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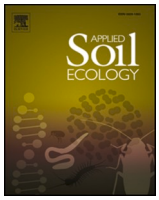

Soil age and soil phosphate content shape microarthropod communities of Dutch forest ecosystems

Joren Bruggink [a,*], Marie-Charlott Petersdorf [a], Wilco C.E.P. Verberk [a], Henk Siepel [a,b]

[a] Department of Ecology, Radboud Institute for Biological and Environmental Sciences, Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, the Netherlands
[b] Wildlife Ecology and Conversation, Wageningen University, Droevendaalsesteeg 3a, 6708 PB Wageningen, the Netherlands

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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 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.

1. 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 detrivores 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, 1975; 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 Edmonds, 1998). Many

* Corresponding author.
E-mail address: joren.bruggink@ru.nl (J. Bruggink).

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microarthropod species are additionally poor colonizers (Ojala and Huhta, 2001; Lehmitz et al., 2012; 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. (2013) for Dutch forests. The poor ability to colonize new sites in non-phoretic Acari differs from that of Collembola which are able to 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., 2018). Soil acidification additionally creates the formation of insoluble aluminum phosphates (Stoddart, 1909; 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 as fungivorous species due to plants being less stable in their N:P ratio as 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 (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).

2. Methods

2.1. 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. 1). A list of all locations is shown in Supplementary Table S1. These 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.

These old forest soil stands are the same as described in Siepel (1996b) and have been forest 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 top soil 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.

2.2. 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 de 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*, *Mesophorura*, 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

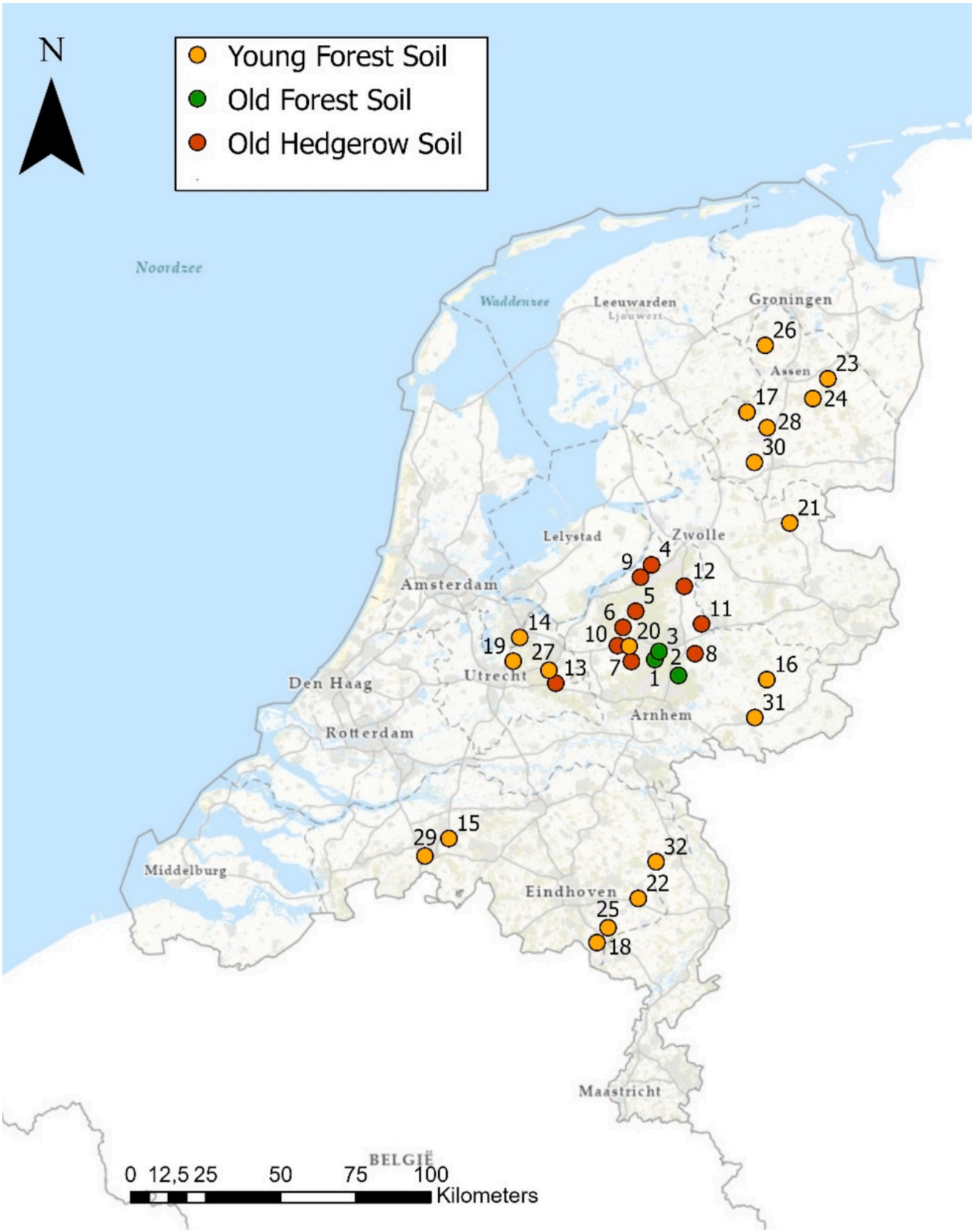

Fig. 1. The forest sites included in the project with a label for the site ID (see Table S1 for more info). The colours indicate different categories with young forests in yellow, old hedgerows in red and old forest soils in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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.

2.3. 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.

2.4. 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 interested 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 aluminum trays (40.5 ml), completely filled to determine soil density and reweighted after oven-drying (48 h, 60 °C). The fraction of organic matter was calculated by loss on ignition (LOI). Dried soil material was incinerated for 4 h 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 g 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 min 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).

2.5. 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.

$$\text{expected number of unique taxa} = \sum \left(\frac{\text{Fcat(N)}}{\text{T(N)}} \right) \tag{1}$$

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

$$\text{Old Forest (N)} = \frac{3!}{(3-\text{N})!\text{N}!} \tag{3}$$

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

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

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).

3. Results

3.1. 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. 2A, Supplementary Table S2.1 and Supplementary Table S3.1). In contrast, there was no significant difference in springtail taxa richness between forest categories (ANOVA, $F_{2.29} = 1.31$, $P = 0.284$; Fig. 2B, Supplementary Table S2.1 and Supplementary Table S3.1). Differences

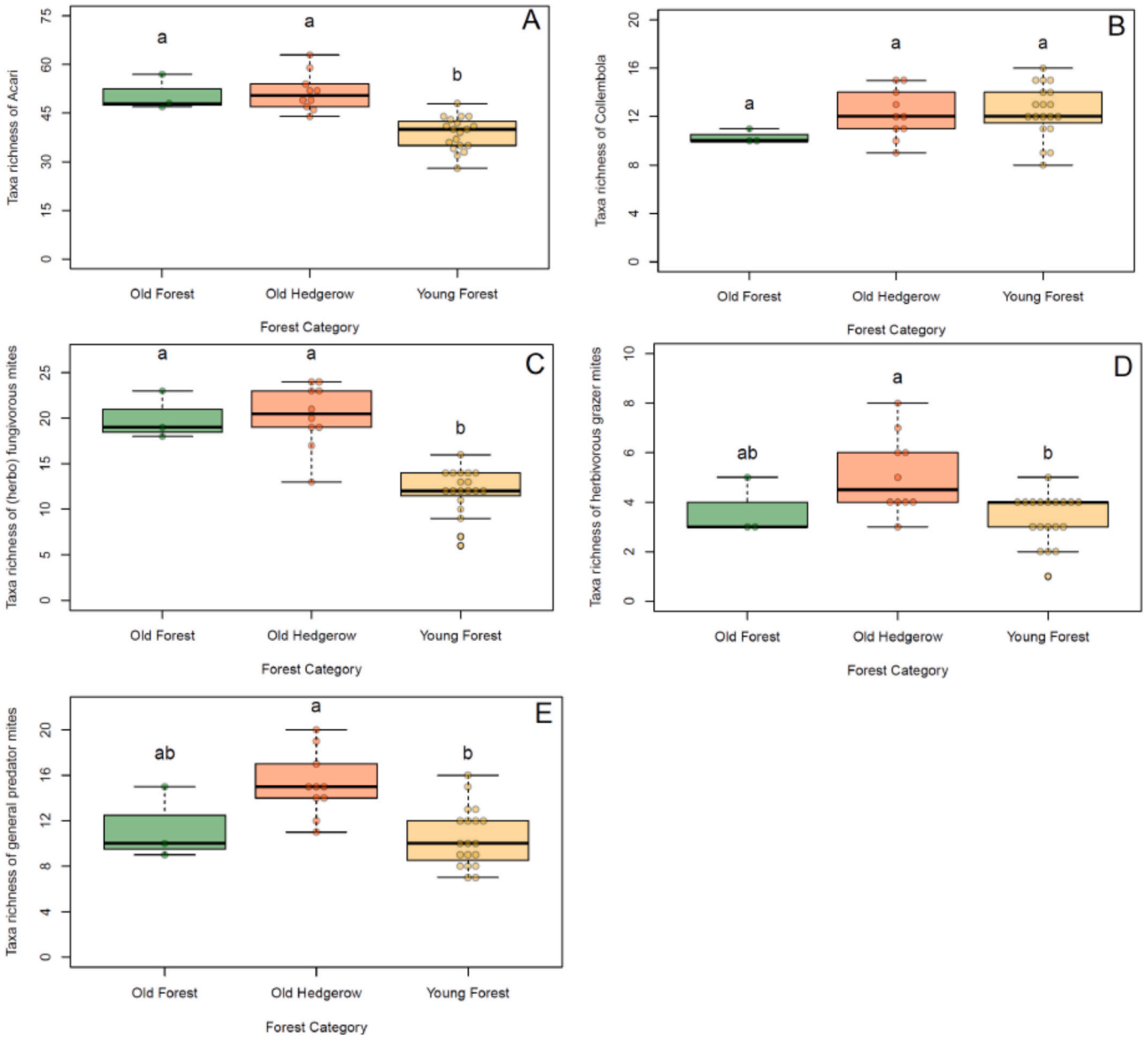

Fig. 2. Mean Acari taxa richness (A), mean Collembola taxa richness (B), mean (herbo)fungivorous mite taxa richness (C), mean herbivorous grazer mite taxa richness (D) and mean general predator mite taxa richness (E) in each forest category. Significance levels are tested with a Tukey HSD test and illustrated with different letters.

in 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. 2C, Supplementary Table S2.1 and Supplementary Table S3.1). 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. 2D, Supplementary Table S2.1 and Supplementary Table S3.1; General predators, ANOVA, $F_{2,29} = 9.77$, $P < 0.001$; Fig. 2E, Supplementary Table S2.1 and Supplementary Table S3.1). For both feeding guilds, the taxa richness was significantly higher in old hedgerow soils than in young forest soils (Herbivorous grazers, Tukey HSD, $P = 0.002$; General predators, Tukey HSD, $P < 0.001$, Supplementary Table S3.1).

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 S4). 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, d.f = 2, $\chi^2 = 98.28$, $P < 0.001$). When focussing on separate feeding guilds within the mites (Fig. 3; Supplementary Table S4), 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

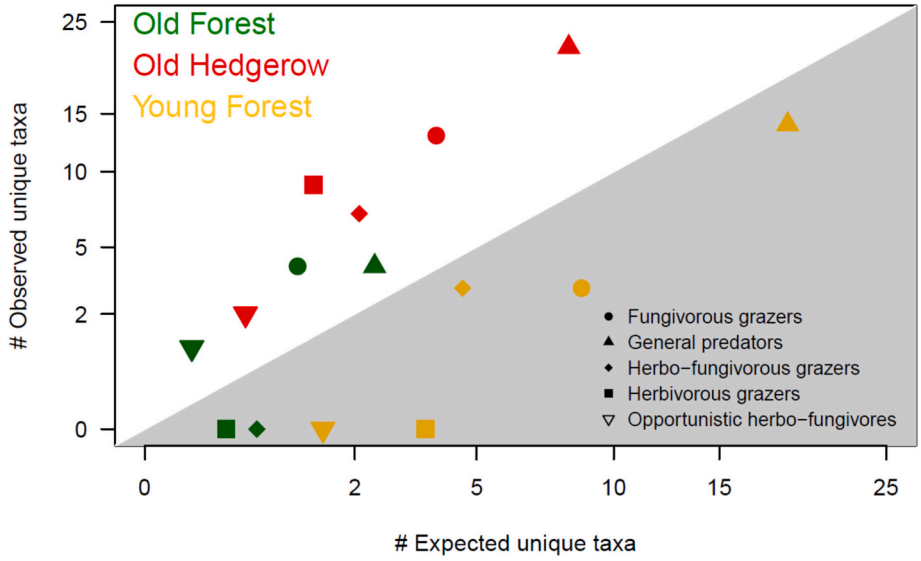

Fig. 3. The number of unique and expected taxa for all three forest categories with several of the more common feeding guild groups of mites. The axis is square root transformed. The line y = x shows the expected values if the number of observed taxa is exactly as would be expected if taxa were randomly dispersed. Colours are used to indicate the forest type and shapes to indicate mite feeding guilds.

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.

All species that were unique for old forest soils are shown in Supplementary Table S5. 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

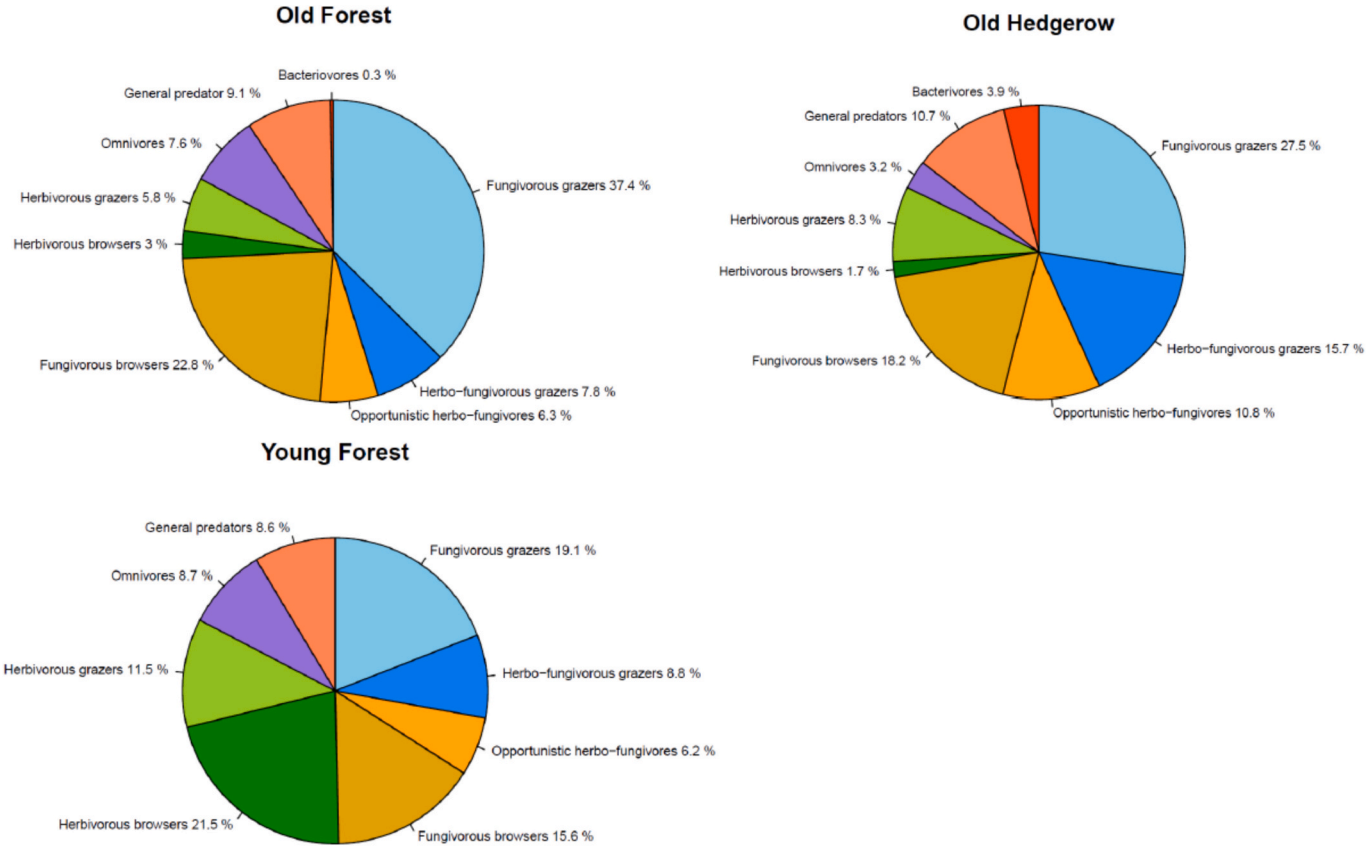

Fig. 4. The average Acari feeding guild distribution of forest locations. Colours are used to indicate the feeding guilds. The blue signifies (herbo) fungivorous grazers, orange and brown the fungi feeders that only feed on cell content, green for herbivorous mites and purple and red for omnivorous and predatory feeding guilds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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 S6). 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 S7).

3.2. 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. 4, Supplementary Table S2.2 and Supplementary Table S3.2). 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. 4, Supplementary Table S2.2 and Supplementary Table S3.2), 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 3225.57 (±1032.65) ind./m^2 in old forests and 14,769.70 (±9165.44) ind./m^2 in old hedgerows. Despite old hedgerow soils hosting the most species of herbivorous grazers (Fig. 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

3.3. 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 S2.3 and Supplementary Table S3.3). Mites smaller than 900 μm were more numerous than large mites, but their densities did not differ across soil categories (Fig. 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. 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. S1, Supplementary Table S2.4 and Supplementary Table S3.4).

4. 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 we used a (slightly) larger auger 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, 2001; 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 also 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., 2022). 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 were 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

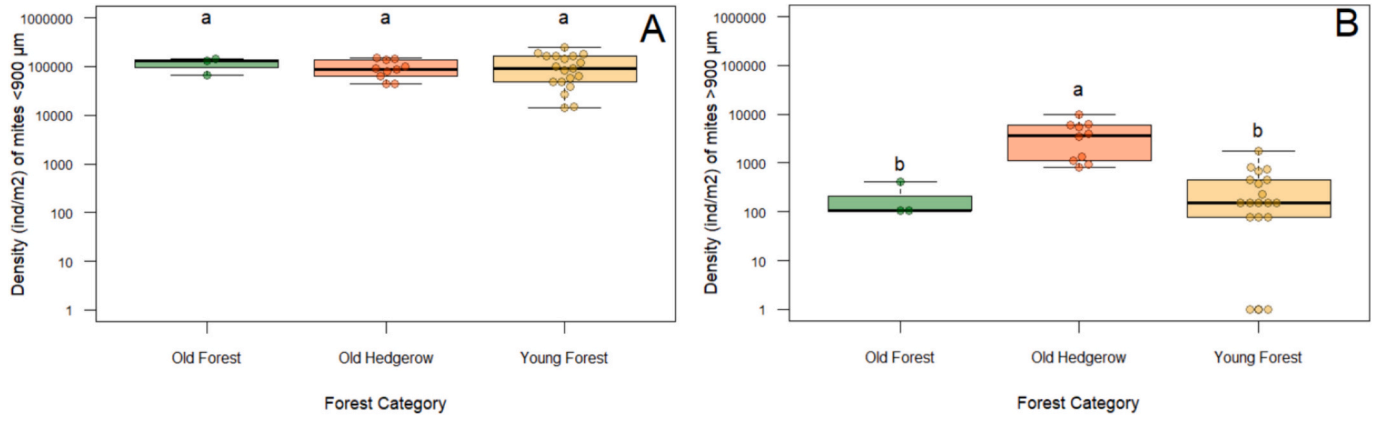

Fig. 5. The density (ind/m^2) of mite taxa smaller than 900 μm (A) and mite taxa larger than 900 μm (B) grouped by forest category. The axis is log10 transformed. Significance levels are illustrated with letters (A is significantly higher as B, AB is no significance).

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. S1).

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 in order 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 colonization 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.

CRediT authorship contribution statement

Joren Bruggink: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Marie-Charlott Petersdorf:** Writing – review & editing, Conceptualization. **Wilco C.E.P. Verberk:** Writing – review & editing, Visualization, Software, Conceptualization. **Henk Siepel:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.105918.

Data availability

The data is available on Zenodo using this link: https://doi.org/10.5281/zenodo.14651285

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