an accumulation of soil organic matter (Hommel et al. 2020). We will start studying the microarthropod community throughout the decomposition process in litterbags and then relate the richness of fungivorous grazing species to the decomposition of leaf litter.

Hereby we expect that herbivorous microarthropod species dominate the earlier stages of the litterbag experiment. Their main contribution to decomposition will be in this phase since many these species preferably feed on leaf litter with a higher nutrient content (Petersdorf et al. 2024). In the later stages of the litterbag experiment, where only the more recalcitrant litter material is left, it is expected that the abundance and diversity of fungivorous grazing species will increase due to the more prominent role of fungi in this stage of decomposition. We expect that there will be no inhibition in the early phase of decomposition since nitrogen and phosphorus levels in the leaves will determine the speed. However, we do expect a stagnation of decomposition of the recalcitrant lignified compounds later in the process due to the expected low fungivorous grazer richness as a result of long-term N-deposition (Gan et al. 2013). Lastly, we hypothesize that higher richness of the fungivorous grazer groups (fungivorous grazers and herbo-fungivorous grazers) will stimulate the leaf mass loss in later stages of decomposition.

Methods

Site information

This study took place in 9 different forest locations scattered around the Veluwe (Fig. 4.1). Five of those locations are on the old acidophilous oak forest habitat (Bosbergerweg, Eikenheg, Hoog Baarlo, Keulerbosch and Kootwijkerdijk; H9190 in the EU habitats directive), four are in the Atlantic acidophilous beech-oak forests (H9120 in the EU habitats directive), from which three are on public forests (Hoenderloo, Loenen and Ugchelen) and the final one Nieuw Dennenlust is on a private forest estate. All litterbags were placed in oak (*Quercus)* tree stands. The H9190 locations are thought to especially be influenced by long-term N-deposition and have a low average pH_{NaCL} of 2.74 ± 0.08 in the litter layer and a pH_{NaCL} of 2.99 ± 0.04 in the top 10 cm of the soil, while the public H9120 locations are on a slightly better buffered soil and have an average pH_{NaCL} of 2.70 ± 0.13 in the litter layer and a pH_{NaCL} of 3.17 ± 0.01 in the top 10 cm of soil. On the Nieuw Dennenlust location we measured a pH_{NaCL} of 2.90 in the litter layer and a pH_{NaCL} of 2.83 in top 5 cm of the soil.

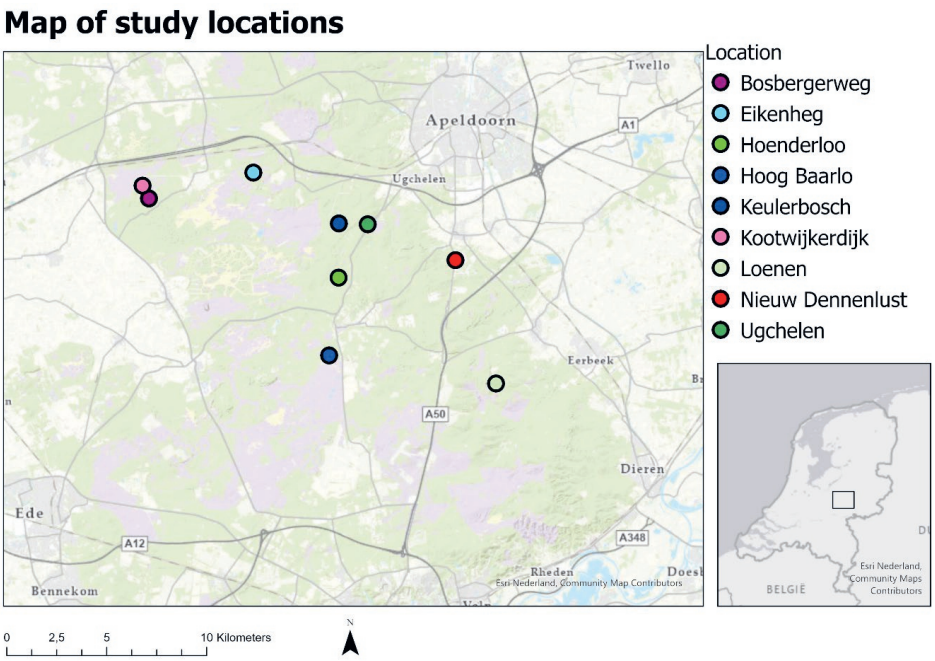


Fig. 4.1: *Map of the study locations in this experiment. Locations in the old oak forest habitat (H9190) are shown in blue and purple colours, locations in the beech-oak location (H9120) are shown in green colours and the Nieuw Dennenlust location in red.*

According to the weather data from the royal Dutch institute of meteorology, the years 2022-2024 were extremely warm with resp. a mean temperature of 11.6 °C, 11.8°C and 11.8 °C (average since 1999: 10.84 °C); mean annual rainfall varied considerably over these years with 2022 being a dry year with 729 mm annual rainfall and the subsequent years being very

wet with resp. 1060 mm and 986 mm annual rainfall (average since 1999: 819.88 mm) (KNMI 2023; KNMI 2024; KNMI 2025). The autumn of 2022, when the litterbags were set out, was relatively warm and with regular amount of rainfall.

Litterbag handling

Leaves used in this study were collected from *Quercus robur* branches at the height of 1.5-2 meter in the Veluwe forest in September 2022 (52°05'31.5"N 5°57'09.2"E) and were then oven dried for 24 hours at 70 °C. 60 litterbags for each location were hand-crafted from a non-decomposable polymide plastic material using a mesh size of 2 mm in order to exclude macroarthropods. Each litterbag was filled with approximately 5-gram dried leaf material and then closed with a stapler. Each litterbag was weighed before being used in the field to get the full dry weight. In each location all 60 litterbags were buried next to each other under the litter layer.

Litterbags were placed on 1 November 2022. Sampling took place 1, 3, 6, 9, 12, 15, 18 and 21 months after placement. During each sampling moment 5 litterbags were randomly taken at each location. This means that 40 litterbags were used in total for each location. The extra 20 were to ensure random sampling at each moment. The rest of the unused litterbags were removed from the soil at the end of the experiment.

Microarthropod extraction and litterbag.

After collection a Tullgren extraction was used with standard protocol (Siepel and Van de Bund, 1988). The microarthropods were collected in 70% ethanol and taken to the lab for further analyses. Litterbags were put in dry stove at 70°C for 24 hours and were then weighted again to get the remaining mass in dry weight. Roots and soil particles were removed prior to weighing the litterbags. CNS elemental analyser was then used to obtain the nutrient variables in the remaining *Quercus robur* litter.

Microarthropods were transferred to object slides with 30% lactic acid for clarification and identification. Almost all individuals were identified to species level, except for a few juveniles and individuals of the *Mesaphorura* genus (identified to genus level). Unidentified juveniles were omitted for richness analyses. Identification followed e.g. Weigmann (2006) for Oribatida, (1993) for Gamasina, Karg (1989) for Uropodina, and several family and generic keys for Prostigmata, Endeostigmata, and Astigmatina. All individuals were assigned to a feeding guild following the method of Siepel and de Ruiter-Dijkman (1993). This method is based on the activity of three enzymes: cellulase (for plant cell walls), chitinase (for fungal cell walls) and trehalase (for fungal cell contents). The following feeding guilds are distinguished: bacterivore, fungivorous browser (feeding of fungal cell content only), fungivorous grazer (feeding on fungal cell wall and content), general predator (predatory species), herbivorous browser (feeding on plant cell content only), herbivorous grazer (feeding on plant cell wall and content), herbo-fungivorous grazer (feeding on plants and fungi, both cell wall and cell content), opportunistic herbo-fungivore (feeding on plant cell walls and contents and fungal cell content) and omnivore.

Data analysis

The abundance and species richness of microarthropods between locations and sample month was analysed using a type I ANOVA model with a logarithmic fit. Abundance was $\log_{10}$ transformed to improve normality. Either species richness or abundance was used as the response variable with an interaction between location and the log of sample month used as predictor variables. The same analysis was used for the species richness of the feeding guilds herbivorous grazer, herbo-fungivorous grazer and fungivorous grazer.

While testing differences in abundance of feeding guilds, the proportion of each feeding was calculated in each litterbag to account for differences in abundance between samples. A logit transformation was then performed on the proportion to improve normality of proportional data (Warton and Hui 2011). The change of the fungivorous grazer and herbivorous grazer proportion over time was then analysed using a linear mixed effect model with a logarithmic fit using the nlme package in R (Pinheiro et al. 2025). Either fungivorous grazer or herbivorous grazer proportion was the response variable and the natural log of sample month was the predictor variable. Location was used as a random effect.

The development of the microarthropod community in the litterbag experiment was tested with a canonical correspondence analysis (CCA) using the vegan package in R (Oksanen et al. 2025). Seasonal effects were analysed using a transformed Julian day using formula 1. This transformation was used to ensure that the winter months had similar values. Location and sample month were used as constrained variables and Julian day as a conditional variable to account for seasonal effects. The significance of location and sample month was tested with the permutation test provided by the R package vegan using 999 permutations. The result of the permutation tests was then analysed using a type I ANOVA model.

$$Transformed\ Julian\ day\ = \sin(\frac{2\pi * Julian\ day}{365}) * 0.5 * 365 \quad (1)$$

The litterbag decomposition was fitted using a segmented regression (Segmented R package, Muggeo 2008). The proportion of remaining weight was used as response variable and the location and sample month were used as predictor variables including an interaction term between both variables. Additionally, an interaction between sample month and the transformed Julian day (formula 1) was added to correct for a potential seasonal effect of sample month. Initial breakpoints were set at 6 and 15 months based on observation of scatterplots. A type I ANOVA model was then used on the segmented regression model to analyse differences between locations.

The decay rate was calculated by the single exponential decay model (Olson 1963, formula 2) where M is the remaining mass in %, k is the decay constant and t is the time expressed in the sample month. Since we aimed to separate the decomposition in the early and late stage of decomposition, we calculated separate decay rates for the first 6 months and the decomposition after 6 months. 6 months was used as the separation point as we found both a shift in microarthropod community and a change in litter mass loss rate at this point of time. For the first decay rate of the first 6 months the mass remaining in each litterbag was

scaled as a proportion of the initial 5 grams. For the decay rate after 6 months, we calculated the mean litter weight at 6 months in each location and then scaled the litter mass from 9 to 21 months as a proportion of the mean weight at 6 months.

$$M = e^{-k*t} \quad (2)$$

The effect of leaf nitrogen content, leaf phosphorus content, fungivorous species richness and herbo-fungivorous species richness on the leaf mass loss were analysed using linear mixed effect models with location as random factor. Interactions of each variable with the sample month was tested, but only included in the model if it were significant to avoid overcomplicated models. This model was split for early (first 6 months) and late decomposition (mass loss after 6 months).

Results

Abundance and species richness of microarthropods over time

The absolute number of microarthropods retrieved from the litter bags depended both on the location and the sample month (interaction term: ANOVA, $F_{8, 331} = 5.798$, $P<0.001$, Fig. 4.2). The species richness of microarthropods increased rapidly in the first 9 months and then levelled of (ANOVA, $F_{1,331} = 259.11$, $P<0.001$; Fig. 4.3, Supplementary Table S3.1.1). There were significant differences between locations in microarthropod species richness (ANOVA, $F_{8, 331} = 6.126$, $P<0.001$) with no significant interaction between location and sampling month (ANOVA, $F_{8, 331} = 0.661$, $P = 0.726$).

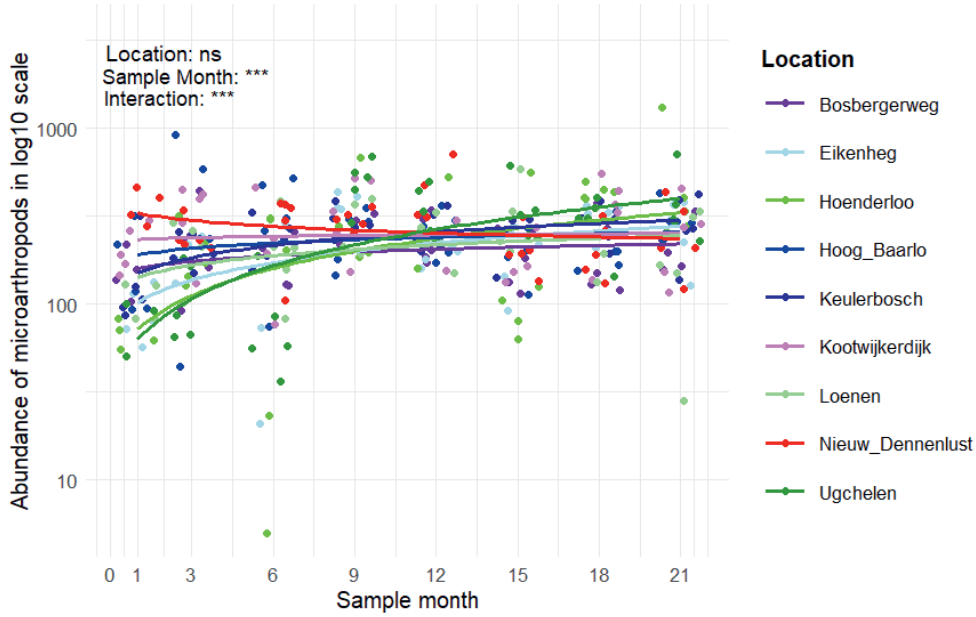


Fig. 4.2: *Abundance of microarthropods in litterbags using a logarithmic curve (log_{10} Abundance ~ Sample month * Ln(location). Note that the y axis is shown logarithmically. Locations in the old oak forest habitat (H9190) are shown in blue and purple colours, locations in the beech-oak location (H9120) are shown in green colours and the Nieuw Dennenlust location in red.*

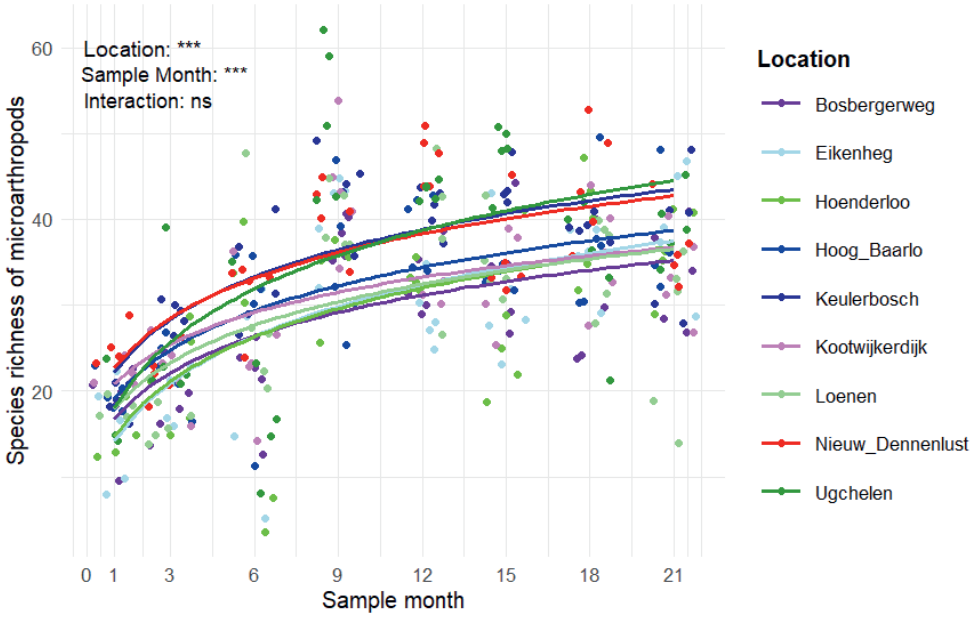


Fig. 4.3: *species richness of microarthropods in litterbags using a logarithmic curve (Species richness ~ Sample month * Ln(location). Locations in the old oak forest habitat (H9190) are shown in blue and purple colours, locations in the beech-oak location (H9120) are shown in green colours and the Nieuw Dennenlust location in red.*

The development of the microarthropod community

The CCA analysis found a significant effect of both sample month (ANOVA, $F_{1, 338} = 17.935$, $P < 0.001$, Fig. 4.4a) and location (ANOVA, $F_{8, 339} = 6.845$, $P<0.001$) on the microarthropod community. The constrained portion in total explained 17.68 % of the CCA. The conditional variable Julian day explained 1.28 % of the variation.

The first axis explains 37.19 % of the constrained eigenvalues and separated the early 6 months with the latter stages of the experiment. The first half year of litterbags were dominated by herbivorous grazers such as *Platynothrus peltifer* and *Lepidocyrtus lanuginosus (*Fig. 4.4b, Supplementary Table S4.2*)*. Other notable species for this period are active predatory mite species such as *Parasitus lunulatus* (CC1 score: -1.45, Supplementary Table S4.2) and *Zerconopsis remiger* (CC1 score: -1.37, Supplementary Table S4.2) and the bacterivore species *Histiostoma sapromyzarum* (Fig. 4.4b). The variation between samples is larger in the samples of the first 6 months compared to latter samples, where the community got more similar between samples. The microarthropod community after the first half year was largely indicated by a gradual increase of fungivorous species. Notable examples include *Oppiella nova*, *Nothrus silvestris* and *Liochthonius hystrinicus* (CC1 score: 0.82, Fig. 4.4b, Supplementary Table S4.2).

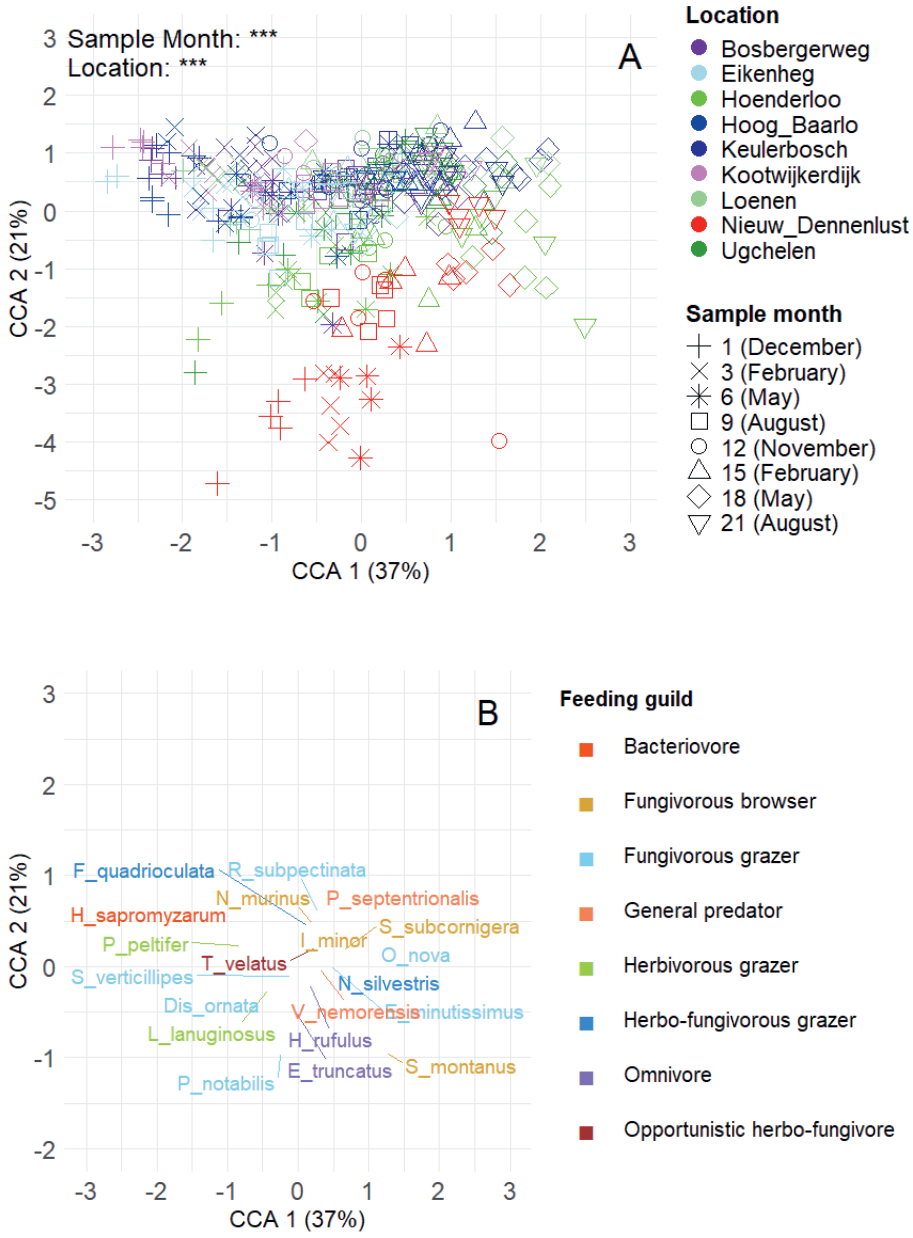


Fig. 4.4: *Canonical correspondence analysis ordination displaying the litterbag samples. Both sample month and location were used as constrained variables and the transformed Julian day as a conditional variable. Plot 4a displays the sample months, which are displayed with different symbols and locations with different colours. Locations in the old oak forest habitat (H9190) are shown in blue and purple colours, locations in the beech-oak location (H9120) are shown in green colours and the Nieuw Dennenlust location in red. Plot 4b shows the 20 most abundant species in litterbag samples. Colours are used to indicate the feeding guild of each species. Note the different values on the y-axis.*

The second axis explained 21.25 % of the constrained eigenvalues and made the separation between Nieuw Dennenlust with the other locations. Particularly in the first 6 months had Nieuw Dennenlust a distinctive microarthropod community compared to the other locations. Over the entire experiment Nieuw Dennenlust had 24 unique species, while the mean number of unique species was only 4 across all other locations (Supplementary Table S4.3).

The development of feeding guilds is reflected into the proportions of herbivorous grazers and fungivorous grazers. Herbivorous grazers start dominant having a mean proportion of 34.04 ± 3.11 % in the first month, but at the end of the experiment only had 6.93 ± 0.88 % (lme, $t_{1, 338} = -14.280$, P<0.001; Fig. 4.5, Supplementary Table S4.1.2). Fungivorous grazers show the opposite pattern starting with a proportion of only 10.81 ± 2.25%, but having a peak of 32.46 ± 2.87 % at 9 months (lme, $t_{1, 339} = 10.460$, P<0.001). The intersection between the two feeding guilds is at 6 months.

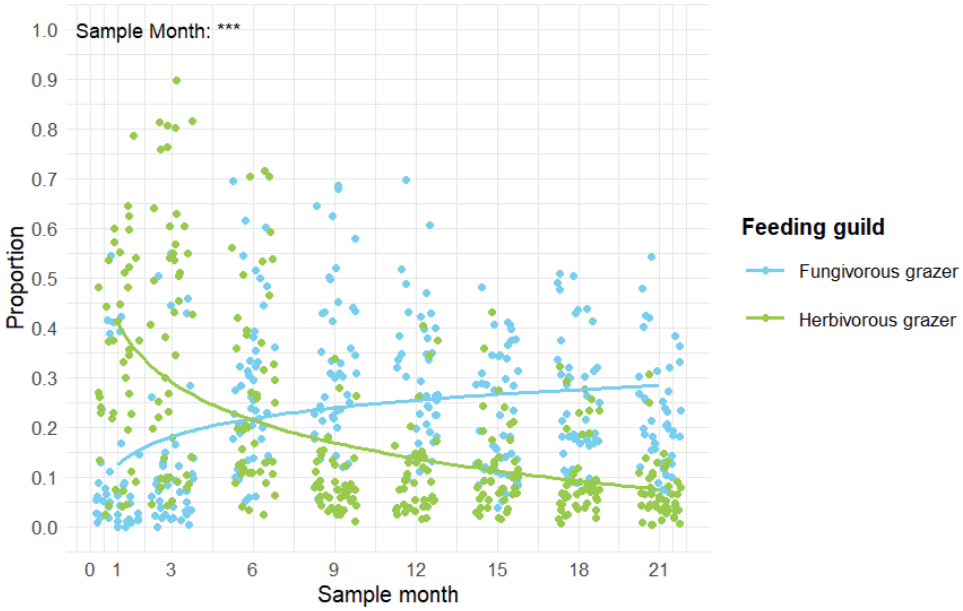


Fig. 4.5: *The proportion of herbivorous grazers (green) and fungivorous grazers (blue) over time in this litterbag experiment. A logarithmic curve was used as a fit (Proportion ~ Location * Ln (Sample month).*

Species richness of herbivores and fungivores

Despite the fact that herbivores dominated by first 6 months in abundance, the species richness of herbivorous grazers still significantly increased over time (ANOVA, $F_{1, 331} = 3.20$, P = 0.002; Supplementary Table S4.1.1). The fungivorous grazing species increased strongly significantly over time (ANOVA, $F_{1, 331} = 285.22$, P<0.001; Fig. 4.6a, Supplementary Table S4.1.1) and differed between locations (ANOVA, $F_{8, 331} = 6.189$, P<0.001). Keulerbosch stands out as the richest location for fungivorous grazers, followed by Bosbergerweg, Hoenderloo,

Nieuw Dennenlust and Ugchelen. Hoog Baarlo and Loenen had the lowest fungivorous grazer species richness. The species richness of herbo-fungivores generally increased significantly over time as well (ANOVA, $F_{1, 331} = 154.59$, $P<0.001$; Fig. 4.6b, Supplementary Table S4.1.1), however there was a significant interaction between the sample month and location (ANOVA, $F_{8, 331} = 4.190$, $P = 0.001$), which is due to the locations Bosbergerweg and Hoenderloo having a smaller increase in herbo-fungivorous species over time compared to other locations (Fig. 4.6b). Hoog Baarlo and Ugchelen were the most species rich locations for herbo-fungivorous grazers with Hoenderloo and Nieuw Dennenlust being the lowest.

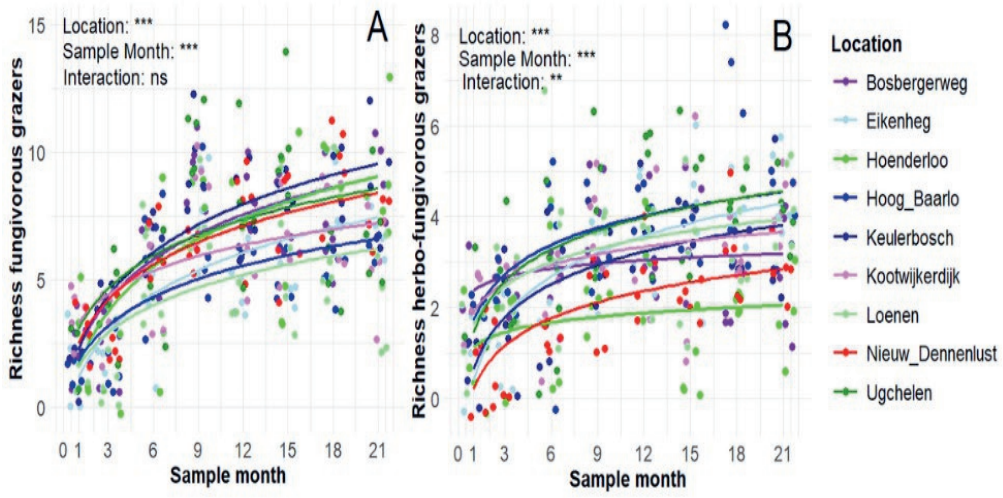


Fig. 4.6: *Species richness of the fungivorous grazers and herbo-fungivorous grazers over time. Locations in the old oak forest habitat (H9190) are shown in blue and purple colours, locations in the beech-oak location (H9120) are shown in green colours and the Nieuw Dennenlust location in red. Figure 6a shows the fungivorous grazer species richness using a logarithmic curve (Species richness fungivorous grazers ~ Location * Ln (Sample month). Figure 6b shows the species richness of the herbo-fungivorous grazers using a logarithmic curve (Species richness herbo-fungivorous grazers ~ Location * Ln (Sample month). Note the different axis values on the y-axis.*

The decomposition of leaf litter

The segmented regression found breakpoints at 6.00 ± 1.85 and 15.13 ± 2.27 ($R^2 = 0.77$, Fig. 4.7). The rate of decomposition significantly differs between locations (ANOVA, $F_{8, 311} = 4.841$, $P <0.001$, Fig. 4.7, Supplementary Table S4.1.3). Hoog Baarlo was overall the quickest decomposing location and Hoenderloo the slowest. There was a significant interaction between location and sample month as well as indicating that the rate of decomposition did not follow the same pattern between locations (ANOVA, $F_{8, 311} = 2.943$, $P=0.003$). Nieuw Dennenlust, for example, had a slow start in decomposition, but ended with a relatively low proportion remaining weight at the end. The transformed Julian day had no significant interaction with sample month, indicating limited seasonal effect in the decomposition pattern (ANOVA, $F_{1, 331} = 0.101$, $P = 0.751$).

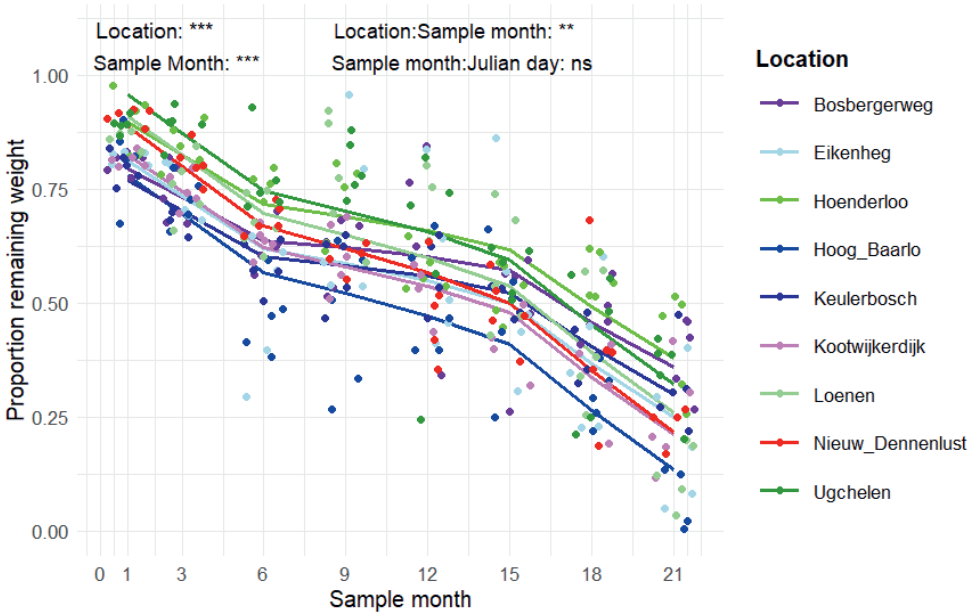


Fig. 4.7: *The decomposition of Quercus robur* litter using a segmented linear regression fit (Maffeo 2008). Initial breakpoints were set at 6 and 15 months. *Locations in the old oak forest habitat (H9190) are shown in blue and purple colours, locations in the beech-oak location (H9120) are shown in green colours and the Nieuw Dennenlust location in red*

The leaf mass loss (g) in the first half year was positively impacted by the N content in leaves (lme, $t_{8,136} = 3.163$, P = 0.002; Supplementary Table S4.1.4). The average decay rate constant k during this period was 0.083 (Table 4.1). After the early phase of decomposition there is a period of stagnating decomposition (mean litter mass loss is 0.629 ± 0.161 gram between month 6 and month 15, Fig. 4.7). This has resulted in a mean reduction of 60.01% in the decay rate k (Table 4.1). Hoog Baarlo and Eikenheg had the largest inhibition in decomposition between months 6-15. Differences in leaf mass loss in this latter period was associated with leaf P content (lme, $t_{1, 201} = 2.956$, P = 0.004), but with no significant effect of leaf nitrogen content anymore (lme, $t_{1, 201} = 1.023$, P = 0.308). The effect of fungivorous grazer species richness within locations on the leaf mass loss in the late decomposition phase depended on the sample month (interaction term: lme, t = -3.428, P = 0.001, Fig. 4.8a). Between month 9-12 a higher fungivorous grazer richness within that particular month was associated with a higher mass loss in leaves, however at month 18-21 leaves with higher mass loss had lower fungivorous grazer species richness. The opposite pattern was found for herbo-fungivorous grazer species richness (interaction term: lme, t = -2.663, P = 0.008, Fig. 4.8b): for months 9-12 higher herbo-fungivorous grazer species richness was associated with lower leaf mass loss, while for months 18-21 higher herbo-fungivorous grazer species richness was associated with higher leaf mass loss.

Table 4.1: *The decomposition rate variables in each location. The litter decay constant (k) was taken from this formula: $M = e^{k*t}$. Where M is the remaining mass (%) and t is the time in months. For the k of 0-6 months, we scaled the proportion of remaining mass to the initial 5 gram. For the k after 6 months, we used the average weight at month 6 in each location and then scaled the remaining mass to that value.*

Location	K 0-6 months	K after 6 months	Reduction decay rate k (%)	Mass loss between 6-15 months (g)
Bosbergerweg	0.087	0.017	81.46	0.670
Eikenheg	0.119	0.022	80.67	-0.126
Hoenderloo	0.052	0.020	59.62	1.077
Hoog Baarlo	0.126	0.046	62.70	-0.054
Keulerbosch	0.101	0.018	80.19	0.390
Kootwijkerdijk	0.083	0.035	56.63	1.135
Loenen	0.072	0.040	43.06	0.536
Nieuw Dennenlust	0.066	0.033	48.48	1.002
Ugchelen	0.044	0.031	27.27	1.022
Mean	**0.083**	**0.030**	**60.01**	**0.629**

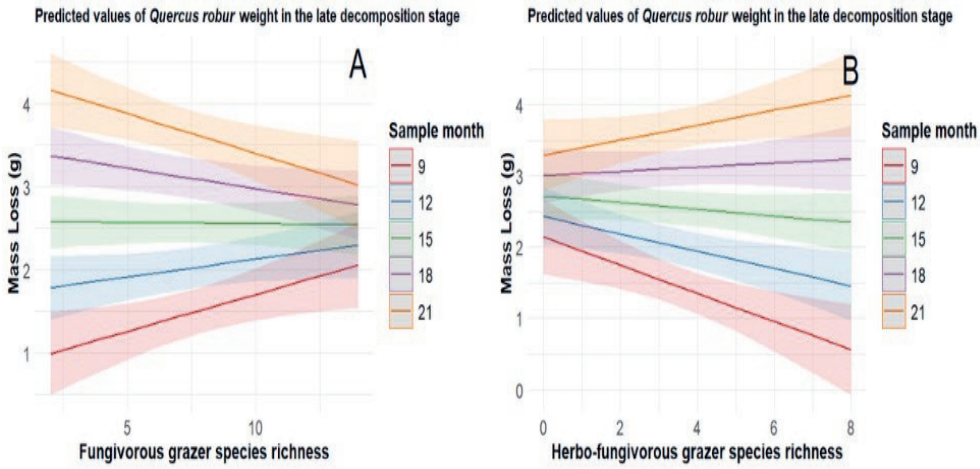


Fig. 4.8: *interaction between fungivorous grazer (a) and herbo-fungivorous grazer (b) with the mass loss (g) in Quercus robur leaves between months 9-21*. Each line represents the correlation of fungivorous grazer species richness and leaf mass loss within that particular month.

Discussion

The development of the microarthropod community

The species richness of microarthropods rapidly increased until 9 months, after which the number of species plateaued. There is still, however, species turnover in the latter period of the experiment, as can be seen by the CCA analysis. Similar patterns of microarthropod colonization in litterbags was found in other studies (Anderson 1975b; Siepel 1990; Hansen and Coleman 1998). We found a clear separation in the microarthropod community between the first 6 months of the litterbag studies and the microarthropod community between 9 and 21 months. The study of Osler et al. (2004) found a strong seasonal effect in the early phase of litterbag colonization of microarthropods, however we found only a small effect of Julian day. Osler's study was carried out in a Mediterranean climate with dry hot summers, while ours was more temperate. In our study the time since deployment was a much more important factor in determining the microarthropod community in litterbags.

The separation between early and late decomposition

We used 6 months as the dividing line between early and late decomposition since that was the point where the microarthropod community shifted from being dominated by herbivores to a period of increasing fungivore dominance. Additionally, we saw a change in decay rate of litter where 6 months was the start of a period of stagnating decomposition. The dividing line of 6 months between early and late decomposition compares reasonably well with how other studies defined the change from early to late decomposition (Berg and Matzner 1997; Parton et al. 2007; Zhou et al. 2008). This change in decomposition rate cannot be attributed to solely a seasonal factor of the summer since there was no significant interaction of the transformed Julian day and the sample month. Many soil microarthropods are vulnerable to effects of drought (Siepel 1996a) and while there was a dry period in May 2023, the rest of the summer was very wet, so we do not expect that drought is a contributing factor.

Early phase of decomposition

As hypothesized, the early phase of the decomposition was dominated by herbivorous grazing species. These are species that have cellulase enzyme activity and primarily feed on plant material (Siepel and de Ruiter-Dijkman 1993). We found a particular high abundance of a couple of species in the early 6 months, such as *Platynothrus peltifer* and *Lepidocyrtus lanuginosus,* that can colonize and reproduce quickly in the litterbags. Herbivorous grazing species are benefitted by feeding on high nutrient leaf material that is present in the earlier phases of decomposition (Petersdorf et al. 2024). Even though herbivorous grazing species are dominant in proportion in the early phase, the species richness of this group peaked between months 9-15, indicating that some species arrive later in the process. Those species might be adapted to feed on either lower nutrient level leaf material or on the roots that develop in the litterbags in later stages.

The decomposition rate in the early phase was predominantly determined by the nitrogen content in the leaves, which is a commonly found to be an important factor for the

decomposition rate in early phases (Berg and Matzner 1997; Parton et al. 2007). Additionally, litter with high nitrogen content will increase the productivity of herbivorous microarthropods (Petersdorf et al. 2024). The initial decomposition rate was relatively quick in this experiment compared to some other litterbag studies using *Quercus robur* (Newsham et al. 2001; Davey et al. 2007; Farská et al. 2009; Rawlik et al. 2021; Wang et al. 2021), which could be due to relatively high initial N concentration in our litter. Additionally, we collected leaves from the forest edge, which Remy et al. (2017) showed to decompose more quickly than leaves collected in interior forest. N content could not explain mass loss in the later phase of decomposition, which indicates that the stimulating effect only occurs during the early phase.

Late phase of decomposition

The microarthropod community after 6 months was indicated by an increasing level of colonization by fungivorous species. During this phase the slower decomposition of recalcitrant lignin-bound materials is prevalent and fungi play an important role in the breakdown of these compounds (Hammel 1997; Osono 2017; Bani et al. 2018). Ectomycorrhizal fungi, for instance, have been found to have significant N accumulation ability in later stages of decomposition (Pena et al. 2013). The fungal community undergoes several changes throughout the decomposition process (Voříšková and Baldrian 2013) and the fungal richness increases in later stages of the decomposition process (Purahong et al. 2016). It is no surprise then that this phase of higher fungi activity and richness is accompanied by a higher proportion and species richness of fungivorous microarthropods in this study.

We found a much slower decomposition rate in this phase compared to the first six months. Particularly, between 6 and 15 months there was a stagnation of decomposition for many locations, which has been measured before in some studies using *Quercus robur* litter (Bocock et al. 1960; Slade and Riutta 2012; Kamczyc et al. 2019; Pieristè et al. 2019), although this is not the case in all *Q. robur* litterbag studies (Alvarez et al. 2008; Vesterdal et al. 2016). Among those latter studies, both Alvarez et al. (2008) and Vesterdal et al. (2016) were done in forests with much higher pH compared to our experiment. Bocock et al. 1960 attributed this period of stagnation to drought effects in the summer, but this was not the determining factor in our experiment and the study of Kamczyc et al. (2019) reported a stagnating period starting in October. We hypothesize that acidification effects of nitrogen deposition is also a potential factor in causing this stagnation, since it is known that excess nitrogen will supress the decomposition of lignin-bound compounds (Berg and Matzner 1997; Whittinghill et al. 2012).

Higher species richness of fungivorous grazers was associated with a higher leaf mass loss (g) during this period of stagnation in months 9 and 12, which is an indication that the presence of these species helped the breakdown of tougher compounds. In the final months of the study higher fungivorous grazer species richness was associated with low leaf mass loss, which is likely because the quickest decomposing litterbags had not much leaf material left so there was not much food sources for these species. Herbo-fungivorous grazers showed the opposite pattern of fungivorous grazers having a positive correlation of their species

richness and leaf mass loss in the final months (18 and 21) of the study. This is likely a reflection of the different ecology in these groups. Herbo-fungivorous grazers are litter feeders and feed primarily on dead fungal biomass, next to plant organic matter (Siepel et al. 2019), and thus become more active in very late phases of decomposition, while fungivorous grazers are exclusive fungivores and need to be able to feed on living fungi, in addition to old fungal material, to obtain all their nutrients.

The relationship of fungivorous grazers and litter decomposition is illustrated by Hoog Baarlo and Eikenheg, the two locations with the largest inhibition of decomposition in months 9 and 12 (Table 4.1), also having a low richness of fungivorous grazers compared to other locations. Both did have a high richness of herbo-fungivorous grazers which is reflected in a rapid decomposition in months 18 and 21. There are exceptions to this pattern, however. Keulerbosch, notably, had a high richness of fungivorous grazers compared to other locations, but had relatively slow decomposition in months 9 and 12 so there are other factors influencing the pattern of decomposition as well.

Implications for the decomposition process

While elevated nitrogen levels stimulate the early decomposition of easily degradable compounds, it slows down the decomposition of recalcitrant material in later phases (Berg and Matzner 1997; Whittinghill et al. 2012). Often, this slower decomposition rate due to high nitrogen levels is attributed to an inhibition of microbial activity or down-regulating enzymes from fungi that are important in the decomposition of lignin (Edwards et al. 2011; van Diepen et al. 2016). Additionally, it is important to note that excess nitrogen deposition has been found to decrease fungi biodiversity, particularly of ectomycorrhizal species (Arnolds 1991; Treseder et al. 2004; Suz et al. 2014; Morrisson et al. 2016). Our results suggest that the species richness of fungivorous grazing microarthropod species is an additional factor steering litter decay rate in latter phases of decomposition. It has been shown that that fungivorous species decline in response to excess nitrogen deposition as well (Gan et al. 2013) and indeed the species richness in this experiment was quite low compared to previous measurements in the same forests (Bruggink et al. 2025). We found that the richness of fungivorous grazers correlate to higher leaf mass losses and therefore there is risk of inhibition of decomposition in forests with low fungivorous grazer richness. For habitat recovery purposes this means that, in addition to recovering the soil chemistry and fungal activity, attention to the restoration of the fungivorous microarthropod community is necessary to restore the decomposition processes in forests.

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Author contributions

Joren Bruggink: Conceptualization, Data curation, Formal Analysis, Visualization, Writing – original draft. **Marie-Charlott Petersdorf**: Conceptualization, Writing – review and editing. **Wilco C.E.P Verberk**: Conceptualization, Supervision, Writing – review and editing. **Henk Siepel:** Conceptualization, Supervision, Writing – review and editing

Chapter 5

The effect of two restoration methods (silicate rock powder and soil inoculation) on the forest soil microarthropod community.

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Submitted

Abstract

The soil microarthropod community plays an important role in the decomposition process of forest soil ecosystems, but this community is under pressure in many sites due to nitrogen-induced acidification. To explore restoration steps, we tested the effectiveness of two potential restoration methods: 1) the addition of silicate rock powder (SRP) to increase soil pH and replenish soil minerals to improve the microarthropod habitat and 2) soil inoculation from a donor site with higher microarthropod species richness to enrich the microarthropod community itself. A full factorial experiment in five forest sites was established, combining three rock powder treatments (control, Eifelgold and Soilfeed) with two inoculation treatments (inoculated, null).

SRP application was found to be effective in increasing soil pH, soil minerals and enhanced biological activity, as it resulted in an increase in the proportion of fungivorous browsers and predatory microarthropods. These groups reflect increased fungal biomass and prey availability, respectively. Soil inoculation did not significantly increase overall species richness, but it did slightly enhance the richness of herbo-fungivorous grazers, a group important for decomposing fungal biomass. However, this effect was limited and unlikely to substantially accelerate decomposition.

In conclusion, SRP application seems to be a promising method of restoring soil ecosystems and stimulating soil biota. Long-term restoration, however, requires reducing nitrogen deposition and stimulating the richness of soil microarthropods by identifying suitable donor sites from areas with lower nitrogen deposition levels.

Introduction

Forest soils are under pressure in many nutrient poor, acidic soils in Western Europe due to the accumulative deposition of nitrogen. Excess nitrogen deposition alters soil chemistry through multiple pathways (Bobbink et al. 2010; de Vries et al. 2014). For one, it causes soil acidification that leads to the release of aluminium (Al^{3+}) that is toxic to plant roots (Shortle and Smith 1988; de Graaf et al. 1997; Rout et al. 2001; Rahman and Upadhyaya 2020). This is often measured using the aluminium/calcium (Al:Ca) ratio in the soil solution, which is a good indicator of the risk of adverse impacts on plant growth and nutrition (Cronan and Grigal 1995). Soil acidification additionally causes the leaching of base cations, such as calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^{+}) (Wellbrock and Bolte 2019). The base saturation as a result can be low in nitrogen-impacted forests, especially in acid sensitive and nutrient poor sandy soils, which indicates a reduced buffering capacity and potential nutrient imbalances for plants. Furthermore, excess nitrogen disrupts the ecological stoichiometry of carbon: nitrogen: phosphorus (C:N:P) ratios in plant material (Aber et al. 1995; Sardans et al. 2012; Vogels et al. 2023), often leading to P-limitation (Penn and Camberatio 2019), an element necessary for the growth and reproduction of fauna. The soil acidification, leaching of base cations and P-limitation induced by excess nitrogen deposition are all stress factors of the arthropod community (Vogels et al. 2013; Nijssen et al. 2017; Vogels et al. 2017).

The microarthropod community is an important arthropod group of the forest soil ecosystem (Wallwork 1983). This group of animals is often highly abundant and species rich on local scale (Anderson 1975a; Siepel 1996b; Zaitsev and Berg 2001) and play a key role in nutrient cycling and organic matter decomposition (Seastedt 1984). Fungivorous grazing species are especially important since their grazing on fungi breaks down the chitin in cell walls of (old) fungi, which releases nutrients from the (also senescent) fungal cell walls into the soil system, and thereby stimulates the activity of microbes that have a role in the decomposition of organic matter (van der Drift and Jansen 1977; Hanlon and Anderson 1979; Siepel and Maaskamp 1994; Filser 2002). The effect of excess nitrogen deposition on soil microarthropods remains understudied, however there is evidence that in particular fungivorous species are vulnerable to nitrogen deposition effects (Gan et al. 2013). Without these animals that graze on fungi, there is a risk that dead fungal biomass is not broken down sufficiently, leading to an inhibition in the decomposition of soil organic matter. Therefore, it is critical that the species richness of this group can be restored.

However, there are two main challenges with the restoration of the soil microarthropod community: 1) the current soil conditions (pH and the nutrient balance) are unsuitable for many species and need to be improved before effective restoration is possible, and 2) many microarthropod species have poor dispersal ability (Ojala and Huhta 2001) which means that even if soil conditions are improved, species richness might not be restored without additional help (Bruggink et al. 2025).

For the first challenge, several restoration methods have been used in the past, including sod cutting and liming, however these methods have not proved to be suitable. Sod cutting (i.e.,

removing the topsoil layer) was often used in heathlands and occasionally in forests as well (Boxman and Roelofs 2006). Sod cutting was used to remove excess N in the soil, however it removed P as well, further inducing P-limitation (Siepel et al. 2018), while additionally being a disturbance factor for soil microarthropods, as was found in studies done in heathlands (Guo 2021). Liming, while effective in restoring the soil pH (Haynes 1982; Boxman and Roeloefs 2006; Court et al. 2018; Jansone et al. 2020), accelerated the N mineralisation and thereby N availability, due to its fast dissolution and quick effect on pH, and therefore increased the stoichiometric imbalance and intensified P-limitation (Burke and Raynal 1998). Liming, for this reason, has been found to decrease the fauna richness in the long-term (Siepel et al. 2019; Vogels et al. 2021). An alternative method is the addition of silicate-based rock powder (also known as rock dust/flour) (SRP). The benefit of SRP application is that it has a lower acid neutralizing capacity and weathers more slowly than lime, which makes it more suitable as a long-term solution (Taylor et al. 2021; van der Bauwhede et al. 2024a; Findeisen et al. 2025; van der Bauwhede et al. 2025). Moreover, it can replenish the soil with base cations (Ca, K and Mg) that have been previously lost due to soil acidification and thereby increase the base saturation (Lewis et al. 2021; van der Bauwhede et al. 2024b). The soil fauna community has not often been included in experiments using SRP (Vicca et al. 2021) so the effect of this method on soil microarthropods is still unknown.

For the second challenge, since the microarthropod community cannot easily reestablish itself after disturbance, microarthropod species may need to be brought into the habitat proactively by soil inoculation. The use of soil inoculation has been successful for restoring plant richness (Vécrin and Muller 2003; Carbajo et al. 2011; Pywell et al. 2011; Bulot et al. 2014; Wubs et al. 2016), but studies restoring soil fauna are more rare and present inconclusive results (Parreira et al. 2022). Brady et al. (2002) reported a change in community composition and an increase in Shannon-diversity of macroinvertebrates following soil inoculation and Benetková et al. (2020) reported a change in nematode community following soil inoculation, while Kardol et al. (2009) found that soil inoculation did not significantly alter the nematode community. Experiments that include soil microarthropods reported increased abundance or Shannon-diversity following inoculation (Wubs et al. 2016; Moradi et al. 2018; van der Bij et al. 2018), however the effect on the species richness is rarely quantified and therefore remains a key knowledge gap.

An important advantage of quantifying species richness is that it enables the use of species traits to find generality in patterns (McGill et al. 2006; Verberk et al. 2013). To evaluate the effect of soil microarthropods on the decomposition processes, we need to be able to separate their feeding strategy. We can distinguish feeding strategies of soil microarthropods using a few different methods including carbohydrase activity (Siepel and de Ruiter-Dijkman 1993) or stable isotope analysis (Schneider et al. 2004). The carbohydrase method not only separates microarthropods into fungivores and herbivores, but it can distinguish browser and grazer strategies. Fungivorous grazers have chitinase activity and can therefore feed on fungal cell walls, while fungivorous browsers only feed on fungal cell content.

To address the knowledge gaps in the mentioned restoration methods, we established a full factorial experiment in five old oak forests as replicates in the Netherlands, using SRP

application and microarthropod soil inoculation. We chose the old oak forest habitat (H9190 in the EU habitats directive) because this habitat occurs naturally in poor, acidic soils with low buffering capacity, making these forests particularly vulnerable to negative effects of excess nitrogen deposition (Simkin et al. 2016; Hommel et al. 2020). Each plot received either a soil inoculation from a local donor site or remained uninoculated (null treatment), and one of three SRP treatments (Eifelgold, Soilfeed, or a control).

For this experiment we have developed two main hypotheses:

- Hypothesis 1: SRP addition will improve the pH in the soil, which in turn will increase soil biological activity. We will focus on the proportion of fungivorous browsers, which are indicators of fungal activity since they only feed on cell content and therefore need to feed on living fungi, and the proportion of predatory microarthropods since they are an indication of higher prey (nematodes, microarthropods and insect larvae) availability.
- Hypothesis 2: Soil inoculation will increase the species richness of microarthropods by introducing new species into the system. Important is whether fungivorous grazing species (fungivorous grazers and herbo-fungivorous grazers) can be introduced to restore the soil decomposition processes (fungivorous browsers are often phoretic, thus are not dispersal limited Siepel (1994)).

Method

Site information

We replicated this experiment in 5 forest sites, all situated in the old acidophilous oak forest habitat (Fig. 5.1; H9190 in the EU habitats directive). Soil measurements in 2020 for these 5 locations indicated a high level of soil acidification with a low average pH_{NaCL} of 3.07 ± 0.06 and a base saturation of 11.69 % ± 1.31 in the top 10 cm of mineral soil.

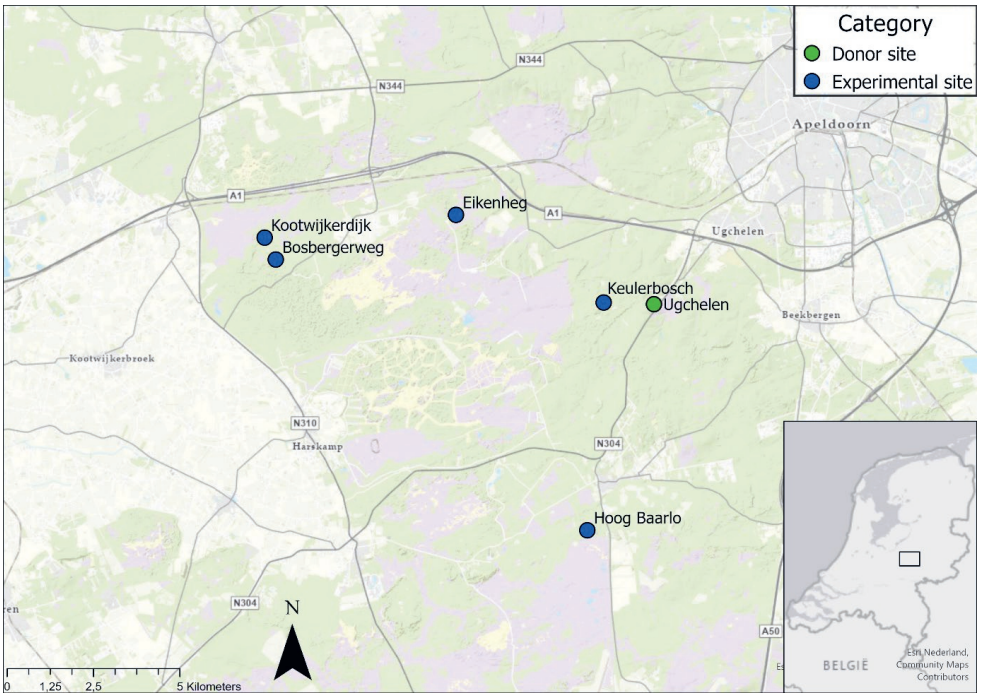


Fig. 5.1: *Map of the experimental sites and the donor location. The five experimental sites in blue are in the H9190 old oak forest habitat and the donor location in green is in the H9120 beech-oak forest. The donor location moreover represents an old forest soil site, having been forested since at least the Middle Ages.*

The donor site, Ugchelen, is from the similar beech-oak forest habitat (H9120 in the EU habitats directive) that is originally slightly better buffered against acidification (Evans 2006), however it currently is in an acidified state as well. Here, we measured a pH_{NaCL} of 3.18 in 2024 and an even lower base saturation of 4.98 % in the top 10 cm of mineral soil. We nevertheless identified this location as the best local donor site since it is an old forest location that has been known to be forested since at least the Middle Ages and it was previously measured in 1994, among two other old forest sites, to have a very high species richness of microarthropods (Siepel 1996b; Bruggink et al. 2025). We took new soil samples in the three old forest locations in 2023 and in all locations there was a decline in the species richness of soil microarthropods. Ugchelen was chosen among the three locations based on

the species richness of fungivorous grazers + herbo-fungivorous grazers (Fig. S5.1a) and the percentage of fungivorous grazers + herbo-fungivorous grazers (Fig. S5.1b).

Experimental set up

At each old oak forest location an experiment was set up with three 0.5 hectare plots (70x70 meter) to test the impact of SRP application on the forest ecosystem. The three plots were then randomly assigned to one of three treatments: (1) the addition of 10 t ha^{-1} Eifelgold, (2) the addition of 10 t ha^{-1} Soilfeed, or (3) an untreated control plot. The mineralogy of the SRP is provided in Supplementary Table S5.1 (Dar-Tech, Inc. n.d; Servaplant 2019; Swoboda et al. 2021). Eifelgold as a basalt-based SRP is expected to weather more quickly than the fiodite-based Soilfeed (van der Bauwhede et al. 2024a) and Eifelgold additionally contains more P than Soilfeed. SRP was manually applied between mid-February and mid-April 2020. Within each 0.5 hectare plot, we created two smaller 2x2 meter plots where soil inoculation was applied in one plot (inoculated), while the other remained untreated (null). These subplots were located within 6x6 meter exclosure areas with a 2 meter high fence to prevent disturbance of large grazing animals. We visualized this experimental set up in Fig. 5.2.

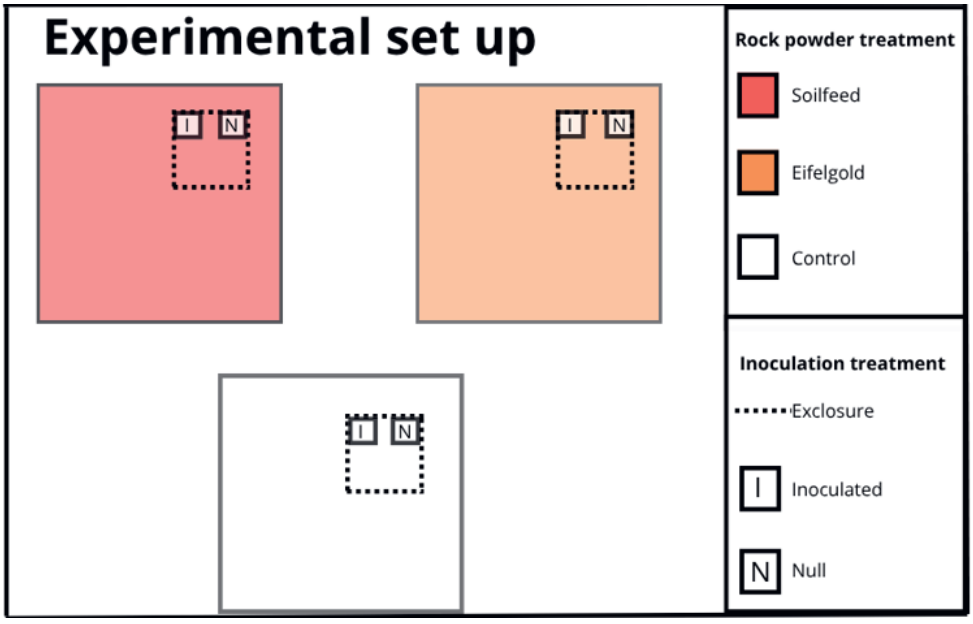


Fig. 5.2: *Example of the experimental set up in a site. The bigger squares visualize the 70x70 meter rock powder plots. The smaller dotted square is the 6x6 meter exclosure and the small squares within that exclosure are the 2x2 meter inoculation plots. Three soil samples for microarthropods were taken in each 2x2 meter plot, adding up to 18 samples per location and therefore 90 in total. Note that this figure is not done to scale.*

Soil inoculation was applied in October 2023. 2 m^2 of the FH layer + the top 5 cm of mineral soil (litter layer was not included) from the donor location Ugchelen was added to each inoculated plot and spread evenly across the plot. This means that 30 m^2 soil was collected

from the donor location in total. Donor soil was taken using spades and spread across a wide area to minimize disturbance at the donor site.

Data collection

Three soil samples in each 2x2 meter subplot were taken in March 2025, one and half year after the soil inoculation was applied. All soil samples were taken using a soil auger to 5 cm mineral soil depth (+ the FH and litter layer) and 5 cm Ø. Microarthropods were extracted from the soil samples using Berlese-Tullgren funnel following standard protocol (Siepel and van der Bund 1988) and collected in 70% ethanol. Species were then mounted to microscope slides in 30% lactic acid for species identification. All identification was done to species level, except for some juveniles and individuals from the *Mesaphorura* (Collembola) genus. Unidentified juveniles were omitted only from richness analysis. Identified species were then assigned a feeding guild according to Siepel and de Ruiter-Dijkman (1993). This method is based on the activity of three enzymes: cellulase (for plant cell walls), chitinase (for fungal cell walls) and trehalase (for fungal cell contents). The following feeding guilds are distinguished: bacterivore, fungivorous browser (feeding of fungal cell content only), fungivorous grazer (feeding on fungal cell wall and content), general predator (predatory species), herbivorous browser (feeding on plant cell content only), herbivorous grazer (feeding on plant cell wall and content), herbo-fungivorous grazer (feeding on plants and fungi, both cell wall and cell content), opportunistic herbo-fungivore (feeding on plant cell walls and contents and fungal cell content) and omnivore.

After soil microarthropod extraction, the three soil samples of each plot were pooled together to create a single soil sample per plot for soil chemical analyses. The soil chemical analysis followed Findeisen et al. (2025). In short, exchangeable cation concentrations (e.g. Ca, K, Mg) and pH were measured with a NaCl-extraction and the base saturation with a strontium extraction.

Statistical analysis

The effects of inoculation and SRP treatment on microarthropod density, species richness and soil chemical variables were analyzed using linear mixed-effects models, with site as a random effect and the interaction between inoculation and SRP treatment as fixed factors. Models were fitted using the lme4 package (Bates et al. 2015) in R studio (R Core Team 2025) and Type III ANOVA tables were generated with the lmerTest package (Kuznetsova et al. 2017) using the Kenward–Roger method to approximate degrees of freedom (Kenward and Roger 1997). Pairwise comparisons were done on models with significant results using estimated marginal means with TukeyHSD adjustment using the emmeans package (Lenth and Piaskowski 2025). The density of soil microarthropods, the total species richness of all soil microarthropods and the fungivorous grazer species richness were $\log_{10}$ transformed to improve normality. This same model was used for the proportion and species richness of feeding guild and the abundance of individual species that have a total abundance of ≥ 50 individuals. The proportions of feeding guilds were logit transformed to improve normality of proportional data (Warton and Hui 2011). The base saturation was logit transformed for the same reason.

To examine whether soil inoculation was successful in introducing new species in the old oak forest locations, we analyzed the number of unique species in the inoculated and null plots (unique is defined as a species that occurs either only in inoculated or null plots). We compared the number of observed unique species with the number of unique species that would be expected if every species were distributed randomly using a χ^2 test. The expected sum of unique species was calculated by using formula 1. The total permutations a species can occur across all samples was calculated using formula 2 and the total permutations a species can occur in one category using formula 3.

A detrended correspondence analysis (DCA) analysis with 26 segments was to compare the inoculated and null samples to the soil samples taken in Ugchelen in 2023. The DCA was performed using the vegan package (Oksanen et al. 2025). Ellipses for treatment groupings were created using a multivariate t-distribution with a confidence level of 95% (Friendly et al. 2013).

Expected # unique species = $\sum \frac{Category\ (N)}{Total\ (N)}$ (1)

Total (N) = $\frac{90!}{(90-N)!N!}$ (2)

Category (N) = $\frac{45!}{(45-N)!N!}$ (3)

All plots were created using ggplot2 (Wickham 2016) and ggbeeswarm (Clarke et al. 2023).

Results

Soil chemical analysis

SRP treatment significantly increased the soil pH of the top 5 cm mineral soil (+ FH layer) by a mean value of 0.3 in both Eifelgold and Soilfeed treatment (ANOVA, $F_{2, 20} = 7.390$, P = 0.004, Table 5.1, Supplementary Table S5.2 and S5.3). The base saturation was about 6% higher in Eifelgold compared to the control and 4% higher in inoculated plots compared to the null, although neither was statistically significant (ANOVA, SRP effect: $F_{2, 20} = 2.318$, P = 0.124; inoculation effect $F_{1, 20} = 2.050$, P = 0.168). Eifelgold application was able to significantly increase the salt-extracted K (ANOVA, $F_{2, 20} = 3.761$, P = 0.041) and Mg (ANOVA, $F_{2, 20} = 9.136$, P = 0.002) concentrations compared to the control plots. The salt-extracted P concentration, in contrast, was not significantly different between the SRP treatments (ANOVA, $F_{2, 20} = 0.799$, P = 0.464). Soilfeed-treated plots and inoculated plots had a higher level of manganese (Mn) compared to control and null plots, although neither was significant (ANOVA, SRP effect: $F_{2, 20} = 1.775$, P = 0.195; inoculation effect: $F_{1, 20} = 3.566$, P = 0.074). While soil inoculation did not affect the pH, it did significantly decrease the Al:Ca ratio (ANOVA, $F_{2, 20} = 4.971$, P = 0.037).

***Table 5.1**: Mean values (± SE) of soil chemical parameters measured in experimental plots. pH, Al and Ca, K, Mg, Mn and P were measured using a NaCl solution and the base saturation using a strontium extraction. The chemical analysis was done on the same soil samples used for soil microarthropod extraction, after pooling the three soil samples of each 2x2 m experimental plot together. The soil samples are of the top 5 cm mineral soil + LFH layer and 5 cm Ø.*

Treatment	pH (NaCl)	Base saturation (%)	Al:Ca ratio	Ca (µmol/L)	K (µmol/L)	Mg (µmol/)	Mn (µmol/L)	P (µmol/L)
Silicate Rock Powder treatment								
Control	2.91 ± 0.06	29.11 ± 3.40	0.48 ± 0.13	2879.0 ± 219.15	974.58 ± 107.32	1069.6 ± 166.41	177.0 ± 18.26	54.2 ± 12.26
Eifelgold	3.20 ± 0.09	35.10 ± 4.49	0.38 ± 0.09	3674.8 ± 501.13	1337.7 ± 126.16	2309.7 ± 390.58	227.1 ± 40.60	33.8 ± 10.60
Soilfeed	3.19 ± 0.06	26.88 ± 8.50	0.49 ± 0.09	3278.5 ± 371.39	1140.3 ± 99.01	1082.8 ± 150.79	256.9 ± 29.15	44.1 ± 17.15
Inoculation treatment								
Inoculated	3.07 ± 0.07	32.56 ± 3.28	0.37 ± 0.07	3683.8 ± 355.79	1217.2 ± 96.61	1561.0 ± 246.69	253.4 ± 27.82	43.5 ± 10.87
Null	3.13 ± 0.07	28.16 ± 3.07	0.53 ± 0.09	2871.1 ± 261.68	1084.5 ± 95.62	1413.7 ± 271.43	187.3 ± 20.94	44.5 ± 11.42

Hypothesis 1: The effect of SRP application on the fungivorous browsers and predatory microarthropods

The density of all soil microarthropods was not significantly different between the SRP treatments (ANOVA, $F_{2, 80} = 0.659$, $P = 0.420$) or the soil inoculation treatments (ANOVA, $F_{2, 80} = 0.199$, $P = 0.820$).

SRP application changed the composition of the soil microarthropod community. The proportion of fungivorous browsers differed strongly between SRP treatments (ANOVA, $F_{2, 80} = 10.089$, $P < 0.001$, Fig. 5.3a, Supplementary Table S5.2 and S5.3). Eifelgold plots had a 1.5-fold higher proportion of fungivorous browsers (0.32 ± 0.03) than both the control (0.18 ± 0.02) and Soilfeed plots (0.20 ± 0.02). This higher proportion of fungivorous browsers in the Eifelgold plots can be seen in the density of several species. The most prominent example is the prostigmatid species *Pediculaster mesembrinae*, which had a mean density of 25,465 ± 9,214 ind./m^2 in the Eifelgold plots, while it had a mean density of only 1,468 ± 1,289 ind./m^2 in the control plots and 1,136 ± 662 ind./m^2 in the Soilfeed plots (ANOVA, $F_{2, 80} = 6.518$, $P = 0.002$) . Other species that were significantly more abundant in Eifelgold plots compared to control and/or Soilfeed plots include *Schwiebea talpa* and *Protaphorua armata* (Supplementary Table S5.3).

The effect of SRP addition on the fungivorous browser proportion had nearly significant interaction term with the soil inoculation treatment (ANOVA, $F_{2, 80} = 2.890$, $P = 0.061$). The control plots were negatively affected by soil inoculation (inoculated: 0.12 ± 0.01; null: 0.23 ± 0.04), although there was no overall significant effect of soil inoculation on the fungivorous browser proportion (ANOVA, $F_{1, 80} = 2.223$, $P = 0.140$).

The proportion of predatory mite species differed significantly between SRP treatments (ANOVA, $F_{2, 80} = 4.840$, $P = 0.010$, Fig. 5.3b, Supplementary Table S5.2 and S5.3). Soilfeed plots had a 1.5-fold higher proportion (0.09 ± 0.02) of predatory species than both control (0.06 ± 0.01) and Eifelgold plots (0.06 ± 0.01). The most prominent difference between SRP treatments was for the mesostigmatid species *Pachylaelaps bellicosus*, which had significantly higher density in Soilfeed plots (Soilfeed: 1,375 ± 326 ind./m^2, control: 626 ± 153 ind./m^2 and Eifelgold plots: 713 ± 183 ind./m^2; ANOVA, $F_{2, 80} = 3.134$, $P = 0.041$; Supplementary Table S5.4) compared to the control plots. The proportion of Collembola, an important food source of many predatory microarthropods, did not differ between SRP treatments (ANOVA, $F_{2, 80} = 0.220$, $P = 0.803$).

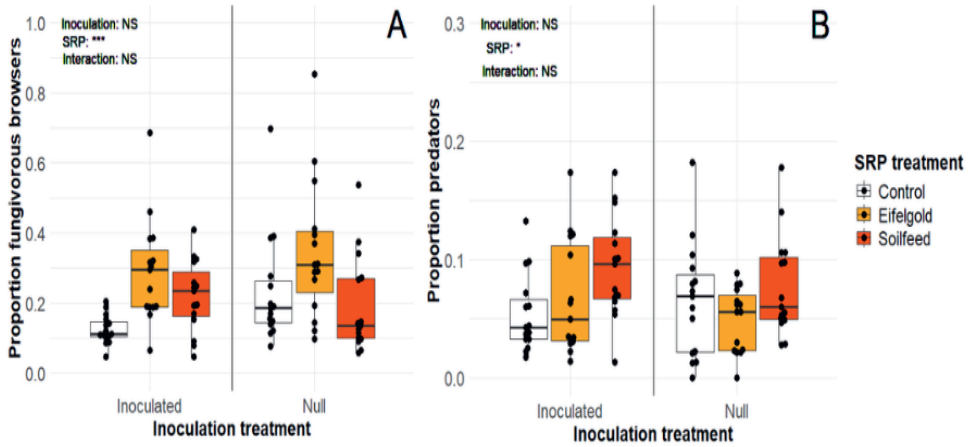


Fig. 5.3: *The proportion of fungivorous browsers (a) and predatory microarthropods (b) in the experimental plots as affected by either soil inoculation (inoculated and null) or rock powder treatment (control, Eifelgold and Soilfeed). Note the different values on the y-axis.*

Hypothesis 2: The effect of soil inoculation on the species richness of microarthropods

The species richness of microarthropods was not significantly affected by either soil inoculation (ANOVA, $F_{1, 80} = 1.739$, P = 0.191, Fig. 5.4a, Supplementary Table S5.2) or SRP treatment (ANOVA, $F_{2, 80} = 0.142$, P = 0.868). No effect of inoculation was found on the fungivorous grazer species richness either (ANOVA, $F_{1, 80} = 1.279$, p = 0.261). Inoculated plots did have a higher herbo-fungivorous species richness than null plots (inoculated: 4.00 ± 0.20; null: 3.49 ± 0.17, Fig. 5.4b, Supplementary Table S5.1), which was nearly significant (ANOVA, $F_{1, 80} = 3.869$, P = 0.053). This higher species richness of herbo-fungivorous species is reflected in multiple species that had a higher occurrence in inoculated plots compared to the null plots: *Nanhermannia nana* (inoculated: 25 samples, null: 18 samples), *Microtritia minima* (inoculated: 23 samples, null: 16 samples) and *Pergalumna nervosa* (inoculated: 8 samples, null: 2 samples). The effect of inoculation on herbo-fungivorous species richness differed between SRP treatments, with control plots having the strongest effect (inoculated 4.47 ± 0.31; null: 3.40 ± 0.29) and Eifelgold plots having no effect of inoculation (inoculated: 3.40 ± 0.41; null: 3.40 ± 0.34), however the interaction term of inoculation and SRP treatment was not statistically significant (ANOVA, $F_{2, 80} = 1.412$, P = 0.250).

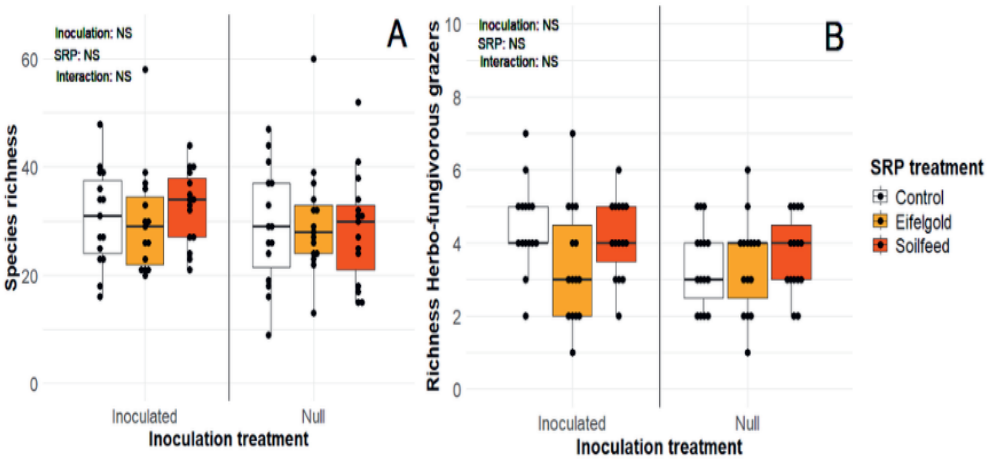


Fig. 5.4: *The total species richness (4a) and species richness of herbo-fungivorous grazers (4b) in the experimental plots as affected by either soil inoculation (inoculated and null) or rock powder treatment (control, Eifelgold and Soilfeed).*

The inoculated plots had 24 unique species in comparison to 15 unique species in null plots (20.64 unique species would be expected in both categories if species were randomly distributed), however the difference between expected and observed unique species was not statistically significant (χ^2 test, $\chi^2 = 2.098$, P = 0.148; Supplementary Table S5.5). All the unique species were uncommon in the dataset with the most distinctive unique species in the inoculated plots, *Oribatula tibalis*, only occurring in 3 out 45 inoculated samples.

The DCA analysis shows that soil inoculation brought the soil microarthropod community closer to the Ugchelen soil samples. This effect of soil inoculation was not clear in the Eifelgold and Soilfeed plots, likely due to a strong signal of the SRP application (Fig. 5.5a), however the effect in the control plots was clearer (Fig. 5.5b). The axis-length of DCA1 is 3.504 and the eigenvalue is 0.358, while DCA2 had an axis-length of 2.656 and an eigenvalue of 0.241. The species (only considering species with a total abundance of at least ≥50 individuals) most strongly associated with Ugchelen were *Oppiella nova* (DC1 = 1.67), *Microtydeus subterraneus* (DC1 = 1.67), *Berniniella sigma* (DC1 = 1.63), *Suctobelbella subcornigera* (DC1 = 1.56) and *Microtritia minima* (DC1 = 1.53). These species were all more abundant in inoculated plots compared to null plots (Supplementary Table S5.4). The species *Oppiella nova* additionally stands out because it had the highest difference in occurrence between inoculated plots (38 samples) and null plots (26 samples) among all species.

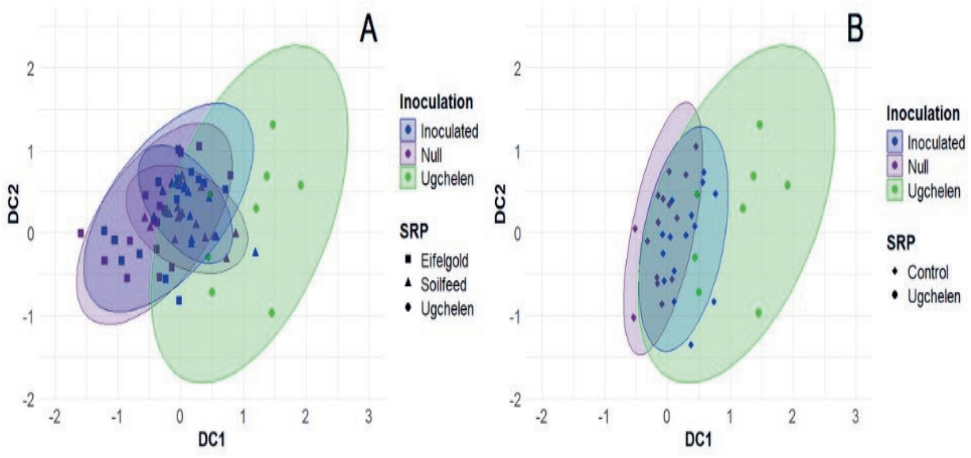


Fig. 5.5: *Detrended component analysis showing the inoculated and null plots in comparison to the soil samples of the donor location Ugchelen. Figure a show the SRP treatment (Eifelgold and Soilfeed) while (b) shows the control treatments with no SRP application. Ellipses were created using a multivariate t-distribution (Friendly et al. 2013) with a confidence level of 95%*

Discussion

The effect of SRP application

SRP application significantly increased soil pH and, despite no significant change in base saturation, points to a slightly improved buffering capacity of the top 5 cm mineral soil and the LFH layer. This increase coincided with an increase in soil activity as we measured with the proportion of fungivorous browsers and predatory microarthropod species. Fungivorous browsers were more abundant in the Eifelgold treated plots compared to Soilfeed and control plots. These species generally have a quick life cycle (Siepel 1994) and depend on living fungal cells for their nutrients. Their observed increase therefore suggests enhanced fungal activity in Eifelgold plots. The species most common in Eifelgold plots *Pediculaster mesembrinae* is a known pest for mushroom cultivation (Hernández-Abarca et al. 2005; Szafranek and Lewandoski 2017) and is an example of a species that can quickly take advantage of the increased food availability. Previous findings by Findeisen et al. (2025) reported increased abundance of saprotrophic fungi following Soilfeed application in heathlands, although we did not find a clear effect of Soilfeed on the fungivorous browsers.

The increased fungal activity in Eifelgold plots could be related to the quicker effect on the base cations (Mg and K) concentration that was measured in Eifelgold plots. Additionally, an increase in P availability would explain increased fungal growth (Beever and Burns 1981; Elser et al. 1996). While the salt-extracted P was not significantly different between the SRP treatments, previous research on foliar P concentration in *Quercus robur* showed that Eifelgold application increased the P-uptake (Sitters et al. 2026), which would not show in the P-NaCl measurements, apparently a higher flux while in the same (low) soil concentration remains.

The increase in predatory mite species in the Soilfeed plots can also be seen as an indication of increased biotic soil activity since it signals a higher prey availability. The species that had the higher increase in abundance in Soilfeed plots, *Pachylaelaps bellicosus*, is a known nematode predator (Karg 1993; Szymkowiak 2001) and is therefore an indication of higher nematode abundance. Nematodes have short life cycles and the abundance of nematodes is positively correlated to the primary production in an ecosystem (Yeates 1979). Microbial feeding nematodes additionally contribute to the stimulation of microbial respiration and therefore the nutrient flow in soil ecosystems (Freckman 1988; Bardgett et al. 1999; Yeates 2003). The response of nematodes to SRP application therefore requires further attention to get a better picture in how the composition and richness of this group is affected. Interestingly, the bacterivore mite species *Histiostoma sapromyzarum* had a higher abundance in Soilfeed plots compared to Eifelgold and control plots (Supplementary Table S4) so this could be an indication of higher bacteria activity in Soilfeed plots. Soilfeed plots additionally had a slightly higher Mn concentration. The study of Vogels et al. (2024) showed that Mn-limitation is a limiting factor for field cricket (*Gryllus campestris* L.) fitness, although this has not been tested for soil microarthropods yet.

The effect of soil inoculation

Soil inoculation could not increase microarthropod species as we had hypothesized. However, it did increase the richness of herbo-fungivorous grazers, which is a group that plays an important role in the soil decomposition process by feeding on dead fungal matter (Siepel and Maaskamp 1994; Siepel et al. 2019). This increase is relatively minor and seems to be potentially impeded by SRP treatment as Eifelgold treated plots did not have an increase in herbo-fungivore species richness. Given that herbo-fungivorous grazers have a slow life cycle (Siepel 1994; Ermilov and Łochyńska 2008; Wehner et al. 2014) it is possible they need more time to establish their population since inoculation.

While the inoculated plots had a higher number of unique species than the null plots, this difference was not statistically significant from the expected distribution. Moreover, since all the unique species were rare in the dataset, no species was introduced in a consistent manner. Thus, while some species were helped with soil inoculation, introduction of species was not sufficient. Since the group of fungivorous grazers was unaffected by soil inoculation, we do not expect that there was a sufficient increase in species richness to restore soil decomposition processes.

A possible explanation is that the donor site did not contain a sufficiently diverse microarthropod community to effectively restore recipient sites. We chose the best local location available as donor location as we wanted to stay within the same biotope (oak forests) and a similar habitat within the same geographic area. Apparently, this site has deteriorated due to excess and continuous nitrogen deposition as well, which can be seen in the low pH and base saturation that was measured in 2024 and the declined soil microarthropod richness compared to 1994. This indicates a worrying situation of the state of forests in the Netherlands and a strong reduction of nitrogen deposition is needed.

Conclusion

SRP application is able to improve buffering of the soil and thereby increases the biological activity in the soil, in terms of increased fungal biomass and abundance of nematodes, which is expected to improve the decomposition at least on a short term. Interestingly, soil inoculation decreased the soil Al:Ca ratio, which is due to Ugchelen soil having a higher calcium content in comparison to the old oak forest locations. This shows that the combination of both strategies can mitigate some of the soil acidification effects of nitrogen deposition. However, since the richness of fungivorous grazing microarthropods has not been effectively restored, the issue of soil organic matter being stored in dead fungal biomass might not be solved, which will impede decomposition on a longer term.

We chose a local site for soil inoculation because we know that it has similar habitat and climate, however, it seems that Dutch nature areas have been degraded too much to facilitate the restoration process. Therefore, locations from the same habitat in other European countries, that have lower level of nitrogen deposition compared to the Netherlands, could be explored. For this it is critical that interactions of soil inoculation and SRP (or other soil improvement methods) are taken in account.

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Author contributions
Joren Bruggink: Conceptualization, Data Curation, Formal Analysis, Software, Visualization, Writing – Original Draft. **Marie-Charlott Petersdorf:** Conceptualization, Writing – Review and Editing. **Wilbert L.P. Jansen:** Data Curation, Writing – Review and Editing. **Judith Sitters:** Data Curation, Writing – Review and Editing. **Maaike J. Weijters:** Data Curation, Project Administration, Writing – Review and Editing. **Wilco C.E.P. Verberk:** Conceptualization, Supervision, Writing – Review and Editing. **Henk Siepel:** Conceptualization, Resources, Supervision, Writing – Review and Editing.

Chapter 6

Synthesis

Restoration of forest ecosystems following the negative effects of excess nitrogen deposition is an increasingly important topic. The high level of nitrogen deposition negatively impacts forest ecosystem functioning as it causes soil acidification (De Vries et al. 2014; Chen et al. 2023), creates stoichiometric imbalances in plant tissue (Aber et al. 1995), including phosphorus (P) shortages (Sardans et al. 2012), which subsequently lead to loss of plant and invertebrate species (Cleland and Harpole 2010; Nijssen et al. 2017; Vogels et al., 2024). Nitrogen-impacted forests, for these reasons, are in a degraded state with declining tree vitality and an accumulation of soil organic matter. The group of soil microarthropods are important for the decomposition of litter (Seasedt 1984; Siepel and Maaskamp 1994; McCary and Schmidt 2021) and are therefore expected to have a key role in the restoration of these forest ecosystems. However, their response to excess nitrogen deposition is not well-studied yet. In my thesis I therefore set out to study the status and role of soil microarthropods in old oak forest, an example of a habitat type that is strongly impacted by nitrogen deposition and thereby contribute to the restoration of this habitat and other forest ecosystems in nutrient poor, acidic soils.

Since the role of microarthropod species in litter decomposition depends on their feeding strategy, I used feeding guilds to categorize species into groups. The feeding guilds are based on the carbohydrase activity of cellulase (plant cells), chitinase (fungal cells) and trehalase (fungal cell content) and not only separates herbivores from fungivores, but it can make the distinction between grazers (feeding on both cell wall and cell content) and browsers (feeding on cell content only) (Siepel and de Ruiter-Dijkman 1993). While fungivorous grazers positively stimulate the decomposition process, fungivorous browsers negatively impact decomposition since they can only feed on living cells and leave the cells senescent (Siepel and Maaskamp 1994).

In this synthesis chapter I will discuss the most important findings in my research using three main aims: 1) evaluating the status and threats for soil microarthropod communities in Dutch forest ecosystems, 2) discuss the importance of soil microarthropods in litter decomposition processes and the implications of species loss and 3) evaluating the effectiveness of restoration methods (silicate rock powder, SRP, application and soil inoculation) for restoring the characteristic soil microarthropod community.

Research aim 1: The status of the soil microarthropods community in Dutch forest ecosystems

In chapters 2 and 3 I evaluated the status of the soil microarthropod community in Dutch forests on sandy soils and identified three key threats to the species richness: high disturbance which is problematic due to poor dispersal ability of many species, P-limitation and the decline of fungi availability, which is an important food source for many species. I will discuss all those factors in more detail below.

Dispersal limitation

It has been known that many soil microarthropods, particularly many oribatid species, are poor dispersers: they are small, have limited mobility and have a relatively slow life cycle

(Siepel 1994; Ojala and Huhta 2001). What was not well studied is how such poor colonisation ability shapes microarthropod richness in forest soils differing in age. Therefore, I set out to compare the species richness of soil microarthropods in old forest soils (forested since at least the Middle Ages), old hedgerow soils (remnants of old forests, but adjacent to agricultural land) and young forests (afforested from heathland between 1880 and 1930). What I found was that the species richness of mites was much lower in young forest soils compared to both old forest soils and old hedgerows. Not only was the overall species richness much higher in old forests soils, these older soils also harboured more unique species compared to younger soils. The species richness of springtails, in contrast, was not significantly different between young forests and old forests, which concurs with the hypothesis that springtails are not limited by dispersal due to their furcula granting them better colonisation ability (Santorufo et al. 2021). Taken together this indicates that many mite species have not yet been able to colonise young forest soils, despite young forests being over 100 years in age.

The difference in species richness between old forest soils and young forest soils was the strongest for the fungivorous grazers and herbo-fungivorous grazer guilds, which are oribatids that both are typically vulnerable to disturbance in the soil and do not seem have the dispersal ability for recolonisation. Both of those guilds can break down the chitin in fungal cell walls and are therefore important for litter decomposition processes in the soil (Siepel and Maaskamp 1994). Their lower richness in younger forests soils therefore suggests a less efficient decomposition in younger forest soils.

P-limitation induced by excess nitrogen deposition

The limitation of phosphorus (P) in nitrogen-impacted forests is a potentially large threat for animals as it skews the N:P ratio. Previous studies on insects revealed that higher, unbalanced, N:P ratios in plant material resulted in a lower fitness of species (Vogels et al. 2017; Vogels et al. 2024). Research on soil microarthropods and stoichiometry has been limited. Since the old hedgerows I studied in my second chapter are adjacent to agricultural land and therefore have been subjected to continued phosphorus influx in the system, I was able to test how P availability affects the soil microarthropod community.

While old hedgerows and old forests were comparable in terms of the species richness of fungivorous species, old hedgerows had a higher number of herbivorous species compared to old forest soils. Plants are less homeostatic in their N:P ratio compared to fungi (Beever and Burns 1981) leading to herbivorous species being more susceptible to P-limitation than fungivorous species (Elser et al. 2000; Siepel et al. 2018). Old hedgerows furthermore had a much higher density of soil microarthropods of the large size class (defined as species that are >900 µm) compared to both old forest and young forest souls, while there was no difference in density in the smaller size class (<900 µm). Larger species need more P to complete their reproduction and growth and are therefore more vulnerable to P-limitation (Elser et al. 1996; Mulder 2010). In conclusion, I found evidence for P-limitation, especially in herbivorous and in large microarthropod species.

Decline of food sources induced by excess nitrogen deposition

In my third chapter I analysed the status of soil microarthropods in the old oak forest habitat in the Netherlands. The old oak forest habitat (H9190 in the EU Habitats Directive) is present on nutrient poor and acidic sandy soils and is therefore vulnerable to high nitrogen deposition effects (Evans 2006; Hommel et al. 2020). From abiotic measurements we know indeed that this habitat is in a poor state which low pH_{NaCl} (2.99 ± 0.04) and low base saturation (12.89 ± 1.23 %).

I found in my research that this poor state is also reflected into the soil microarthropod community, which had a very low abundance and species richness compared to the old forest locations studied in Chapter 2. Specifically, I found a strong decline in the fungivorous mite community (both fungivorous browsers and fungivorous grazers, which rely fully on fungi for their nutrition). In contrast, declines were much less pronounced for mites that can feed on both fungi and plants (herbo-fungivorous grazers and opportunistic herbo-fungivores).

This pattern indicates issues with fungal food sources in our forests. We know from the literature that ectomycorrhizal (ECM) fungi are in decline due to excess nitrogen deposition (Arnolds 1991; Suz et al. 2014). When plants can obtain sufficient N from their environment themselves, they provision less carbon (C) to the symbiotic mycorrhiza, which has been linked to a strong decline in fungal biomass (Treseder 2004). Additionally, soil acidification and other abiotic stresses lower the species richness of ECM by eliminating non-acidophilous and non-nitrophilous fungi species (Suz et al. 2014). ECM fungi are likely to be an important food source for many fungivorous microarthropod species: the study of Remén et al. (2018) found a decrease in the abundance of the fungivorous grazer species *Oppiella nova, Eulohmannia ribagai* and *Scheloribates pallidulus* after a reduction in ECM availability. This information let me to hypothesize that observed decreases in fungivorous species in oak forests are caused by a reduction in fungal food sources. Similarly, the study of Gan et al. (2013) reported the strongest decline in microarthropod species that rely on fungal hyphae as a food source in a long-term nitrogen addition experiment.

Current status of the soil microarthropod community

It is clear that the soil microarthropod community is under a lot of pressure due to the excess nitrogen deposition. In the old oak forest habitat, I measured an extremely low richness of soil microarthropods, which makes it clear that many species have not been able to persist this degradation process. The degraded habitat might also select for certain more favourable traits. For example, one of the herbivorous species that was still common in the old oak forests is *Platynothrus peltifer*. As I found out in Chapter 4, this species is present during very early phases of litter decomposition, which means that they can take advantage of the still relatively nutrient rich litter material. While herbivores that arrive later in the decomposition process are predicted to be more strongly affected by the lower litter quality and high N:P ratio.

The study of Siepel (1996b) proposed that loss of species is not random but instead can be predicted from a species' traits. This approach has been followed in my research too. In the

old oak forest habitat, fungivorous species were filtered out likely due to a loss of their preferred food resource. In a similar manner, P-limitation functions as a filter for herbivores and large species. This means that the species that manage to survive in the nitrogen-impacted forests are more likely to be species with a more generalist lifestyle and therefore can make more efficient use of the type of resources that are still present. For example, species that can feed both on plants and fungi were still doing relatively well compared to specialist species that only feed on fungi in the old oak forests.

Research aim 2: The consequences of low microarthropod richness on litter decomposition processes

It has been often observed that the accumulation of organic matter is a common issue in areas with excess nitrogen deposition, and this has been noticed in the old oak forests as well (Hommel et al. 2020). Since it is known that soil microarthropods play important roles in the nutrient cycling process (Seastedt 1984; Siepel and Maaskamp 1994; Carrillo et al. 2021), the decline of this community I observed in Chapter 3 could give us more insight in this problem of accumulating organic matter. To further explore this relationship, I set out a large-scale litterbag experiment in 9 different forests in the Veluwe and studied the soil microarthropod community for 21 months in relation to the litter decay of *Quercus robur*.

The process of litter decomposition and the role of soil microarthropods

The process of litter decomposition is influenced by many factors including climate and litter quality (Aerts 1997; Bonanomi et al. 2013). There is an important faunal component as invertebrates contributing to increasing the surface area via shredding of leaf material (McCary and Schmitz 2021). In addition, specifically for soil microarthropods, fungivorous grazers contribute by stimulating fungal activity (Siepel and Maaskamp 1994).

Different phases can be recognised for litter decomposition (Berg and Matzner 1997). In the early stage of decomposition, it is mostly the decomposition of easily degradable cellulose components, which is where the decomposition rate is at its quickest. After this phase there is a slower decomposition of lignin-bound compounds. These compounds are recalcitrant and require certain fungi species to be broken down (Hammel 1997; Osono et al. 2007).

I found that in the early stage of the experiment (first 6 months) the microarthropod community was dominated by a few ubiquitous herbivorous grazer species (*Lepidocyrtus lanuginosus* and *Platynothrus peltifer*) that can colonise the litterbags quickly and take advantage of the higher nutrient level of the litter in this phase. After 6 months there is a shift from an herbivorous to fungivorous dominated microarthropod community. During this time there was a reduction in the litter decay rate, which signifies that this phase is dominated by the decomposition of lignin-bound compounds which are more recalcitrant to decomposition. During the later decomposition phases, between months 6 and 15, I observed an inhibition of decomposition with many forest sites having a stagnation in litter decay, which was not explained by the summer season or drought. Therefore, I propose that this inefficient breakdown of the lignin-bound compounds is related to the decrease of fungivorous grazers that was observed in Chapter 3.

Fungivorous grazers

I found indeed that the richness of both fungivorous grazers and herbo-fungivorous grazers correlated to differences in the litter decay rate in the later decomposition phase (after 6 months), although this pattern is different between the groups. Fungivorous grazers seem most important at month 9-12, which is the beginning of the lignin-decomposition phase and it can be expected that the fungi community is at its most active here and fungivorous grazers require the presence of living fungi, in addition to dead fungal mass, to acquire their necessary nutrients. Herbo-fungivorous grazers, in contrast, are most important in the final months of the study. This group feeds on plant matter and when they are feeding on fungi it is mostly on dead fungal matter (Siepel et al. 2019).

As I have discussed previously, ECM fungi are likely to be an important food source for many fungivorous microarthropod species. ECM fungi in contrast to many saprotrophic fungi, are generally slow-growing fungi. Therefore, ECM fungi have recalcitrant secondary metabolites to protect themselves against fungivores (Kempken and Rohlfs 2010). Regardless, there is evidence that ECM fungi are an important food source for many fungivorous grazing species (Remén et al. 2008), which serves as an indication that some fungivorous microarthropod species are adapted to feed on ECM despite the secondary metabolites. For this reason, I hypothesize that there is a degree of specialism in microarthropod fungivore feeding, which would explain the extremely high richness of fungivores grazers that is common in well-developed forest soils.

If the above hypothesis is proven to be true, it suggests that fungivorous grazer species loss means that certain ECM fungi species might not be able to be grazed sufficiently by microarthropods. While ECM fungi are also declining due to the high nitrogen deposition (Suz et al. 2014), they are better in re-establishing themselves when conditions improve (van Diepen et al. 2017), which could result in a mismatch of ECM species present and the presence of soil microarthropods species to graze on them. Indeed, we saw that higher species richness of fungivorous grazers is correlated to higher rates of litter decomposition during the decomposition of lignin compounds. Loss of fungivorous grazer species therefore means that the stimulation of decomposing fungi is not enabled enough.

Research aim 3: Restoration of soil microarthropods

In chapters 2-4, I discussed the pressures for the microarthropod community and how a reduction of the fungivorous species could affect the nutrient cycling processes in forest ecosystems. It becomes clear that is crucial to restore the microarthropod community to the best of our ability. In Chapter 5 I therefore studied the effectiveness of two methods that could help: silicate rock powder (SRP) application to improve the soil abiotic conditions and soil inoculation from a richer forest site to restore the species richness of soil microarthropods. To test both methods, I have set out a full factorial experiment in 5 old oak forest locations with plots using three treatments of rock powders (Eifelgold, Soilfeed and a control with no application) and two treatments of soil inoculation (inoculated or null).

Use of silicate rock powder

SRP's are becoming an increasing point of interest in terms of the restoration of acidified nature areas. SRP's are fine rock particles that are usually by-products of mining in mountainous areas and are used to increase the pH of the soil (Ramos et al. 2022; van der Bauwhede et al. 2024a). SRP's additionally contain many nutrients such as magnesium (Mg), potassium (K) and calcium (Ca) that have been lost in nitrogen-impacted forests due to soil acidification. However, since this method is still quite new, it has not been extensively scientifically studied yet, and we do not know well how it impacts the fauna in soils (Vicca et al. 2021).

The silicate rock powder addition in my experiment was successful in improving the abiotic status and improved the pH of the soil by 0.3 units. This has resulted in a higher biological activity, which I measured using the proportion of fungivorous browsers and predatory microarthropods. The response of these two groups depended on the SRP, however. In the Eifelgold treated plots I found a much higher proportion of fungivorous browsers. Fungivorous browsers are species that generally have a quick life cycle and are depended on living fungi to obtain their nutrients. Their high abundance in Eifelgold plots therefore suggests a much higher fungal activity. This is exemplified with the high abundance of *Pediculaster mesembrinae*, a species that is known to be a pest for mushroom cultivation (Hernández-Abarca et al. 2005; Szafranek and Lewandoski 2017). Eifelgold weathers more quickly than Soilfeed and we measured a higher level of magnesium and potassium there in comparison to Soilfeed plots. While the biologically available salt-extracted P was not significantly higher than the control or Soilfeed plots, from analysis of foliar P concentration of *Quercus robur* we know that Eifelgold increases P-uptake (Sitters et al. in 2026)

In Soilfeed treat plots I instead found an increase in the proportion of predatory mites. Predatory mite species abundance is a reflection for the abundance of their prey and the species that had the clearest increase in abundance in Soilfeed plots, *Pachylaelaps bellicosus*, is known to feed on nematodes (Karg 1993; Szymkowiak 2001). This species is therefore a good indicator of a higher nematode abundance in Soilfeed plots. Nematodes are diverse in their feeding strategy, with many being bacterivores, but also including predation on fungivorous species. Interestingly, the bacterivore mite species *Histiostoma sapromyzarum*, had also the highest abundance in Soilfeed plots so this could serve as an indication of higher bacteria activity.

Restoring soil microarthropod richness using soil inoculation

As I have discussed earlier in this chapter, the lack of dispersal ability of many soil microarthropods is one the largest challenges for the restoration of a disturbed forest ecosystem. In Chapter 2 I found that young forests soils are much impoverished in species richness than old forest soils. Furthermore, I found out in Chapter 3 that the old oak forests in the Veluwe have an impoverished soil microarthropod community. While SRP discussed above can help with the abiotic soil conditions, this dispersal limitation means that many species would be not be able to recover without help. For this reason, we took soil from one

of the old forest locations discussed in Chapter 2, Ugchelen, and spread that soil in plots of 5 old oak forest locations.

Soil inoculation was used with the expectation that it could help restoring the soil microarthropod community, however we found that this not work as well as we had hypothesized. Soil inoculation did help the herbo-fungivorous community, which is important for the litter decomposition, as we have seen in Chapter 4. This increase was relatively minor, however, and the fungivorous grazer group was unaffected. Regardless, this study does show promise that it is possible that microarthropod richness could be improved using soil inoculation. The likely issue in this experiment was that the old forest site has been already degraded too much due to the excess nitrogen deposition in the Netherlands.

Evaluation of the restoration methods

The main aim of these two restoration methods is to restore the soil decomposition processes in excess nitrogen-impacted forests and stop the accumulation of soil organic matter. Silicate rock powder application looks promising as a short-term solution since it improves the abiotic conditions in the soil and promotes the activity of fungi and soil fauna. However, it is important to note that the abiotic conditions are still not sufficient, so it cannot be the only solution. The expectation is that a lot of the increased fungi biomass is fast-growing saprotrophic fungi, as was found to be the case in response of SRP application by Findeisen et al. (2025), that may have a role in the breakdown of litter. SRP application therefore is expected to stimulate decomposition in the short term, however, with its relatively small effect and continued nitrogen deposition the effect is likely too short-lived since there is still a constant influx of N, and the soil will get acidified again.

Long-term restoration will also require a restoration of the fungivorous grazer community, which we hoped to accomplish with soil inoculation, however it appears that even the historically richer forest sites in the Netherlands now have declined too much. Without the restoration of the fungivorous microarthropod community there is risk that not all dead fungal biomass is broken down efficiently, as I found in Chapter 4.

Conclusions

In my thesis I can conclude that the soil microarthropod community is under a lot of stress in the old oak forests in the Netherlands. I found out in Chapter 2 and Chapter 3 that issues of soil disturbance, excess nitrogen and the following soil acidification and phosphate limitation have profound threats on the richness and community composition of soil microarthropods. The group of fungivorous grazers are particularly vulnerable to many of these issues and this group is extremely important in maintaining the nutrient cycling processes in forests. The results in Chapter 4 led me to hypothesize that not only the abundance, but also the species richness of this group is vital for the litter decomposition and that in the current situation of the Veluwe forests the decomposition is inhibited. Unfortunately, the restoration of this group is proving to be a difficult task. As I found in Chapter 5, silicate rock powder will help the abiotic soil conditions a little bit, but its effect is too small and it cannot restore the richness of microarthropods, which will impede its success on the long term. Soil inoculation to improve the species richness did not work as well as we had hypothesized, however

taking soil from other Western European forests, with a lower nitrogen deposition, could be considered since these forests might still be in a better state. The negative effects of nitrogen deposition are well-known, and my thesis offers additional context in how it disrupts ecosystems and that the restoration of nitrogen-impacted forests is proving to be a difficult task. It is therefore vital that the current level of nitrogen deposition is reduced below critical levels in the Netherlands to have any chance of successful restoration of the forest ecosystems.

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Samenvatting

Veel natuurgebieden in Nederland staan onder grote ecologische druk als gevolg van de hoge stikstofdepositie. Deze toename in stikstof beschikbaarheid verstoort ecosystemen op verschillende manieren. Ten eerste stimuleert het hoge stikstofgehalte de productiviteit van planten en daarmee de groei van snelgroeiende plantsoorten. Dit zorgt voor een asymmetrische competititie en uiteindelijk een verlies in soortenrijkdom. Daarnaast veroorzaakt een te hoge stikstofdepositie ook bodemverzuring en daarbij de uitspoeling van basische kationen (calcium (Ca^{2+}), kalium (K^{+}) en magnesium (Mg^{2+})) en het vrijkomen van aluminium (Al^{3+}), dat giftig is voor plantwortels. Overmatige stikstofdepositie veroorzaakt bovendien een verstoring van de stikstof-fosforverhouding (N:P) in de bodem. Dit zorgt voor een limitatie van fosfor in plantmateriaal en vervolgens in de opname van herbivoren. Fosfor is een cruciaal element voor de groei en reproductie van organismen en deze limitatie zorgt daarom voor een reductie in de fitness van veel soorten.

Om deze redenenen zijn veel Nederlandse bossen in een slechte staat. Vooral bossen op mineraalarme zandbodems zijn kwetsbaar. In deze bossen observeren we een afname in boomvitaliteit en een accumulatie van organisch materiaal vanwege een verstoring in de afbraakproces. Het herstel van deze gebieden is daarom een belangrijke aandachtspunt in Nederlandse natuurbeheer. Recentelijk wordt daarom steeds vaker steenmeel gebruikt als een potentieel middel als mitigatie voor de nadelige stikstofeffecten. Steenmeel draagt namelijk bij met het bufferen van de bodem-pH en voegt ook belangrijke basische cationen weer toe aan de bodem. De eerste resultaten van deze methode zijn veelbelovend, maar het effect op de bodemfauna is nog nauwlijks onderzocht.

Mijn proefschrift richt zich daarom op een belangrijke bodemfauna groep: de bodemmicroarthropoden. Deze groep bestaat uit mijten, springstaarten en een aantal andere diergroepen binnen de grootteklasse tussen 0,1 en 2 mm. Bodemmicroarthropoden hebben een hoge abundantie en soortenrijkdom op een lokale schaal wat het een interessante groep maakt voor ecologische studies. Daarnaast hebben vele soorten een belangrijke rol in de strooiselafbraak die zo moeizaam verloopt in deze bossen. De specifieke rol van soorten in dit proces hangt af van hun voeding. Vandaar dat het belangrijk is om onderscheid te maken tussen herbivoren, schimmeleters en roofdieren. Daarnaast maak ik onderscheid tussen grazers en browsers. Grazers voeden zich zowel met de celwand als de celinhoud, terwijl browsers alleen op celinhoud voeden. Schimmelgrazende soorten hebben de voornaamste rol in de strooiselafbraak, omdat bij de afbraak van dode schimmelcelwanden belangrijke voedingstoffen vrijkomen in de bodem wat de groei van andere schimmels bevordert. Zonder deze microarthropoden is er kans dat schimmels niet goed genoeg afgebroken worden en dat de voedingstoffen vastzitten in het systeem. Schimmelbrowsers daarentegen kunnen alleen op de levende schimmels voeden en hebben daarom een negatieve impact op de strooiselafbraak.

Bodemmicroarthropoden zijn dus belangrijk, maar deze groep is ook erg kwetsbaar bij verstoring in de bodem. Daarom is het de verwachting dat de sterke effecten van stikstof op het bodemsysteem grote gevolgen hebben voor deze gemeenschap. Daarnaast hebben veel soorten een bijzonder slechte dispersie, waardoor deze soorten ook niet goed in staat zijn om locaties te herkoloniseren als ze verdwijnen.

Het doel van mijn proefschrift heeft 3 stappen:

Doel (1) een evaluatie van de huidige stand van zaken met betrekking tot de bodemmicroarthropoden gemeenschap in Nederlandse bossen op arme zandbodems.

Doel (2) een evaluatie van de rol van de bodemmicroarthropoden gemeenschap in de strooiselafbraak process in deze bossen.

Doel (3) onderzoeken of we de microarthropoden gemeenschap kunnen herstellen door middel van steenmeelapplicatie en bodementing (als een hulpmiddel voor de dispersielimitatie).

In Hoofdstuk 2 en 3 heb ik het Doel (1) van mijn proefschrift behandeld. In **Hoofdstuk 2** deed ik dit doormiddel van een dataset analyse van 32 Nederlandse bossen om te onderzoeken hoe bosbodemleeftijd en fosfor-limitatie de bodemmicroarthropoden gemeenschap beïnvloed. Van deze bossen waren 3 locaties oude bosbodems, 10 locaties waren oude houtwallen en 19 locaties waren jonge bosbodems. De oude bosbodems en oude houtwallen zijn al bebost sinds ten minste de Middeleeuwen, terwijl de jonge bossen onstaan zijn uit heide tussen 1880 en 1930. De oude houtwallen zijn restanten van de oude bossen, maar bevinden zich langs landbouwgronden wat deze locaties een constante influx van fosfor hebben gegeven door middel van kunstmest. In mijn onderzoek vond ik een signficant hogere soortenrijkdom van microarthropoden in oude bosbodems en houtwallen in vergelijking met de jonge bosbodems, wat er op wijst dat de dispersie-limitatie er voor heeft gezorgt dat meerdere soorten de jongere bossen nog niet hebben gekoloniseerd. In specifiek waren de schimmelgrazende mijten soortenrijkdom veel lager in jonge bossen, wat een mogelijke hiaat in de strooiselafbraak indiceert. Hoewel de algemene soortenrijkdom van oude bossen en houtwallen vergelijkbaar was, vond ik wel een verschillende opmaak in de microarthropoden gemeenschap tussen deze boscategorieën. De houtwal locaties had een hogere soortenrijkdom van herbivore soorten en ook een hogere dichtheid van grotere mijtsoorten (>900 µm) wat te verklaren is met de hogere fosfor waardes in houtwallen. Planten zijn minder stabiel in hun N:P waarde vergeleken met schimmels waardoor herbivoren sterker geraakt worden, terwijl de allometrische effect van fosfor-limitatie ervoor zorgt dat ook grotere soorten gevoeliger zijn.

De oude eikenbossen habitat is een voorbeeld van een habitattype wat sterk onder druk staat van hoge stikstofdepositie en de daarop volgende bodemverzuring. In **Hoofdstuk 3** onderzocht ik daarom de status van de microarthropoden gemeenschap in de habitat oude eikenbossen en vergeleek deze met de gemeenschap van oude bosbodemlocaties behandeld in Hoofdstuk 2 als een referentie. Sinds Hoofdstuk 2 ook duidelijk heeft gemaakt dat vooral schimmeleters gevoelig lijken te zijn voor verstoring, was deze groep ook het richtpunt van dit onderzoek. De resultaten lieten zien dat inderdaad de aantal

schimmeltetende mijten een stuk lager waren in de oude eikenbossen in vergelijking met de referentielocaties, zowel in soortenrijkdom als in abundantie, en dit effect was ook duidelijk sterker in deze groep vergeleken met herbivoren soorten. Uit literatuur is bekend dat schimmels achteruitgaan als een gevolg van stikstofdepositie, met vooral een sterke effect op de ectomycorrhiza groep. Ectomycorrhiza is waarschijnlijk een belangrijke voedselbron voor vele mijtsoorten en vandaar mijn hypothese dat de afname van deze voedselbron ook een belangrijke factor is in het verdwijnen van schimmeleters bij stikstofdepositie en de geobserveerde afname in strooiselafbraak.

Voor deze reden onderzocht ik in **Hoofdstuk 4** Doel (2): de relatie tussen de microarthropoden gemeenschap en de bladafbraak doormiddel van een litterbagstudie. In 8 meetmomenten heb ik totaal 40 litterbags verzameld per locatie en zowel het blad drooggewicht gemeten en de microarthopoden in de litterbag verzameld. De eerste 6 maanden was de vroege fase van afbraak waarbij de makkelijker afbreekbare cellose stoffen in het blad redelijk snel afgebroken worden. In deze fase is er een dominantie van herbivore soorten die gebruik maken van de relatief hogere nutrient waardes in het blad. Na 6 maanden is er en verandering van herbivore soorten naar een dominantie van schimmeletende soorten. Tijdens deze fase wordt de moeilijker afbreekbare lignine stoffen afgebroken. Schimmels hebben een belangrijke rol in deze afbraak wat de hogere aantal schimmeleters verklaard. De afbraaksnelheid vertraagd ook sterk in deze periode. In mijn studie was er ook een periode met een inhibitatie van de afbraak tussen maand 9 en 15, wat een indicatie is voor een inefficiënte afbraak van bladmateriaal. Tijdens de latere periode is ook een significante associatie van schimmelgrazer soortenrijkdom en de afbraaksnelheid in het blad, wat aangeeft dat de afname in schimmelters van Hoofdstuk 3 ook een verklaring is van een afname in afbraaksnelheid.

Het is duidelijk uit Hoofdstukken 2-4 dat de huidige status van de bodemmicroarthropoden gemeenschap niet goed genoeg is voor een gezonde ecosysteem. In **Hoofdstuk 5** richt ik me daarom Doel (3): het herstel van deze gemeenschap. In een full factorial experiment heb ik twee methodes voor herstel getest: steenmeel toevoeging en bodementing. Voor steenmeel zijn twee verschillende merken getest (Eifelgold en Soilfeed + een controle groep) met als doel om de abiotische omstandigheden in de bodem te verbeteren. Voor de bodementing behandeling is materiaal van de oude bos locatie Ugchelen verzameld en enting heeft het doel om te helpen met de dispersie limitatie van microarthropoden. Steenmeel was inderdaad succesvol in het verbeteren van de bodemkwaliteit: zowel Eifelgold als Soilfeed verhoogde de pH waarde met 0,3, terwijl de Ca, K en Mg waardes ook stegen. Dit heeft geleid tot een hogere bodemactiviteit wat te zien is aan de hogere aantal schimmelbrowsers bij Eifelgold plots en hogere aantal roofdieren bij Soilfeed plots. Schimmelbrowsers zijn soorten met een korte levencyclus en voeden zich alleen met nog levende schimmelcellen en zijn daarom een goede indicatie voor hogere schimmelactivteit. Hoger aandeel van roofmijten is een indicatie voor hogere prooi beschikbaarheid. In specifiek was de soort *Pachylaelaps bellicosus* meer algemeen in Soilfeed plots and deze soort is een gespecialiseerde nematode eter. Bodementing, in tegenstelling, had maar een klein effect op de soortenrijkdom van bodemmicroarthropoden. Er was wel een marginale stijging in de

soortenrijkdom van herbo-fungivore grazers die belangrijk zijn voor de strooiselafbraak. Echter is de verwachting dat deze verhoging te klein is voor een grote impact op de afbraak.

In mijn synthese (Hoofdstuk 6) evalueer ik de drie onderzoeksdoelen die ik gesteld heb in mijn proefschrift. Voor Doel (1) werd het duidelijk dat de bodemmicroarthropoden gemeenschap onder hoge druk staat in Nederlandse bossen. De limitatie van P en afname in voedselbeschikbaarheid heeft een significante reductie in de soortenrijkdom veroorzaakt, terwijl verloren soorten waarschijnlijk niet in staat zijn om terug te keren vanwege hun gelimiteerde kolonisatiemogelijkheden. Vooral de afname van schimmelgrazende soorten is zorgwekkend voor de afbraakprocess in bossen. Dit zag ik inderdaad in Doel (2} waar mijn resultaten lieten zien dat de soortenrijkdom van schimmelgrazers in verband staan met de afbraaksnelheid van zomereikblad en een reductie in de soortenrijkdom kan daarom de accumulatie van organisch materiaal verklaren. Voor Doel (3) ondervond ik ook dat de herstel van de microarthropoden gemeenschap een ingewikkeld verhaal wordt. Hoewel steenmeeltoevoeging met succes de abiotische waardes kon verbeteren, zijn de waardes voor pH en bazenverzadiging nog steeds onder de kritieke waarde en de vraag is of de stijging in biologische activiteit ook voor de lange termijn succesvol is. Zeker sinds we niet succesvol waren om soorten in de oude eikenbossen te introduceren via bodementing. De donorlocatie Ugchelen was helaas ook te ver achteruitgegaan om andere locaties te helpen, maar mogelijk kan enting vanuit andere West-Europese landen met lagere stikstofdepositie een oplossing bieden. Mijn onderzoek laat zien dat de hoge stikstofdepositie in Nederland grote consequenties heeft voor de biodiversiteit van bosbodems en het is cruciaal om de stikstofuitstoot onder de kritieke waardes te krijgen voor een kans om deze habitats te herstellen.

Summary

Many nature areas in the Netherlands are currently under high ecological pressure due to long-term excess nitrogen deposition. This increase in nitrogen availability disrupts ecosystems through many pathways. For one the higher nitrogen availability stimulates the productivity of plants and thereby facilitates the growth of fast-growing species, which causes asymmetrical competition and eventually a loss in species. Additionally, nitrogen deposition causes soil acidification, which leads to the leaching of important base cations (calcium (Ca^{2+}), potassium (K^{+}) and magnesium (Mg^{2+})) and the release of aluminium (Al^{3+}), which is toxic for plant roots. Excess nitrogen deposition also causes ecological imbalance of nitrogen: phosphorus (N:P) ratio's in the soil, that cascades up to plants. This leads to a P-limitation in plants and subsequently for the herbivores. P is a crucial element for the growth and reproduction of organisms and this limitation will affect the fitness of consumers.

As a result of these effects, many forests in the Netherlands are in a degraded state. Sites on mineral poor, sandy soil are particularly vulnerable. In these forests we observe a decline in tree vitality and an accumulation of soil organic matter in the form of plant litter. Restoration of these vulnerable habitats has been an important issue for nature management. The application of silicate rock powder (SRP's) has recently emerged as a potential restoration method. SRP's are expected to assist with soil buffering by increasing the pH in the soil and with replenishing the soil with base cations. The early results of this method are promising, but its effect on the fauna community has barely been studied.

My thesis will therefore focus on an important soil animal group: the soil microarthropods. Soil microarthropods consist mainly of mites (Acari) and springtails (Collembola), but also feature other animal groups within the size class of 0.1 to 2 mm. Soil microarthropods are an extremely numerous and species rich group of fauna on a local scale, which makes them an interesting target for ecological studies. Furthermore, this group of animals has an important role in decomposition processes in the soil. The exact role of various species depends on their feeding strategy, however. Therefore, I do not only distinguish between herbivores, fungivores and predators, but I will also separate species into grazer and browser strategies. Grazers can feed on both fungal cell walls and the cell content, while browsers only feed on cell content. Fungivorous grazing species in specific are important for decomposition since they can breakdown (old) fungal cell walls, which releases nutrients that stimulate the growth of other fungi. Without these fungivorous grazers there is risk that fungal matter is not decomposed well and that those nutrients stay locked in the system. Fungivorous browsers, in contrast, can only feed on living fungal cells and therefore negatively impact decomposition.

Soil microarthropods are generally vulnerable to disturbance of the soil, however, and the large changes in the ecosystem caused by nitrogen deposition could therefore have detrimental effects on the species richness in these forests. Moreover, many species have

poor dispersal ability, meaning that they cannot recover after species-loss, even if conditions improve.

The aims of my thesis therefore are three-fold:

Aim (1) to study the current status of soil microarthropod richness in Dutch forests on mineral poor, sandy soil.

Aim (2) to evaluate how the current soil microarthropod community functions in relation to litter decomposition in these forests.

Aim (3) to study the effectiveness of two restoration methods: SRP application and soil inoculation with microarthropods (to help with dispersal).

Aim (1) I worked on in Chapters 2 and 3. In **Chapter 2** I conducted a dataset study of 32 forests in the Netherlands to determine how soil age and P availability shape the soil microarthropod community. Of these 32 forests, 3 were old forest soils, 10 sites were old hedgerows and the other 19 were young forest soils The old forests and hedgerows have been forested since the Middle Ages, while young forests have been established from heathlands between 1880 and 1930. Old hedgerows are remnants of old forests, but adjacent to agricultural land and therefore have been subjected to a constant P-influx from fertilization. In my research I found a significantly higher species richness of soil microarthropods in the old forest locations compared to young forest soils, showcasing the dispersal limitation. Specifically, the fungivorous grazer species that are important for decomposition, were much lower in younger forests. While the overall species richness of old forests and old hedgerows were similar, the shape of the community was still different, which I attribute to the different P levels. Old hedgerows had a higher species richness of herbivorous mite species and a higher density of larger species. Plants are less stable in their N:P compared to fungi leading to herbivores being more vulnerable to P-limitation and furthermore the allometric effect of P-limitation means that larger species are more vulnerable as well.

In **Chapter 3** I researched the status of soil microarthropods in the old oak forest habitat in the Veluwe and compared it to the old forest sites studied in Chapter 2 as a reference. Since Chapter 2 established that many fungivorous species seem to be vulnerable to disturbance, I focused on this group during this study. Both the species richness and abundance of fungivorous mites was indeed low in the old oak forests compared to the reference data. From literature it is known that the species richness and biomass of fungi also decline from nitrogen deposition with a specifically strong effect on the ectomycorrhiza group. Since ectomycorrhizal fungi are likely an important food source for many fungivorous species, I hypothesize that this decline in food source is an important reason for the decline of fungivorous mites and to the slowing down of decomposition that has been noted in this habitat.

In **Chapter 4** I therefore studied Aim (2) with the use of a 21-month litterbag study conducted in 9 forest sites. Using 8 different sampling dates, I collected 5 litterbags in each location and studied the soil microarthropod community in relation to the decomposition of

Quercus robur leaves. The first 6 months were in the early phase of decomposition, which consists of a rapid decomposition of the easily degradable compounds. During this period there was a dominance of herbivorous species that take advantage of the still relatively high nutrient levels in the leaves. After 6 months there was a shift from herbivores to a dominance of fungivorous species. The decomposition speed slows down during this period as it mostly concerns the decomposition of recalcitrant lignin-bound compounds during this point. Fungi play an important role of the decomposition of these compounds, which explains the increase in fungivores. I found an inhibition of decomposition at this point, signalling a potentially inefficient breakdown of recalcitrant material. There was a significant association of fungivorous grazer species richness and decomposition speed during this period as well, which suggests that noted decline of fungivorous grazers in Chapter 3 could also partly explain the reduction in decomposition.

In Chapters 2, 3 and 4 it becomes clear that the current status of the soil microarthropod community in these forests is not sufficient for a healthy ecosystem. In **Chapter 5,** therefore, I focused on Aim (3) to study if two restoration methods can help the soil microarthropod community. In 5 old oak forest sites I established a full factorial experiment using either three SRP treatments (Eifelgold, Soilfeed and Control) and two inoculation treatments (inoculated or null). The old forest site Ugchelen was used as donor site for inoculation since it was in the best state from the three old forest sites. SRP was indeed able to improve the abiotic soil conditions by increasing the pH by 0.3 units and increased the Ca, K and Mg levels. This resulted in a higher biological activity with Eifelgold having higher proportion of fungivorous browsers and Soilfeed having higher proportions of predators. Fungivorous browsers are species with a generally quick life cycle and only feed on living fungi cells and are therefore a good indication of higher fungal activity. Predatory species are indication of higher prey availability. The species with the highest abundance increase, *Pachylaelaps bellicosus*, is a known nematode predator. Soil inoculation, however, only had limited effect on the species richness. There was a small increase in herbo-fungivorous grazers, which are important for decomposition, however this increase is likely too small to have a significant effect on the decomposition

In my synthesis (**Chapter 6**) I evaluated the three research aims using the results from the previous research chapters. For research Aim (1) it is clear that the soil microarthropod community is under high pressure in many Dutch forests. The limitation of P and the reduction in food source have lowered the species richness, while the dispersal limitation prevents natural restoration of the community. Particularly the decline in fungivorous species is worrying for the decomposition process. Which leads me to research Aim (2) where my results indeed show that the reduction in fungivorous grazers can be linked to a decline in decomposition speed of plant litter, which provides an explanation for the accumulation of soil organic matter in acidified forests. For research Aim (3) I found that the restoration of the soil microarthropod community is a difficult challenge as well. While SRP application helps the abiotic status, the pH and base concentration is still under critical levels, and the question is whether the increase in biological activity is successful in the long-term. Especially since we have not been able to improve species richness with soil inoculation. The old forest locations in the Netherlands have declined too strongly due to

nitrogen deposition as well. Potentially, forests of the same habitat in other European countries can be considered as donor instead. Regardless, my thesis gives more data on the negative impact of excess nitrogen deposition and reducing the deposition to below critical levels will be essential for any hope in restoration of these forest habitats.

Dankwoord

Daar is het dan. Het voelt nog steeds een beetje raar dat het nu toch echt af is. Ik kijk terug naar een mooie vierenhalf jaar bij het Radboud. Het was een heel leerzame ervaring en ik heb mijzelf toch wel echt gevonden in deze jaren. Natuurlijk wil ik ook graag mensen bedanken waarmee ik deze jaren heb meegemaakt.

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Curriculum Vitae

Joren Bruggink was born in April 29 in Hoorn, The Netherlands where he would spend his entire childhood. From an early age he was fascinated by nature and particularly any creeping, crawling creatures he could find.

This passion brought him to study Biology at the Aeres University of Applied sciences after his graduation from OSG Westfriesland in 2014. During his first internship at the University of Amsterdam he would discover two of his current interests: science and mites. Joren graduated from his bachelors in 2019, after which he would study Ecology and Evolution at the Vrije Universiteit Amsterdam. One of this master thesis was centred around the intraspecific and interspecific variation in clutch size of terrestrial isopods for which he travelled across the country to collect species. This would lead to an interest in soil ecology that would continue in his PhD.

Joren graduated from his master in 2021 and would start his PhD at the Radboud University in October 2021. His doctoral research was focused on the role of soil microarthropods in the restoration of forests in the Dutch Veluwe area. He would set up multiple long-term field experiments during his PhD and was often found in the lab as well to prepare microscope slides and to identify species. During his PhD he would supervise internship students and was involved in teaching for Animal Ecology and Ecology courses where he found an interest in teaching. In the Ecology department he was involved in organising the research meetings and volunteered to give ecological advice to the biodiversity work group at the Radboud campus.

His research interests include analysis of invertebrate biodiversity, soil ecology and nature restoration, all of which he would to contribute to his follow-up career. He is also passionate to be involved in education and he has great enjoyment in doing data analysis.

Supplementary Materials

Supplementary for Chapter 2

Table S2.1 The site information for each location. The forest type was estimated through satellite google images. The soil type was taken from de Bodemkaart, part of the Basisregistratie Ondergrond (BRO) and supplied by Wageningen Environmental Research (Alterra).

ID	Location	Forest category	Sample year	Forest type	x (wgs84)	y (wgs84)	Soiltype
1	Hoenderloo	Old forest	1994	Deciduous	52.14028	5.87645	Podzol soil
2	Loenen	Old forest	1994	Deciduous	52.0922	5.99085	Podzol soil
3	Ugchelen	Old forest	1994	Deciduous	52.1642	5.89818	Podzol soil
4	Aperloo	Hedgerow	1997	Deciduous	52.42417	5.86359	Enkeerd soil
5	Elspeet	Hedgerow	1997	Deciduous	52.28597	5.785	Enkeerd soil
6	Garderen	Hedgerow	1997	Deciduous	52.23733	5.72301	Podzol soil
7	Harskamp	Hedgerow	1997	Deciduous	52.13515	5.76362	Enkeerd soil
8	Klarenbeek	Hedgerow	1997	Deciduous	52.15705	6.07229	Enkeerd soil
9	Nunspeet	Hedgerow	1997	Deciduous	52.38757	5.81024	Enkeerd soil
10	Stroe	Hedgerow	1997	Deciduous	52.182	5.695	Enkeerd soil
11	Twello	Hedgerow	1997	Deciduous	52.24597	6.10604	Enkeerd soil
12	Vemde	Hedgerow	1997	Deciduous	52.35901	6.02309	Enkeerd soil
13	Woudenberg	Hedgerow	1997	Deciduous	52.07068	5.39473	Peat soil
14	De Vuursche	Young forest	2000	Mixed	52.20718	5.21897	Vaag soil
15	Dorst	Young forest	2000	Coniferous	51.604	4.88199	Podzol soil
16	Ruurlo	Young forest	2000	Deciduous	52.07694	6.41999	Podzol soil
17	Smilde	Young forest	2000	Coniferous	52.87933	6.34087	Vaag soil

18	Buulderbroek	Young forest	2000	Deciduous	51.293	5.59221	Enkeerd soil
19	De Leyen	Young forest	2000	Mixed	52.13613	5.18856	Vaag soil
20	De Schotkamp	Young forest	2000	Coniferous	52.18067	5.75273	Podzol soil
21	Diffelerveld	Young forest	2000	Mixed	52.54499	6.54445	Podzol soil
22	Galgenberg	Young forest	2000	Coniferous	51.42462	5.78978	Podzol soil
23	Gasselterveld	Young forest	2000	Coniferous	52.97519	6.74346	Podzol soil
24	Grollooërveld	Young forest	2000	Coniferous	52.9167	6.66879	Podzol soil
25	De Pan	Young forest	2000	Mixed	51.33785	5.64408	Podzol soil
26	Langeloër	Young forest	2000	Coniferous	53.07804	6.43626	Vaag soil
27	Leusderheide	Young forest	2000	Coniferous	52.10933	5.36239	Podzol soil
28	Lheebroeker	Young forest	2000	Mixed	52.83177	6.43925	Vaag soil
29	Mastbos	Young forest	2000	Mixed	51.55131	4.76723	Podzol soil
30	Oldenhaver	Young forest	2000	Coniferous	52.72851	6.37534	Podzol soil
31	Slangenburg	Young forest	2000	Deciduous	51.96331	6.35917	Podzol soil
32	Vredepeel	Young forest	2000	Coniferous	51.53395	5.87723	Podzol soil

Table S2.2.1: ANOVA tests on the difference in species richness between old forest soils, old hedgerow soils and young forest soils.

Model	df	Sum Sq	Mean Sq	F-Value	P-value
Acari	2	1221.4	610.7	21.06	**<0.001**
Collembola	2	11.32	5.660	1.31	0.284
(Herbo)fungivorous grazer	2	529.83	264.91	32.41	**<0.001**
Herbivorous grazer	2	21.05	10.523	6.99	**0.003**
General predator	2	144.9	72.44	9.77	**<0.001**
Fungivorous browser	2	0.60	0.303	0.16	0.850
Opportunistic herbo-fungivores	2	22.65	11.33	14.78	**<0.001**

Table S2.2.2 : ANOVA tests on the difference in mite feeding guild proportions between old forest soils, old hedgerow soils and young forest soils.

Model	df	Sum Sq	Mean Sq	F-Value	P-value
(Herbo) fungivorous grazer %	2	0.23	0.11	7.57	**0.002**
Fungivorous grazer %	2	0.17	0.08	5.72	**0.008**
Herbo-fungivorous grazer %	2	0.08	0.04	3.17	0.057
Opportunistic herbo-fungivorous %	2	0.06	0.03	1.37	0.270
Fungivorous browser %	2	0.03	0.02	1.42	0.259
Herbivorous browser %	2	0.76	0.38	14.43	**<0.001**
Herbivorous grazer %	2	0.04	0.02	0.86	0.433
Omnivorous %	2	0.08	0.04	7.86	**0.002**
General predator %	2	0.01	0.003	0.40	0.677

Table S2.2.3 : ANOVA test on the difference in density of mites of different size classes between old forest soils, old hedgerow soils and young forest soils.

Model	df	Sum Sq	Mean Sq	F-Value	P-value
Category	2	13.39	3.50	17.73	**<0.001**
Size	1	11.87	93.89	31.44	**<0.001**
Category * Size	2	6.44	3.22	8.53	**<0.001**

Table S2.2.4 : ANOVA tests on the difference in Olsen-P content classes between old forest soils, old hedgerow soils and young forest soils.

'Model	df	Sum Sq	Mean Sq	F-Value	P-value
Log_{10} Olsen-P content	2	0.69	0.34	8.812	**0.003**

Table S2.3.1: Tukey HSD results of the ANOVA tests with differences species richness. Diff is the difference in value between the categories, lower and upper show the 95% confidence interval ranges. P adj. is the correct P-value after adjustment for multiple corrections. Values in bold signify significant effects.

Main test	Two categories tested	Diff	Lower	Upper	P adj.
Collembola	Hedgerow – old forest	1.87	-1.51	5.24	0.371
Collembola	Young forest – old forest	2.09	-1.10	5.27	0.254
Collembola	Young forest – hedgerow	0.22	-1.78	2.22	0.960
Acari	Hedgerow – old forest	0.83	-7.92	9.59	0.970
Acari	Young forest – old forest	-11.93	-20.19	-3.67	**0.004**
Acari	Young forest –hedgerow	-12.76	-17.96	-7.57	**<0.001**
(Herbo)fungivorous grazer	Hedgerow – old forest	0.30	-4.35	4.95	0.987
(Herbo)fungivorous grazer	Young forest – old forest	-8.05	-12.44	-3.67	**<0.001**
(Herbo)fungivorous grazer	Young forest –hedgerow	-8.35	-11.11	-5.59	**<0.001**
Herbivorous grazer species	Hedgerow – old forest	1.43	-0.56	3.43	0.196
Herbivorous grazer species	Young forest – old forest	-0.35	-2.23	1.53	0.890
Herbivorous grazer species	Young forest – hedgerow	-1.78	-2.97	-0.60	**0.002**
General predator	Hedgerow – old forest	3.87	-0.56	8.29	0.096
General predator	Young forest – old forest	-0.81	-4.99	3.37	0.883
General predator	Young forest – hedgerow	-4.67	-7.30	-2.05	**<0.001**
Fungivorous browser	Hedgerow – old forest	-0.37	-2.57	1.84	0.912
Fungivorous browser	Young forest – old forest	-0.09	-2.17	1.99	0.994
Fungivorous browser	Young forest – hedgerow	0.28	-1.03	1.59	0.859
Opportunistic herbo-fungivore	Hedgerow – old forest	-2.07	-3.49	-0.64	**0.003**
Opportunistic herbo-fungivore	Young forest – old forest	-2.88	-4.22	-1.53	**<0.001**
Opportunistic herbo-fungivore	Young forest – hedgerow	-0.81	-1.66	0.03	0.061

Table S2.3.2: Tukey HSD results of the ANOVA tests with differences in the proportion of mite feeding guilds. Diff is the difference in value between the categories, lower and upper show the 95% confidence interval ranges. P adj. is the correct P-value after adjustment for multiple corrections. Values in bold signify significant effects.

Main test	Two categories tested	Diff.	Lower	Upper	P adj.
Herbo(fungivorous)grazer %	Hedgerow – old forest	-0.02	-0.22	0.18	0.973
Herbo(fungivorous)grazer %	Young forest – old forest	-0.19	-0.38	-0.002	0.054
Herbo(fungivorous)grazer %	Young forest – hedgerow	-0.17	-0.29	-0.05	**0.004**
Fungivorous grazer %	Hedgerow – old forest	-0.10	-0.30	0.10	0.431
Fungivorous grazer %	Young forest – old forest	-0.21	-0.40	--0.03	**0.021**
Fungivorous grazer %	Young forest - hedgerow	-0.11	-0.23	0.003	0.058
Herbo-fungivorous grazer %	Hedgerow – old forest	0.11	-0.07	0.30	0.317
Herbo-fungivorous grazer %	Young forest – old forest	0.001	-0.17	-0.18	1.000
Herbo-fungivorous grazer %	Young forest – Hedgerow	-0.11	-0.22	<0.001	0.051
Opportunistic herbo-fungi %	Hedgerow – old forest	0.06	-0.17	0.30	0.782
Opportunistic herbo-fungi %	Young forest – old forest	-0.03	-0.25	0.19	0.942
Opportunistic herbo-fungi %	Young forest – Hedgerow	-0.09	-0.230	0.05	0.239
Fungivorous browser %	Hedgerow – old forest	-0.06	-0.23	0.11	0.673
Fungivorous browser %	Young forest – old forest	-0.10	-0.26	0.06	0.289
Fungivorous browser %	Young forest – Hedgerow	-0.04	-0.14	0.06	0.580
Herbivorous browser %	Old hedgerow – old forest	-0.001	-0.27	0.26	0.997
Herbivorous browser %	Young forest – old forest	0.31	0.06	0.56	**0.013**
Herbivorous browser %	Young forest – hedgerow	0.32	0.16	0.47	**<0.001**
Herbivorous grazer %	Hedgerow – old forest	0.02	-0.21	0.26	0.974
Herbivorous grazer %	Young forest – old forest	0.08	-0.14	0.31	0.628
Herbivorous grazer %	Young forest – Hedgerow	0.06	-0.14	0.20	0.517
Omnivore %	Hedgerow – old forest	-0.09	-0.21	0.02	0.137
Omnivore %	Young forest – old forest	0.02	-0.09	0.13	0.922
Omnivore %	Young forest – Hedgerow	0.11	0.04	0.18	**0.001**
General predator %	Hedgerow – old forest	0.02	-0.14	0.17	0.969
General predator %	Young forest – old forest	-0.02	-0.16	0.13	0.952
General predator %	Young forest – Hedgerow	-0.03	-0.13	0.06	0.656

Table S2.3.3: Tukey HSD results of the ANOVA tests with the log_{10} density of mites, using an interaction between forest category and mite size class. Diff is the difference in value between the categories, lower and upper show the 95% confidence interval ranges. P adj. is the correct P-value after adjustment for multiple corrections. Values in bold signify significant effects.

Model	Two categories tested	Diff.	Lower	Upper	P adj.
Log 10 density small	Hedgerow – old forest	-0.10	-1.29	1.09	1.000
Log 10 density small	Young forest – old forest	-0.14	-1.27	0.98	0.999
Log 10 density small	Young forest – Hedgerow	-0.04	-0.75	0.67	1.000
Log 10 density large	Hedgerow – old forest	1.23	0.04	2.42	**0.039**
Log 10 density large	Young forest – old forest	-0.18	-1.31	0.94	0.997
Log 10 density large	Young forest – Hedgerow	-1.42	-2.12	-0.71	**<0.001**

Table S2.3.4: Tukey HSD results of the ANOVA tests with the log_{10} Olsen-P content. Diff is the difference in value between the categories, lower and upper show the 95% confidence interval ranges. P adj is the correct P-value after adjustment for multiple corrections. Values in bold signify significant effects.

Model	Two categories tested	Diff	Lower	Upper	P adj.
Log 10 Olsen-P	Hedgerow – old forest	0.43	0.09	0.76	**0.013**
Log 10 Olsen-P	Young forest – old forest	0.06	-0.32	0.43	0.919
Log 10 Olsen-P	Young forest –Hedgerow	-0.37	-0.65	-0.09	**0.010**

Table S2.4: The number of observed and expected unique species in each forest category for several microarthropod groups including a chi square test. Significant χ2 values are shown in bold. Other = Non-Acari or Collembola microarthropod.

Group	**Obs. old forest**	**Exp. old forest**	**Obs. old hedgerow**	**Exp. old hedgerow**	**Obs. young forest**	**Exp. young forest**	**χ2**
Total	12	5.80	65	21.28	41	50.61	**98.28**
Fungivorous grazer	4	1.06	13	3.86	3	8.67	**32.40**
Herbo-fungivorous grazer	0	0.57	7	2.09	3	4.59	**12.66**
Opportunistic herbo-fungivore	1	0.10	2	0.46	0	1.44	**14.70**
Fungivorous browser	0	0.20	2	0.86	2	2.46	1.80
Herbivorous browser	0	0.09	0	0.31	1	0.65	0.59
Herbivorous grazer	0	0.30	9	1.29	0	3.58	**49.96**
General predator	4	2.40	22	8.17	14	18.79	**25.70**
Omnivore	0	0.00	0	0.00	0	0.00	n. a
Bacteriovore	0	0.00	0	0.00	0	0.00	n. a
Collembola	3	1.08	10	4.24	17	10.43	**15.38**
Other	0	0.00	0	0.00	1	0.01	n. a

Table S2.5: List of unique old forest species with the associated microarthropod group they belong, their size, number of locations it was found and how many individuals were found in total. The size of mites given are the average size in µm, while Collembola size in µm was taken from Hopkin 2007, which consists of the maximum species length.

No	Group	Feeding guild	Species	Size (µm)	Occur.	Total count
1	Oribatida	Fungivorous grazer	*Berniniella sigma*	200	2	34
2	Oribatida	Fungivorous grazer	*Ceratozetes minimus*	350	1	1
3	Oribatida	Fungivorous grazer	*Damaeus clavipes*	980	1	1
4	Oribatida	Fungivorous grazer	*Eupelops torulosus*	690	1	1
5	Oribatida	Opportunistic herbo-fungivore	*Carabodes femoralis*	660	2	7
6	Mesostigmata	General predator	*Gamasellus*	600	1	1
7	Prostigmata	General predator	*Smaridiidae*	700	1	1
8	Mesostigmata	General predator	*Trachytes pauperior*	475	2	6
9	Mesostigmata	General predator	*Veigaia kochi*	1390	1	2
10	Collembola		*Entomobrya multifasciata*	2000	1	7
11	Collembola		*Micranurida pygmaea*	500	1	1
12	Collembola		*Tomocerus minor*	4500	2	4

Table S2.6: List of unique old hedgerow species with the associated microarthropod group they belong, their size, number of locations it was found and how many individuals were found in total. The size of mites given are the average size in µm, while Collembola size in µm was taken from Hopkin 2007, which consists of the maximum species length.

No.	(Group	Feeding guild	Species	Size (µm)	Occur.	Total count
1	Oribatida	Fungivorous grazer	*Atopochthonius artiodactylus*	200	1	1
2	Oribatida	Fungivorous grazer	*Autogneta willmanni*	320	1	1
3	Oribatida	Fungivorous grazer	*Berniniella conjuncta*	210	1	1
4	Oribatida	Fungivorous grazer	*Berniniella lunaris*	205	3	93
5	Oribatida	Fungivorous grazer	*Hypogeoppia terricola*	215	1	39
6	Oribatida	Fungivorous grazer	*Liacarus coracinus*	875	2	3
7	Oribatida	Fungivorous grazer	*Liacarus subterraneus*	1005	6	21
8	Oribatida	Fungivorous grazer	*Liacarus xylariae*	920	1	2
9	Oribatida	Fungivorous grazer	*Oppiella falcata*	240	9	199
10	Oribatida	Fungivorous grazer	*Pantelozetes paolii*	390	2	12
11	Oribatida	Fungivorous grazer	*Punctoribates punctum*	380	6	32
12	Oribatida	Fungivorous grazer	*Ramusella insculpta*	250	2	130
13	Oribatida	Fungivorous grazer	*Zygoribatula exilis*	380	1	1
14	Oribatida	Herbo-fungivorous grazer	*Acrogalumna longipluma*	750	1	2
15	Oribatida	Herbo-fungivorous grazer	*Chamobates subglobulus*	685	1	2
16	Oribatida	Herbo-fungivorous grazer	*Hermanniella septentrionalis*	610	1	9
17	Oribatida	Herbo-fungivorous grazer	*Nanhermannia sellnicki*	550	1	3

18	Oribatida	Herbo-fungivorous grazer	*Nothrus anauniensis*	755	2	12
19	Oribatida	Herbo-fungivorous grazer	*Nothrus palustris*	1095	3	70
20	Oribatida	Herbo-fungivorous grazer	*Trichoribates novus*	735	1	1
21	Oribatida	Opportunistic herbo-fungivorous	*Carabodes areolatus*	550	1	1
22	Oribatida	Opportunistic herbo-fungivorous	*Carabodes coriaceus*	645	3	2
23	Oribatida	Fungivorous browser	*Gustavia microcephala*	405	1	4
24	Oribatida	Fungivorous browser	*Suctobelba trigona*	250	2	4
25	Oribatida	Herbivorous grazer	*Achipteria willmanni*	625	5	25
27	Oribatida	Herbivorous grazer	*Malaconothrus monodactylus*	450	3	7
28	Oribatida	Herbivorous grazer	*Malaconothrus punctulatus*	400	1	1
29	Oribatida	Herbivorous grazer	*Phthiracarus anonymus*	400	1	51
30	Oribatida	Herbivorous grazer	*Phthiracarus globosus*	560	2	7
31	Oribatida	Herbivorous grazer	*Steganacarus michaeli*	870	2	7
32	Oribatida	Herbivorous grazer	*Xenillus clypeator*	1300	7	57
33	Oribatida	Herbivorous grazer	*Xenillus tegeocranus*	910	3	7
34	Mesostigmata	General predator	*Asca bicornis*	350	3	8
35	Mesostigmata	General predator	*Dinychus perforatus*	885	2	10
36	Mesostigmata	General predator	*Eulaelaps stabularis*	475	1	2
37	Mesostigmata	General predator	*Gamasellolus bicolor*	355	5	11
38	Mesostigmata	General predator	*Geholaspis aeneus*	1050	3	8
39	Prostigmata	General predator	*Ledermuelleria*	300	4	28
40	Mesostigmata	General predator	*Leptogamasus suecicus*	480	1	1
41	Mesostigmata	General predator	*Mixozercon sellnicki*	330	1	2
42	Mesostigmata	General predator	*Pachylaelaps imitans*	935	5	19

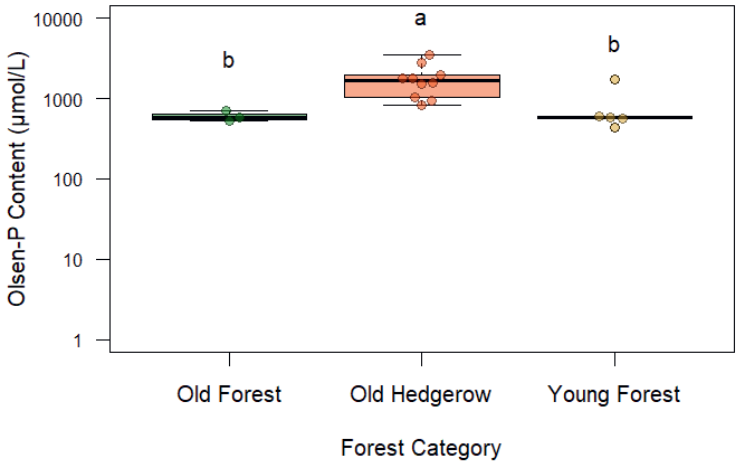
43	Mesostigmata	General predator	*Pachylaelaps laeuchlii*	845	1	1
44	Mesostigmata	General predator	*Pachylaelaps longisetus*	770	1	2
45	Mesostigmata	General predator	*Paragamasus robustus*	1045	1	2
46	Mesostigmata	General predator	*Pergamasus mediocris*	1080	1	1
47	Mesostigmata	General predator	*Pergamasus noster*	1220	1	2
48	Mesostigmata	General predator	*Pergamasus puerilis*	370	4	15
49	Mesostigmata	General predator	*Pergamasus quisquiliarium*	1220	1	4
50	Mesostigmata	General predator	*Pergamasus ruehmi*	1175	1	1
51	Mesostigmata	General predator	*Rhodacarellus silesiacus*	280	1	4
52	Mesostigmata	General predator	*Rhodacarus agrestis*	450	1	17
53	Mesostigmata	General predator	*Rhodacarus roseus*	465	1	4
54	Mesostigmata	General predator	*Trichouropoda ovalis*	985	7	77
55	Mesostigmata	General predator	*Uroobovella*		2	4
56	Mesostigmata	General predator	*Zerconopsis remiger*	615	3	8
57	Collembola		*Entomobrya corticalis*	1400	6	9
58	Collembola		*Heterosminthurus claviger*		1	2
59	Collembola		*Isotomodes productus*	900	1	2
60	Collembola		*Lipothrix lubbocki*	2000	1	1
61	Collembola		*Neanura muscorum*	3500	6	10
62	Collembola		*Protaphorura armata*	2500	1	7
63	Collembola		*Protaphorura pseudovanderdrifti*		3	56
64	Collembola		*Pseudosinella alba*	1000	4	30
65	Collembola		*Sinella curviseta*	2000	2	10
66	Collembola		*Sminthurides parvulus*	300	6	22

Table S2.7: List of unique young forest species with the associated microarthropod group they belong, their size, number of locations it was found and how many individuals were found in total. The size of mites given are the average size in µm, while Collembola size in µm was taken from Hopkin 2007, which consists of the maximum species length.

No.	Group	Mite feeding guild	Species	Size (µm)	Occurr.	Total Count
1	Oribatida	Fungivorous grazer	*Astegistes pilosus*	595	1	1
2	Oribatida	Fungivorous grazer	*Cepheus dentatus*	795	1	1
3	Oribatida	Fungivorous grazer	*Ceratozetes mediocris*	410	14	115
4	Oribatida	Herbo-fungivorous grazer	*Acrotritia ardua*	620	1	8
5	Oribatida	Herbo-fungivorous grazer	*Chamobates pusillus*	520	2	2
6	Oribatida	Herbo-fungivorous grazer	*Trichoribates trimaculatus*	620	4	7
7	Oribatida	Fungivorous browser	*Damaeus auritus*	850	3	3
8	Oribatida	Fungivorous browser	*Oribatella quadricornuta*	505	1	12
9	Prostigmata	Herbivorous browser	*Eriophyidae*	150	1	1
10	Mesostigmata	General predator	*Alliphis halleri*	425	3	3
11	Prostigmata	General predator	*Anystidae*	600	1	2
12	Mesostigmata	General predator	*Asca aphidioides*	290	2	5
13	Prostigmata	General predator	*Cilliba*	700	1	1
14	Prostigmata	General predator	*Erythraeidae*		1	1
15	Mesostigmata	General predator	*Geholaspis mandibularis*	760	2	2
16	Mesostigmata	General predator	*Pachylaelaps multidentatus*	615	12	129
17	Mesostigmata	General predator	*Pachylaelaps tesselatus*	565	1	1
18	Mesostigmata	General predator	*Parazercon radiatus*	340	3	11

19	Mesostigmata	General predator	*Pergamasus misellus*	505	6	20
20	Mesostigmata	General predator	*Pergamasus norvegicus*	1450	1	1
21	Mesostigmata	General predator	*Pergamasus runcatellus*	700	1	3
22	Mesostigmata	General predator	*Pergamasus septentrionalis*	1430	1	2
23	Mesostigmata	General predator	*Zercoseius spathuliger*	430	1	4
24	Collembola		*Allacma fusca*	3500	1	1
25	Collembola		*Brachystomella parvula*	1000	1	2
26	Collembola		*Entomobrya lanuginosa*	2000	1	6
27	Collembola		*Entomobrya nivalis*	2500	2	7
28	Collembola		*Friesea truncata*	1000	8	56
29	Collembola		*Heteromurus nitidus*	3000	1	2
30	Collembola		*Lepidocyrtus violaceus*	1600	1	5
31	Collembola		*Neelides minutus*	1000	2	4
32	Collembola		*Paratullbergia callipygos*	1200	6	21
33	Collembola		*Pogonognathellus flavescens*	6000	3	4
34	Collembola		*Proisotoma minuta*	1100	7	54
35	Collembola		*Pseudachorutes*		6	94
36	Collembola		*Pseudisotoma sensibilis*	1800	2	17
37	Collembola		*Sphaeridia pumilis*	250	16	102
38	Collembola		*Tomocerus vulgaris*	4000	4	11
39	Collembola		*Vertagopus cinereus*	1800	2	2
40	Collembola		*Willemia*		2	4
41	Collembola		Pauropoda		10	135

Fig. S2.1 The Olsen P content (µmol/L) of 3 old forest locations, 10 old hedgerow locations and 5 young forest locations. The axis is on a $\log_{10}$ scale. Significance levels between each group were tested using a Tukey HSD test (details Table S2.3.4) and shown with letters (a is significantly higher as b, ab is no significance).



Supplementary for Chapter 3

Table S3.1: Results of the linear mixed effects models comparing the density of microarthropods between H9190 oak forests and the reference locations. The density is given in individuals/m^2 and log_{10} transformed. Location of the site was used as a random factor. Significant results are given in bold.

Model	**Value**	**S.E.**	**DF.**	**t-value**	**p**
Density models					
Density	-0.445	0.155	7	-2.859	**0.017**
Density fungivorous browsers	-1.136	0.438	7	-2.596	**0.036**
Density fungivorous grazers	-1.114	0.418	7	-2.633	**0.032**
Density herbo-fungivores	-0.644	0.368	7	-1.803	0.114
Density herbivores	-0.238	0.124	7	-1.914	0.097
Density predators	-0.680	0.288	7	-2.362	0.050
Density other	-0.247	0.146	7	-1.691	0.135
Proportion models					
Proportion fungivorous browsers	-0.829	0.346	7	-2.396	**0.048**
Proportion fungivorous grazers	- 0.908	0.352	7	- 2.580	**0.037**
Proportion herbo-fungivores	0.009	0.257	7	0.035	0.973
Proportion herbivores	0.677	0.386	7	1.754	0.123
Proportion predators	- 0.071	0.199	7	-0.360	0.729
Proportion other Acari	0.753	0.562	7	1.339	0.222
Species richness models					
Species richness	-14.578	5.108	7	-2.899	**0.023**
Species richness fungivorous browsers	-4.940	0.995	7	-4.925	**0.002**
Species richness fungivorous grazers	-5.094	1.938	7	-2.629	**0.034**
Species richness herbo-fungivores	-2.422	1.024	7	2.366	0.050
Species richness herbivores	-0.692	0.416	7	-1.666	0.140
Species richness predators	-2.884	1.013	7	-2.846	**0.025**
Species richness other Acari	0.135	0.253	7	0.532	0.611

Table S3.2*:* Data for fungal fruit body inventory done in H9190 locations (KW = Kootwijkerdijk, BW = Bosbergerweg, HB = Heidensberg, MS = Maanschoten (highlighted in red). For each location we used data of one field visit per year in the fall of both 2023 and 2024. In 2023 the sites were visited on 16 and 17 October and in 2024 all sites were visited between 30 September and 2 October. During a field visit a plot of 5000 m^2 was surveyed and each fungi species was morphologically identified by its fruit body. Additionally, species were divided into mycorrhizal species and non-mycorrhizal species.

	BW	HB	KW	MS	**Mean**
Mycorrhiza species richness 2023	8	10	14	11	**10.75**
Mycorrhiza species richness 2024	4	5	7	10	**6.50**
Mycorrhiza species richness mean	6	7.5	10.5	10.5	**8.63**
Non-mycorrhiza species richness 2023	6	5	2	6	**4.75**
Non-mycorrhiza species richness 2024	3	4	2	7	**4.00**
Non-mycorrhiza richness mean	4.5	4.5	2	6.5	**4.38**

Supplementary for Chapter 4

Table S4.1.1: Model results of the Type I ANOVA models used. Location and the natural log of the sample month, and an interaction between these two variables, were used as predictor variables. P-values given in bold are statistically significant.

Model	Variable tested	df	Sum of squares	F-Value	P-value
Abundance	Location	8	0.803	1.737	0.088
	Sample Month	1	2.760	47.79	**<0.001**
	Interaction	8	2.679	5.798	**<0.001**
Species richness	Location	8	2856	6.126	**<0.001**
	Sample Month	1	15099	259.11	**<0.001**
	Interaction	8	308	0.661	0.726
Species richness herbivorous grazers	Location	8	53.8	3.200	**0.002**
	Sample Month	1	89.5	42.55	**<0.001**
	Interaction	8	14.5	0.865	0.547
Species richness Fungivorous grazers	Location	8	226.8	6.870	**<0.001**
	Sample Month	1	1229.80	297.95	**<0.001**
	Interaction	8	47.90	1.450	0.175
Species richness herbo-fungivorous grazers	Location	8	129.0	12.810	**<0.001**
	Sample Month	1	194.6	154.59	**<0.001**
	Interaction	8	33.5	3.325	**0.001**

Table S4.1.2: Model output of the linear mixed effect models analysing how the proportion of Fgs and Hgs change over time. The logit-transformed proportion of either Fg or Hg was used as the response variable and the natural log transformed sample month was used as predictor variable. Location was used as random factor. Significant p-values are given in bold.

Model	Variable	Value	Std. Error	df	T-value	P-value
Proportion Fgs	Sample month	0.449	0.043	339	10.51	**<0.001**
Proportion Hgs	Sample month	-0.628	0.044	339	-14.28	**<0.001**

Table S4.1.3: Model of the segmented regression analysis in an ANOVA type I table. The model is Proportion remaining weight = Location * Sample Month + Sample Month: Julian day. The Julian day is adjusted with the following formula: Julian day adjusted =sin((2π*Julian day)/365) * 0.5 * 365. Significant p-values are given in bold.

Variable	df	Sum of squares	F-Value	P-value
Location	8	0.984	11.075	**<0.001**
Sample month	1	10.567	951.29	**<0.001**
U1 Sample month	1	0.014	1.294	0.256
U2 Sample month	1	0.353	31.801	**<0.001**
Psi1 Sample month	1	0.082	7.363	**0.007**
Psi2 Sample month	1	0.015	1.338	0.248
Location : Sample month	8	0.262	2.943	**0.003**
Sampl Month: Julian day	1	0.001	0.101	0.751

Table S4.1.4: Model output of the linear mixed effect models testing the effect of leaf N and leaf P content on the early decomposition (first 6 months). Proportion remaining weight was the response variable, N and P as the predictor variables and location as random factor. Significant p-values are given in bold.

Variable	Value	Std. Error	Df	T-value	P-value
Early					
Fungivorous grazer species richness	-0.016	0.019	115	-0.864	0.399
Herbo-fungivorous grazer species richness	0.016	0.027	115	0.611	0.542
N	0.027	0.009	115	3.134	**0.002**
P	-0.001	0.003	115	-0.504	0.615
Sample month	0.219	0.019	115	11.578	**<0.001**
Late					
Fungivorous grazer species richness	0.227	0.077	194	3.053	**0.003**
Herbo-fungivorous grazer species richness	-0.425	0.133	194	-3.205	**0.002**
N	0.002	0.002	194	1.020	0.309
P	0.021	0.008	194	2.599	**0.010**
Sample month	0.209	0.051	194	4.094	**<0.001**
Fungivorous grazer richness: Sample month	-0.015	0.005	194	-3.272	**0.001**
Herbo-fungivorous richness: Sample month	0.025	0.008	194	3.043	**0.003**

Table S4.2: Species score output for the canonical correspondence analysis. Constrained variables were location, sample month and a transformed Julian day**.** The Julian day is transformed with the following formula: Julian day corrected=sin((2π*Julian day)/365) * 0.5 * 365. The abundance is the total number of individuals over all litterbags and occurrence is the number of litterbags the species was found. The feeding guilds are used from Siepel and Ruiter-Dijkman 1993 and the given feeding guilds are: B = Bacterivore, Fb = Fungivorous browser, Fg = Fungivorous grazer, General predator = General preadtor, Hfg = Herbo-fungivorous grazer, Hb = Herbivorous browser, Hg = Herbivorous grazer, O = Omnivore and Ohf = Opportunistic herbo-fungivore.

Species	Abundance	Occurrence	Feeding guild	CC1	CC2
Abrolophus rhopalicus	8	5	Gp	0.02	1.02
Acerentomon gallicum	297	37	Fb	0.95	-0.18
Achipteria coleoptrata	98	43	Hg	0.73	0.97
Acrotritia duplicata	967	170	Hfg	0.64	-0.43
Adelphacarus sellnicki	30	23	Fg	0.72	-0.31
Adoristes ovatus	313	134	Ohf	-0.67	0.01
Alicorhagia fragilis	200	69	O	1.11	-0.28
Allacma fusca	1	1	Hfg	0.39	-0.79
Allopauropus danicus	204	34	Ohf	0.19	0.02
Alycus roseus	17	11	Gp	-0.27	1.05
Amblyseius obtusus	5	4	Gp	-0.80	-0.27
Anurida granaria	45	23	Fb	0.74	-0.30
Anurida tullbergi	1	1	Fb	0.16	2.26
Anystis baccarum	4	2	Gp	0.55	2.15
Aphelacarus acarinus	3	3	Fg	-0.98	-0.28
Armascirus cyaneus	314	65	Gp	-0.23	-0.15
Arrhopallites caecus	70	30	Fb	0.42	-0.34
Arrhopallites pygmaeus	79	36	Fb	-0.65	1.19
Asca aphidioides	31	13	Gp	0.06	1.48
Atropacarus striculus	295	89	Hg	0.75	-0.33
Bakerdania comatus	18	16	Fb	0.17	-0.03
Banksinoma lanceolata	404	83	Fg	-0.16	-0.49
Berniniella sigma	37	26	Fg	0.86	0.19
Bimichaelia sarekensis	1	1	Fb	0.16	2.26
Brachychthonius immaculatus	35	21	Fg	-0.39	0.06
Brachychthonius impressus	5	4	Fg	1.51	1.89
Brachystomella parvula	17	8	Hg	-0.03	-0.60
Brachytydeus latiuscula	63	32	Hb	-0.74	0.15
Brachytydeus mirabilis	201	68	Hb	0.50	0.93
Brevipalpus obovatus	4	3	Hb	0.23	-0.34
Caenonychus fallax	1	1	Hb	-1.72	-0.35
Camisia spinifer	2	2	Hg	1.45	0.62
Campodea plusiochaeta	25	16	Hg	0.74	-0.22
Carabodes coriaceus	48	34	Ohf	0.28	-0.12
Carabodes labyrinthicus	2	2	Ohf	-0.91	1.27

Carabodes marginatus	10	10	Ohf	0.78	-0.08
Carabodes willmanni	3	3	Ohf	-1.06	-0.09
Cepheus cepheiformis	20	15	Fg	0.50	1.01
Ceratophysella denticulata	789	68	Hfg	-1.31	-0.34
Ceratoppia bipilis	152	77	Fg	-0.03	-0.16
Chamobates cuspidatus	499	82	Fb	0.28	-0.41
Chamobates pusillus	25	6	Hfg	0.62	-0.46
Chernes hahni	2	2	Gp	1.35	-1.17
Cocceupodes australis	697	94	O	-0.81	1.32
Cosmolaelaps claviger	1	1	Gp	0.55	2.15
Cosmolaelaps miles	1	1	Gp	0.29	-0.33
Cultroribula bicultrata	57	16	Fg	0.75	0.87
Cymbaeremaeus cymba	2	2	Ohf	0.24	-0.53
Cyphoderus albinus	4	3	Fb	-1.10	-0.23
Damaeobelba minutissima	32	27	Fb	0.80	-0.20
Damaeus clavipes	89	38	Fg	-0.35	-0.19
Dicyrtoma fusca	29	22	Hg	-0.43	-0.24
Dicyrtomina ornata	7	6	Hg	-0.98	-0.26
Dicyrtomina saundersi	21	13	Hg	0.04	0.80
Dissorhina ornata	4380	216	Fg	-0.50	0.21
Eniochthonius minutissimus	1938	214	Fg	0.50	-0.01
Entomobrya multifasciata	90	42	Hg	-0.03	0.48
Entomobryoides purpurascens	198	48	Fg	0.46	-0.07
Ephippiochthonius kewi	11	2	Gp	0.02	-0.67
Epicrius canestrinii	58	27	Gp	0.62	-0.80
Ereynetes septentrionalis	148	62	Gp	0.17	-0.04
Eueremaeus oblongus	3	3	Hg	-0.38	-0.61
Eulohmannia ribagai	2	2	Fg	0.55	2.15
Eupelops plicatus	52	29	Fg	0.52	-0.64
Eupodes truncatus	4738	328	O	-0.08	0.44
Eustigmaeus coronaria	1	1	Gp	-0.92	-0.57
Euzetes globulus	9	5	Hfg	0.48	0.51
Eviphis ostrinus	2	2	Gp	-0.86	-0.50
Folsomia fimetaria	3	1	Fb	-0.54	1.17
Folsomia quadrioculata	2514	171	Hfg	0.11	-0.45
Foveacheles terricola	52	18	Gp	-0.44	2.42
Friesea mirabilis	74	37	Fb	0.13	-0.13
Galumna lanceata	248	75	Fg	0.23	0.33
Gamasellodes bicolor	66	22	Gp	0.35	1.73
Geholaspis longispinosus	12	10	Gp	-0.65	0.26
Geolaelaps praesternalis	3	2	Gp	0.26	0.95
Graptoppia paraanalis	2	2	Fg	0.36	2.21
Hemileius initialis	2	1	Fb	-0.54	1.17
Hermannia gibba	53	29	Hfg	0.19	-0.66
Hermanniella granulata	117	64	Hfg	-0.03	-0.45

Heteromurus nitidus	4	2	Hg	-1.37	-0.25
Histiostoma sapromyzarum	4153	200	B	-1.07	-0.37
Holoparasitus calcaratus	148	87	Gp	-0.31	0.02
Hypochthonius rufulus	2774	189	O	0.25	0.23
Hypogastrura manubrialis	1	1	Hg	-0.68	0.08
Iphidozercon gibbus	2	2	Gp	-1.03	-0.46
Isotoma viridis	694	109	Hfg	-1.01	-0.18
Isotomiella minor	4272	245	Fb	0.49	-0.47
Isotomurus palustris	39	12	Fb	-0.99	0.19
Ixodes ricinus	2	2	Gp	-0.13	-0.70
Labidostomma luteum	233	63	Gp	0.75	-0.32
Lamprochernes nodosus	32	16	Gp	0.20	-0.63
Ledermuelleriopsis sp.	71	32	Gp	0.18	1.04
Lepidocyrtus curvicollis	27	5	Hg	-1.67	-0.23
Lepidocyrtus cyaneus	362	80	Fg	-0.53	-0.44
Lepidocyrtus lanuginosus	2696	260	Hg	-0.45	0.26
Lepidoglyphus burchanensis	1	1	Fb	-0.92	-0.57
Leptus molochinus	3	3	Gp	0.88	-0.65
Liacarus coracinus	4	3	Fg	0.16	-0.41
Liacarus subterraneus	12	10	Fg	0.16	-0.26
Liebstadia humerata	1	1	Hfg	-0.91	-0.01
Lineremaeus licnophorus	1	1	Fb	1.35	1.93
Linopodes motatorius	165	76	Gp	-0.47	-0.37
Linotetranychus cylindricus	9	7	Hb	-0.75	0.13
Liochthonius brevis	156	31	Fg	-0.01	-0.56
Liochthonius evansi	28	12	Fg	-0.31	0.33
Liochthonius horridus	2	1	Fg	1.35	1.93
Liochthonius hystrinicus	937	107	Fg	0.82	-0.14
Liochthonius lapponicus	9	5	Fg	0.51	2.16
Liochthonius plumosus	22	13	Fg	1.30	0.05
Lipothrix lubbocki	31	21	Fg	0.08	-0.07
Machuella bilineata	21	15	Fg	0.91	-0.02
Macrocheles carinatus	18	10	Gp	0.91	2.06
Macrocheles glaber	1	1	Gp	-0.41	-0.57
Macrocheles opacus	49	35	Gp	0.21	-0.47
Malaconothrus processus	24	21	Hg	0.21	-0.74
Melanozetes mollicomus	219	195	Fb	0.28	-0.08
Mesaphorura sp.	2	1	Hb	0.99	-0.95
Mesostigmata sp.	448	128	Fb	0.72	-0.03
Metabelba papillipes	1	1	Fg	1.13	-0.75
Micreremus brevipes	4	4	Fg	0.86	-0.03
Microppia minus	100	50	Fg	-0.40	0.19
Microtritia minima	7	7	Hfg	0.77	-0.36
Microtydeus subterraneus	900	145	Hb	0.24	-0.14
Nanhermannia nana	714	141	Hfg	0.43	-0.14
Nanorchestes arboriger	392	104	Hb	0.39	0.00

Neanura muscorum	35	28	Ohf	0.27	-0.47
Neelus murinus	1306	137	Fb	0.15	-0.50
Neobisium carcinoides	87	35	Gp	-0.41	0.04
Nothrus silvestris	1700	195	Hfg	0.99	-0.08
Odontocepheus elongatus	186	99	Ohf	0.44	-0.16
Odontoscirus meridionalis	74	35	Gp	-0.00	-0.15
Ophidiotrichus tectus	10	5	Fg	0.17	-0.36
Oppiella maritima	16	1	Fg	1.06	0.73
Oppiella nova	3793	175	Fg	1.05	-0.11
Orchesella villosa	2	2	Fg	0.37	0.71
Oribatula tibialis	218	40	Fg	0.25	0.34
Pachylaelaps bellicosus	86	40	Gp	0.89	-0.21
Pachylaelaps fuscinuliger	7	5	Gp	1.15	1.20
Palaeacarus hystrinicus	10	6	Fg	0.97	-1.01
Paragamasus alpestris	2	2	Gp	0.36	2.21
Paragamasus robustus	3	2	Gp	-0.58	-0.38
Parasitus lunulatus	94	36	Gp	-1.45	0.13
Parisotoma notabilis	4937	271	Fg	-0.23	0.95
Peloptulus montanus	75	28	Fg	-1.09	-0.24
Pergalumna nervosa	220	30	Hfg	-0.06	-0.59
Pergamasus crassipes	207	111	Gp	-0.54	-0.19
Pergamasus minorleitnerae	11	3	Gp	1.75	1.83
Pergamasus misellus	94	47	Gp	0.55	-0.51
Pergamasus nasellus	26	11	Gp	-1.30	-0.32
Pergamasus puerilis	2	2	Gp	-0.44	-0.21
Pergamasus runciger	1	1	Gp	1.15	1.99
Pergamasus septentrionalis	37	27	Gp	-0.64	2.48
Pergamasus vagabondus	1164	234	Gp	0.56	-0.58
Phauloppia nemoralis	7	6	Hfg	-0.60	1.27
Phthiracarus anonymus	241	107	Hg	0.15	-0.23
Phthiracarus ferrugineus	19	14	Hg	0.03	1.51
Phthiracarus globosus	304	129	Hg	-0.00	-0.11
Phthiracarus laevigatus	3	3	Hg	0.48	1.26
Platynothrus peltifer	8399	317	Hg	-0.87	-0.22
Proctolaelaps jueradeus	8	6	Gp	0.42	2.03
Proisotoma minima	2	2	Fb	0.76	-0.60
Protaphorura armata	521	127	Fb	-0.02	-0.43
Protoribates lophotrichus	26	3	Fg	1.01	-0.95
Prozercon kochi	157	56	Gp	0.33	-0.70
Pseudosinella alba	140	36	Fb	-0.06	0.65
Pseudosinella immaculata	6	6	Fb	-0.34	1.38
Punctoribates punctum	15	11	Fg	-0.44	0.07
Quadroppia maritalis	57	34	Fg	1.04	-0.34
Quadroppia quadricarinata	21	13	Fg	1.17	0.60
Ramusella clavipectinata	1	1	Fg	-0.24	2.37

Rhagidia punkva	219	103	Gp	0.14	0.06
Rhinoppia subpectinata	1517	84	Fg	0.31	-0.60
Rhizoglyphus echinopus	293	33	Fb	0.74	0.96
Rhodacarus coronatus	24	14	Gp	1.19	0.79
Scheloribates ascendens	3	3	Ohf	0.83	1.08
Scheloribates pallidulus	14	8	Fg	0.55	-0.14
Schwiebea lebruni	25	8	Fb	0.45	-0.51
Schwiebea talpa	822	177	Fb	0.46	0.06
Scutacarus acarorum	10	3	Fb	1.05	1.71
Scutacarus montanus	1474	75	Fb	1.32	0.91
Scutacarus parvus	95	35	Fb	0.51	-0.65
Scutacarus quadriangularis	2	2	Fb	0.36	2.21
Sellnickochthonius oesziae	1	1	Fg	0.95	2.04
Sellnickochthonius zelawaiensis	42	15	Fg	1.46	0.42
Shibaia heteropoda	250	116	Gp	-0.45	-0.17
Siteroptes graminis	8	4	Hb	0.16	-0.19
Sminthurides parvulus	4	3	Hb	1.11	-0.86
Sminthurus viridis	1	1	Fg	1.35	1.93
Spatiodamaeus verticillipes	2284	228	Fg	-0.08	0.12
Sphaeridia pumilis	779	210	Fb	-0.37	-0.13
Steganacarus magnus	765	214	Hg	-0.02	0.21
Steneotarsonemus sp.	194	56	Hb	-0.17	-0.35
Stigmaeus insectus	65	29	Gp	0.82	-0.37
Stigmaeus sphagneti	2	2	Gp	0.16	2.26
Subeupodes boerneri	7	3	O	1.35	1.93
Subiasella quadrimaculata	2	2	Fg	0.99	0.19
Suctobelbella acutidens	152	53	Fb	0.44	-0.47
Suctobelbella falcata	18	10	Fb	0.32	-0.54
Suctobelbella forsslundi	4	3	Fb	0.81	-0.99
Suctobelbella hamata	2	1	Fb	1.35	1.93
Suctobelbella lobata	18	8	Fb	0.82	2.08
Suctobelbella subcornigera	2739	224	Fb	0.63	-0.21
Suctobelbella subtrigona	576	116	Fb	0.66	0.38
Suctobelbella vera	14	11	Fb	0.46	0.00
Symphylellopsis subnuda	19	12	Hg	0.56	0.19
Tectocepheus velatus	5027	317	Ohf	0.24	-0.20
Tomocerus vulgaris	141	78	Hg	0.17	-0.10
Trachytes aegrota	449	126	Gp	-0.03	-0.31
Trachytes oudemansi	5	5	Gp	-0.64	-0.63
Trichoribates trimaculatus	1	1	Hfg	0.59	-0.84
Trichouropoda ovalis	177	48	Gp	0.23	1.60
Trombicula canestrinii	1	1	Gp	1.49	-0.66
Tyrophagus similis	21	15	Fb	-0.29	0.35
Urodiaspis tecta	272	48	Gp	0.17	2.03
Uropoda minima	596	197	Gp	0.10	0.07
Uroseius cylindricus	619	68	Gp	0.52	-0.91

Veigaia cerva	45	33	Gp	0.40	-0.34
Veigaia exigua	1	1	Gp	0.52	-0.25
Veigaia kochi	1	1	Gp	0.27	-0.89
Veigaia nemorensis	1836	274	Gp	0.31	0.02
Xenillus tegeocranus	6	5	Hg	0.30	0.50
Xenylla boerneri	138	32	Fb	-0.95	-0.19
Zercon triangularis	215	37	Gp	-0.23	2.28
Zerconopsis remiger	884	98	Gp	-1.37	0.01
Zygoribatula exilis	5	5	Fg	-0.52	-0.06

Table S4.3: List of unique species (unique is defined as a species that only occurred in one of the nine locations). Occurrence is the number of litterbags a species was found.

Location	Species	Occurrence
Bosbergerweg	*Metabelba papillipes*	1
Eikenheg	*Hypogastrura manubrialis*	1
Eikenheg	*Veigaia exigua*	1
Hoenderloo	*Camisia spinifer*	2
Hoenderloo	*Folsomia fimetaria*	1
Hoenderloo	*Geolaelaps praesternalis*	2
Hoenderloo	*Hemileius initialis*	1
Hoenderloo	*Oppiella maritima*	1
Hoog Baarlo	*Allacma fusca*	1
Hoog Baarlo	*Macrocheles glaber*	1
Keulerbosch	*Chernes hahni*	2
Kootwijkerdijk	*Caenonychus fallax*	1
Kootwijkerdijk	*Eustigmaeus coronaria*	1
Kootwijkerdijk	*Lepidoglyphus burchanensis*	1
Kootwijkerdijk	*Veigaia kochi*	1
Loenen	*Melanozetes mollicomus*	1
Loenen	*Protoribates lophotrichus*	3
Loenen	*Trichoribates trimaculatus*	1
Nieuw Dennenlust	*Anurida tullbergi*	1
Nieuw Dennenlust	*Anystis baccarum*	2
Nieuw Dennenlust	*Bimichaelia sarekensis*	1
Nieuw Dennenlust	*Brachychthonius impressus*	4
Nieuw Dennenlust	*Cosmolaelaps claviger*	1
Nieuw Dennenlust	*Eulohmannia ribagai*	2
Nieuw Dennenlust	*Foveacheles terricola*	18
Nieuw Dennenlust	*Graptoppia paraanalis*	2
Nieuw Dennenlust	*Lineremaeus licnophorus*	1
Nieuw Dennenlust	*Liochthonius horridus*	1
Nieuw Dennenlust	*Liochthonius lapponicus*	5
Nieuw Dennenlust	*Macrocheles carinatus*	10
Nieuw Dennenlust	*Paragamasus alpestris*	2
Nieuw Dennenlust	*Pergamasus minorleitnerae*	3
Nieuw Dennenlust	*Pergamasus puerilis*	2
Nieuw Dennenlust	*Pergamasus runciger*	1
Nieuw Dennenlust	*Ramusella clavipectinata*	1
Nieuw Dennenlust	*Scutacarus quadriangularis*	2
Nieuw Dennenlust	*Sellnickochthonius oesziae*	1
Nieuw Dennenlust	*Sminthurus viridis*	1
Nieuw Dennenlust	*Stigmaeus sphagneti*	2
Nieuw Dennenlust	*Subeupodes boerneri*	3
Nieuw Dennenlust	*Suctobelbella hamata*	1
Nieuw Dennenlust	*Suctobelbella lobata*	8

Ugchelen	*Cosmolaelaps miles*	1
Ugchelen	*Liebstadia humerata*	1
Ugchelen	*Trombicula canestrinii*	1

Supplementary for Chapter 5

Table S5.1: Mineralogy of the two rock powder types used in this experiment. The values for Soilfeed were taken from Dar-Inc (n.d) and the values for Eiffelgold were taken from Servaplant (2019) and Swoboda et al. (2021).

Element	Typical value Soilfeed (%)	Typical value Eifelgold (%)
SiO_2	46	43
Al_2O_3	18	14
Na_2O	5	3
K_2O	5	3.5
CaO	8	12
MgO	4	9
TiO_2	3	2.5
Fe_2O_3	11	11
BaO	<0.2	0.1
P_2O_5		0.5
SO_3		0.1
MnO		0.25
Cl		<0.1
ZnO		<0.1
V_2O_5		<0.1
SiO		<0.1

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Table S5.2: Model output of the type III ANOVA tables used in this study. SRP and soil inoculation were used as predictor variables with the experimental site as random factor. Each model was fit using a linear mixed-effect model and used the Kenward-Rogers method to approximate degrees of freedom for the ANOVA table. The density of soil microarthropods, the total species richness and the species richness of fungivorous grazers were log_{10} transformed to improve normality. The feeding guild proportions and the base saturation were all logIt transformed to improve normality of proportional data. Soil pH, Ca, K, Mg and Al:Ca ratio were determined using a NaCl solution, base saturation using a strontium extraction.

Model	Parameters	Sum Sq.	NumDF	DenDF	F-value	P-value
Abundance and species richness models						
log_{10} Density	Inoculation	0.061	1	80	0.659	0.420
	SRP	0.037	2	80	0.199	0.820
	Interaction	0.059	2	80	0.320	0.727
Proportion Collembola	Inoculation	0.055	1	80	0.133	0.716
	SRP	0.182	2	80	0.220	0.803
	Interaction	1.023	2	80	1.236	0.296
log_{10} Species richness	Inoculation	0.035	1	80	1.739	0.191
	SRP	0.006	2	80	0.142	0.868
	Interaction	0.018	2	80	0.455	0.636
log_{10} Fungivorous grazer species richness	Inoculation	0.055	1	80	1.279	0.261
	SRP	0.011	2	80	0.128	0.880
	Interaction	0.003	2	80	0.031	0.970
Herbo-fungivorous grazer species richness	Inoculation	5.878	1	80	3.869	0.053
	SRP	5.356	2	80	1.763	0.178
	Interaction	4.289	2	80	1.412	0.250
Fungivorous browser species richness	Inoculation	4.011	1	80	0.836	0.363
	SRP	20.600	2	80	2.147	0.124
	Interaction	12.869	2	80	1.322	0.272
Opportunistic herbo-fungivorous species richness	Inoculation	0.100	1	80	0.123	0.726
	SRP	3.756	2	80	2.315	0.105
	Interaction	0.200	2	80	0.123	0.884
Herbivorous grazer species richness	Inoculation	0.011	1	80	0.006	0.940
	SRP	6.467	2	80	1.655	0.198
	Interaction	6.956	2	80	1.780	0.175
General predator species richness	Inoculation	2.500	1	80	0.286	0.595
	SRP	31.200	2	80	1.781	0.175
	Interaction	16.800	2	80	0.959	0.388
Feeding guild proportion models						
Fungivorous grazer proportion	Inoculation	0.888	1	80	1.567	0.214
	SRP	3.517	2	80	3.104	0.050
	Interaction	1.607	2	80	1.418	0.248
	Inoculation	0.771	1	80	1.309	0.256

Herbo-fungivorous grazer proportion	SRP	1.254	2	80	1.065	0.350
	Interaction	0.232	2	80	0.197	0.822
Fungivorous browser proportion	Inoculation	1.252	1	80	2.223	0.140
	SRP	11.249	2	80	9.987	**<0.001**
	Interaction	3.255	2	80	2.890	0.061
Opportunistic herbo-fungivore proportion	Inoculation	0.365	1	80	0.867	0.346
	SRP	5.889	2	80	6.993	**0.002**
	Interaction	0.425	2	80	0.505	0.605
Herbivorous grazer proportion	Inoculation	0.799	1	80	1.584	0.212
	SRP	0.608	2	80	0.602	0.550
	Interaction	0.678	2	80	0.673	0.513
Herbivorous browser proportion	Inoculation	0.462	1	80	0.791	0.377
	SRP	7.451	2	80	6.344	**0.003**
	Interaction	1.215	2	80	1.039	0.358
Omnivore proportion	Inoculation	0.483	1	80	1.329	0.252
	SRP	0.302	2	80	0.416	0.661
	Interaction	0.060	2	80	0.083	0.920
General predator proportion	Inoculation	0.220	1	80	0.983	0.324
	SRP	2.167	2	80	4.840	**0.010**
	Interaction	0.316	2	80	0.705	0.497
Soil chemistry models						
pH (NaCl)	Inoculation	0.024	1	20	0.645	0.431
	SRP	0.552	2	20	7.390	**0.004**
	Interaction	0.038	2	20	0.504	0.611
Base saturation (Sr)	Inoculation	0.336	1	20	2.050	0.168
	SRP	0.760	2	20	2.318	0.124
	Interaction	0.138	2	20	0.422	0.622
Ca (NaCl)	Inoculation	4,954,11	1	20	6.497	**0.019**
	SRP	1,583,41	2	20	2.077	0.152
	Interaction	455,625	2	20	0.598	0.560
K (NaCl)	Inoculation	132,005	1	20	1.502	0.235
	SRP	330,431	2	20	3.761	**0.041**
	Interaction	61,485	2	20	0.700	0.508
Mg (NaCl)	Inoculation	162,833	1	20	0.293	0.594
	SRP	5,072,40	2	20	9.136	**0.002**
	Interaction	18,620	2	20	0.968	0.968
Mn (NaCl)	Inoculation	32,751	1	20	3.566	0.074
	SRP	32,598	2	20	1.775	0.195
	Interaction	1,597	2	20	0.087	0.917
Al:Ca ratio	Inoculation	0.176	1	20	4.971	**0.037**
	SRP	0.069	2	20	0.970	0.396
	Interaction	0.054	2	20	0.771	0.476
P (NaCl)	Inoculation	7.30	1	20	0.005	0.941
	SRP	2,083.63	2	20	0.799	0.464
	Interaction	937.71	2	20	0.359	0.703

Table S5.3: Pairwise t-test using TukeyHSD adjustments results of the type III ANOVA models shown in Supplementary Table S2. Only models that had a significant main effect are shown here. Significant p values are shown in bold.

Model	Contrast	Est.	S.E.	d.f.	t-ratio	p-value
Fungivorous browser proportion	Control – Eifelgold	-0.812	0.194	80	-4.190	**<0.001**
	Control – Soilfeed	-0.145	0.194	80	-0.749	0.735
	Eifelgold - Soilfeed	0.667	0.194	80	3.441	**0.003**
Opportunistic herbo-fungivorous proportion	Control – Eifelgold	0.488	0.168	80	2.913	**0.013**
	Control – Soilfeed	0.584	0.168	80	3.488	**0.002**
	Eifelgold - Soilfeed	0.096	0.168	80	0.574	0.834
Herbivorous browser proportion	Control – Eifelgold	0.603	0.197	80	3.054	**0.009**
	Control – Soilfeed	0.615	0.197	80	3.115	**0.007**
	Eifelgold - Soilfeed	0.012	0.197	80	0.061	0.998
General predator proportion	Control – Eifelgold	0.005	0.122	80	0.045	0.999
	Control – Soilfeed	-3.326	0.122	80	-2.672	**0.025**
	Eifelgold - Soilfeed	-0.332	0.122	80	-2.717	**0.022**
pH (NaCL)	Control – Eifelgold	-0.293	0.086	20	-3.391	**0.008**
	Control – Soilfeed	-0.282	0.086	20	-3.264	**0.010**
	Eifelgold - Soilfeed	0.011	0.086	20	0.127	0.991
Al:Ca ratio (NaCl)	Inoculated – Null	-0.153	0.069	20	-2.230	**0.037**
Ca (NaCl)	Inoculated – Null	813	319	20	2.549	**0.019**
K (NaCl)	Control – Eifelgold	-363	133	20	-2.739	**0.033**
	Control – Soilfeed	-166	133	20	-1.250	0.439
	Eifelgold - Soilfeed	197	133	20	1.489	0.317
Mg (NaCl)	Control – Eifelgold	-1240	333	20	-3.722	**0.004**
	Control – Soilfeed	-13.2	333	20	-0.040	0.999
	Eifelgold - Soilfeed	1227	333	20	3.682	**0.004**

Table S5.4: Distribution of species with a total abundance of ≥ 50. Fg is the feeding guild (Siepel and de Ruiter-Dijkman (1993) of each species using the following groups: b (bacterivore), fb (fungivorous browser), fg (fungivorous grazer), gp (general predator), hb (herbivorous browser), hfg (herbo-fungivorous grazer), hg (herbivorous grazer), o (omnivore) and ohf (opportunistic herbo-fungivore. Results of an ANOVA model applied to all those species are shown in the right columns. All models followed the same type III ANOVA tables used in this study. SRP and soil inoculation were used as predictor variables with the experimental site as random factor. Each model was fit using a linear mixed-effect model and used the Kenward-Rogers method to approximate degrees of freedom for the ANOVA table. Significant p-values are given in bold.

Species	**Fg**	**Mean Abundance SRP**			**Mean abundance inoculation**		**Results ANOVA model**		
		Cont.	**Eifel.**	**Soilf.**	**Inocul.**	**Null**	**p SRP**	**p ino.**	**p int.**
Adoristes ovatus	Ohf	17	27	7	22	29	**0.031**	0.443	0.82
Alicorhagia fragilis	O	34	23	23	32	48	0.759	0.447	0.49
Atropacarus striculus	Hg	83	53	19	75	80	0.118	0.894	0.32
Berniniella sigma	Fg	104	8	46	109	49	**0.045**	0.202	0.19
Cocceupodes australis	O	54	70	29	73	80	0.147	0.785	0.77
Dissorhina ornata	Fg	8	54	2	47	17	0.106	0.358	0.35
Eniochthonius minutissimus	Fg	100	69	31	103	97	0.245	0.905	0.21
Eupodes truncatus	O	339	312	460	630	481	0.182	0.154	0.90
Folsomia quadrioculata	Hfg	326	473	272	606	465	0.297	0.387	0.80
Histiostoma sapromyzarum	B	8	11	41	15	45	0.308	0.303	0.19
Hypochthonius rufulus	O	154	137	122	181	232	0.903	0.559	0.31
Isotoma viridis	Hfg	106	16	23	106	39	0.293	0.391	0.29
Isotomiella minor	Fb	260	172	276	411	297	0.197	0.134	0.41
Lepidocyrtus lanuginosus	Hg	19	46	24	54	35	0.075	0.216	0.73
Malaconothrus processus	Hg	41	5	25	58	13	0.489	0.224	0.38

Megalothorax minimus	Fb	110	150	83	216	127	0.172	**0.044**	0.15
Mesaphorura sp.	Fb	90	177	69	209	127	0.188	0.284	0.23
Microtritia minima	Hfg	72	45	46	104	59	0.526	0.178	0.47
Microtydeus subterraneus	Hb	114	53	174	260	81	0.385	0.097	0.68
Nanhermannia nana	Hfg	50	77	58	104	81	0.619	0.508	0.72
Nanorchestes arboriger	Hb	357	243	123	366	357	**0.031**	0.934	0.40
Nothrus silvestris	Hfg	115	110	159	195	189	0.447	0.908	0.19
Oppiella nova	Fg	200	149	751	605	495	**0.017**	0.695	0.67
Pachylaelaps bellicosus	Gp	37	42	81	101	59	**0.041**	0.071	0.11
Parisotoma notabilis	Hfg	471	504	302	717	560	0.422	0.437	0.79
Pediculaster mesembrinae	Fg	87	1500	67	702	952	**0.002**	0.655	0.93
Pergamasus misellus	Gp	18	34	26	47	31	0.626	0.430	0.62
Pergamasus vagabundus	Gp	39	36	16	40	51	0.134	0.468	0.85
Phthiracarus anonymus	Hg	44	31	31	51	55	0.464	0.787	0.35
Phthiracarus globosus	Hg	25	42	22	36	55	0.363	0.360	0.96
Platynothrus peltifer	Hg	864	592	790	1000	1246	0.310	0.243	0.20
Protaphorura armata	Fb	75	224	206	311	194	**0.006**	0.058	0.16
Rhagidia punkva	Gp	30	18	40	47	41	0.639	0.113	0.06
Rhinoppia subpectinata	Fg	10	57	62	80	49	**0.035**	0.247	0.46
Rhodacarus coronatus	Gp	69	29	55	75	78	0.279	0.923	0.49
Rhysotritia duplicata	Hfg	195	48	62	197	108	**<0.001**	0.063	0.54
Schwiebea talpa	Fb	17	71	30	42	76	**0.013**	0.139	0.16
Scutacarus parvus	Fb	24	18	8	19	31	0.600	0.540	0.32

Sphaeridia pumilis	Fb	98	94	58	96	154	0.145	**0.036**	0.92
Steganacarus magnus	Hg	22	48	30	51	49	0.114	0.897	0.44
Subeupodes boerneri	O	49	31	3	32	51	0.060	0.422	0.91
Suctobelbella subcornigera	Fb	26	28	75	77	52	0.167	0.483	0.76
Tectocepheus velatus	Ohf	519	228	149	600	296	**0.029**	0.088	0.59
Uropoda minima	Gp	10	8	10	37	19	0.737	0.133	0.27
Veigaia nemorensis	Gp	46	44	72	75	87	0.133	0.526	0.29

Table S5.5: List of unique species in inoculated and null plots. Feeding guilds are taken from Siepel-de Ruiter-Dijkman (1993). Abundance is the total number of individuals and occurrence the number of samples where the species was found.

Species	Feeding guild	Inoculation	Abundance	Occurrence
Allacma fusca	Herbo-fungivorous grazer	Inoculation	1	1
Amblyseius obtusus	General predator	Inoculation	1	1
Armascirus cyaneus	General predator	Inoculation	1	1
Arrhopallites caecus	Fungivorous browser	Inoculation	1	1
Autogneta longilamellata	Fungivorous grazer	Inoculation	1	1
Brachychthonius berlesei	Fungivorous grazer	Inoculation	1	1
Brevipalpus obovatus	Herbivorous browser	Inoculation	1	1
Carabodes coriaceus	Opportunistic herbo-fungivorous	Inoculation	3	2
Carabodes marginatus	Opportunistic herbo-fungivorous	Inoculation	1	1
Coccorhagidia pittardi	General predator	Inoculation	1	1
Dendroseius reticulatus	General predator	Inoculation	3	1
Dicyrtoma minuta	Herbivorous grazer	Inoculation	1	1
Entomobrya multifasciata	Herbivorous grazer	Inoculation	1	1
Neanura muscorum	Opportunistic herbo-fungivorous	Inoculation	2	2
Neobisium carcinoides	General predator	Inoculation	1	1
Oribatula tibialis	Fungivorous grazer	Inoculation	5	3
Phauloppia rauschenensis	Herbivorous grazer	Inoculation	1	1
Protoribates lophotrichus	Fungivorous grazer	Inoculation	1	1
Rhizoglyphus echinopus	Fungivorous browser	Inoculation	33	2
Suctobelbella vera	Fungivorous browser	Inoculation	1	1
Trichouropoda ovalis	General predator	Inoculation	3	1
Tyrophagus similis	Fungivorous browser	Inoculation	1	1
Urodiaspis tecta	General predator	Inoculation	1	1
Zerconopsis remiger	General predator	Inoculation	1	1
Adelphacarus sellnicki	Fungivorous grazer	Null	1	1
Brachystomella parvula	Herbivorous grazer	Null	1	1

Carabodes labyrinthicus	Opportunistic herbo-fungivorous	Null	1	1
Cepheus cepheiformis	Fungivorous grazer	Null	3	3
Claveupodes delicatus	Omnivore	Null	2	1
Cymberemaeus cymba	Opportunistic herbo-fungivorous	Null	1	1
Dorycranosus acutus	Herbivorous grazer	Null	1	1
Epicrius canestrinii	General predator	Null	2	2
Linotetranychus cylindricus	Herbivorous browser	Null	1	1
Liochthonius evansi	Fungivorous grazer	Null	7	1
Paradamaeus clavipes	Fungivorous grazer	Null	2	2
Phthiracarus ferrugineus	Herbivorous grazer	Null	1	1
Quadroppia quadricarinata	Fungivorous grazer	Null	2	2
Veigaia cerva	General predator	Null	3	2
Veigaia exigua	General predator	Null	1	1

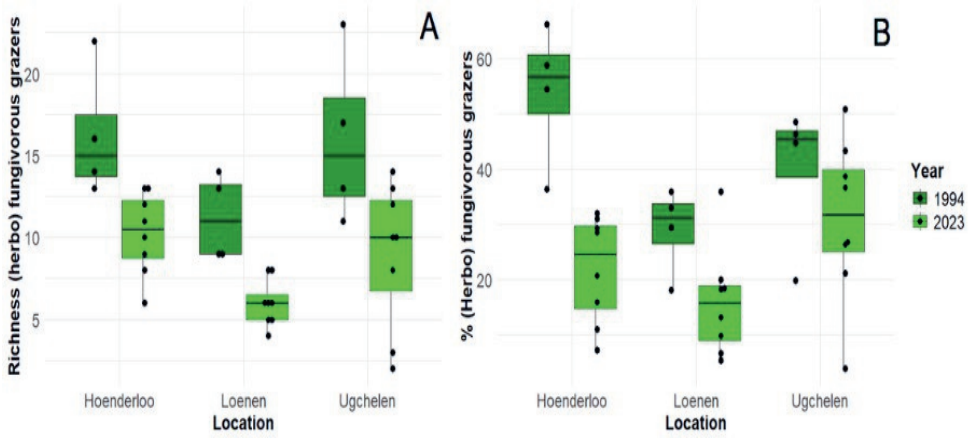


Fig. S5.1: The species richness of herbo-fungivorous grazers + fungivorous grazers (S1A) and % of herbo-fungivorous grazers + fungivorous grazers (S1B) in the three old forest locations. Each location had four soil samples in 1994 (Siepel et al. 1996; Bruggink et al. 2025) and eight soil samples in 2023. Soil samples in 2023 were taken in two separate moments, one sampling moment in February and one sampling moment in August.

Research Data Management

Chapter 2: Data available on Zenodo repository: https://zenodo.org/records/14651286

Chapter 3: Data is on embargo in the Zenodo repository and will be made publicly available upon acceptance in a journal**:** https://zenodo.org/records/18412857

Chapter 4: Data is on embargo in the Zenodo repository and will be made publicly available upon acceptance in a journal: https://zenodo.org/records/18414895

Chapter 5: Data is on embargo in the Zenodo repository and will be made publicly available upon acceptance in a journal: https://zenodo.org/records/18415067

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